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Effect of 2-Mercaptoethanol on enzymes secretion by *Aspergillus niger* in submerged fermentation

Muhammad Irfan and Quratulain Syed*
Food and Biotechnology Research Center (FBRC), PCSIR Laboratories Complex, Ferozpure Road, Lahore, Pakistan

Abstract: The present study was concerned with the production of various enzymes such as amylase, cellulase, xylanase, avicelase and FPase by *Aspergillus niger* in submerged fermentation at 30°C for 72h of fermentation period using Vogel's media and sugarcane bagasse as a substrate. Different concentrations of 2-mercaptoethanol were evaluated on the production of enzymes by *Aspergillus niger*. It was observed that addition of 2-mercaptoethanol to the fermentation medium greatly inhibited the xylanase and α-amylase production by *Aspergillus niger* but addition of 20µl concentration of 2-mercaptoethanol to the fermentation medium favored the production of CMCase, FPase and total protein.

Keywords: Enzyme, *Aspergillus niger*, sugarcane bagasse, 2-mercaptoethanol, submerged fermentation.
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***Author for Correspondence**: mirfanashraf@yahoo.com

INTRODUCTION

A large number of industrial processes utilize lot of enzymes in various industries for example lipases, amylases, proteases, cellulases, xylanases, pectinases and other enzymes[1]. One of the top most enzyme being used in many industries is α-amylase. These enzymes account for 65% of enzyme market in the world. These enzymes are produced by either fungi or bacteria in different fermentation processes. Mostly fungi are utilized for the production of these enzymes, and according to one report *Aspergillus niger* can produce 19 different types of enzymes[2]. For fungal amylase production in developing countries, mostly *Aspergillus* sp is used due to ubiquitous nature and non-fastidious nutritional requirements[3]. For the production of high-priced materials and for the study of biochemical and physiological aspects of the synthesis of microbial metabolites, submerged fermentation (SmF) systems have been extensively used[4]. In this study strain of *Aspergillus niger* is used for the production of various enzymes such as amylase, cellulase, xylanase, avicelase and FPase in submerged fermentation system using sugarcane bagasse as a substrate and the effect of different concentrations of 2-mercaptoethanol were studied on these enzyme production.

MATERIALS AND METHODS

Lignocellulosic biomass

Sugarcane bagasse was purchased from local market of Lahore city, Pakistan which was used as a substrate in submerged fermentation process. The substrate was washed and oven dried at 65°C and then ground to powder form (2mm) by hammer beater mill.

Microorganism

Aspergillus niger was obtained from Microbiology Laboratory, FBRC, PCSIR Labs. Complex Ferozpure road Lahore. It was maintained on PDA slants and revived biweekly.

Inoculum preparation

Inoculum was prepared by adding sterilized distilled water into the 5-day old slant. With the help of inoculating loop the mycelia was mixed and one ml ($2x10^8$) of spore suspension was used as inoculum. Inoculum size was measured with heamacytometer as described earlier[5].

Fermentation methodology

Submerged fermentation was carried out in 250ml Erlenmeyer flask at 30°C. Twenty five milliliter of Vogel's media with different concentration of 2-mercaptoethanol (0, 20, 40, 60, 80 and 100µl) using 2% substrate (sugarcane bagasse) was taken and sterilized at 121°C for 15min. After sterilization, the media was inoculated with 1ml of spore suspension and incubated at 30±1°C with agitation speed of 120rpm for three days of fermentation period.

Estimation of CMCase

CMCase activity was estimated in fermented broth as described earlier[6]. 500µl of the enzyme sample along with 500µl of 1% (w/v) CMC in 50 mM acetate buffer pH 5 was incubated, in a water bath at 50 °C for 30 min. After incubation 3ml of DNS was added and boiled for 5 minutes and absorbance was taken spectrophotometrically at 540nm. The reducing ends liberated were then measured with DNS. One enzyme activity unit (U) was defined as the amount of enzyme releasing 1

µmol of glucose from the substrate under standard assay conditions.

Determination of xylanase activity

The activity of α-amylase was assayed by incubating 0.5mL of the diluted enzyme with 0.5 mL soluble starch (0.5 %, w/v) prepared in 0.1 M sodium Phosphate buffer, pH= 7. After incubation at 60 ºC for 10 minutes the reaction was stopped and the reducing sugars released were assayed colorimetrically by the addition of 1 mL of 3-5-dinitrosalicylic acid reagent[7]. One enzyme activity unit (U) was defined as the amount of enzyme releasing 1µmol of xylose from the substrate under standard assay conditions.

Estimation of FPase

For the estimation of FPase activity, 500µL of culture filtrate was added to test tube containing Whatman No.1 filter paper strip (1x 6cm) incubated at 50°C for 30 minutes. After that 1.5ml DNS were added to test tube and boiled for 10 minutes and reducing sugars liberated were then measured by absorbance which was taken spectrophotometrically at 550nm. One enzyme activity unit (U) was defined as the amount of enzyme releasing 1µmol of glucose from the substrate under standard assay conditions as described earlier[8].

Estimation of avicelase activity

Avicelase activity was measured by incubating 0.5ml of enzyme sample with 0.5ml of avicel (1% avicel prepared in 0.05M acetate buffer pH 5), incubated at 50^{o}C for 30min. After incubation, 1.5 ml DNS were added to test tube and boiled for 10 minutes reducing sugars liberated were then measured by absorbance which was taken spectrophotometrically at 550 nm [7]. One enzyme activity unit (U) was defined as the amount of enzyme releasing 1 µmol of glucose from the substrate under standard assay conditions.

α-Amylase assay

The activity of α-amylase was assayed as described earlier[9]. Reaction mixture containing 0.5mL of the diluted enzyme with 0.5mL soluble starch (0.5%, w/v) prepared in 0.1M sodium Phosphate buffer, pH=7 incubated at 60ºC for 15 minutes. After incubation at 60ºC for 10 minutes the reaction was stopped and the reducing sugars released were assayed colorimetrically by the addition of 1 mL of 3-5-dinitrosalicylic acid reagent. One enzyme activity unit (U) was defined as the amount of enzyme releasing 1 µmol of maltose from the substrate in 1 minute at 60ºC.

Estimation of total proteins

Total proteins in the culture filtrate were estimated by Lowery method[10] using bovine serum albumin as standard protein.

RESULTS AND DISCUSSION

Figures 1 and 2 represented the xylanase and amylase production at different concentrations such as 0, 20, 40, 60, 80 and 100µl of 2-mercaptoethanol using Vogel's media by *Aspergillus niger* is submerged fermentation at 30^{o}C for 72h of fermentation period. Results indicated that addition of 2-mercaptoethanol suppresses the enzyme synthesis. When 2-mercaptoethanol was not added to the medium xylanase yield of 39.0±1.23IU was obtained as compared to the addition of 20µl 2-mercaptoethanol which gave xylanase activity of 18.5±1.01IU. When 100µl of 2-mercaptoethanol was added to the medium xylanase synthesis was completely retards showing activity of 1.2±0.01IU. The alpha amylase production at 0µl and 100µl are 2.0±0.02IU and 0.02±0.001IU respectively.

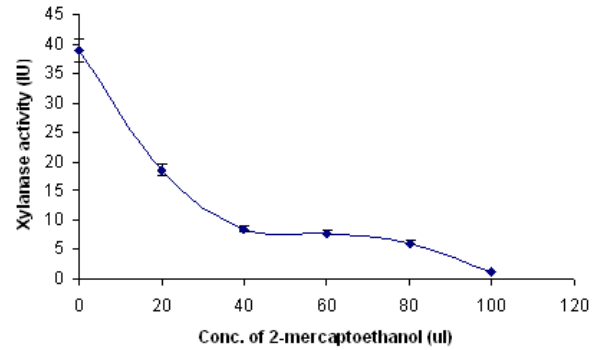

Figure 1: Xylanase production at different concentrations of 2-mercaptoethanol by *Aspergillus niger* in submerged fermentation at 30ºC.

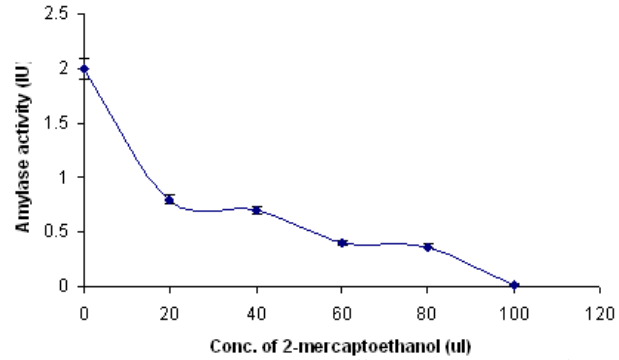

Figure 2: Amylase production at different concentrations of 2-mercaptoethanol by *Aspergillus niger* in submerged fermentation at 30ºC with agitation speed of 120rpm.

2-mercaptoethanol strongly inhibits the enzyme activity indicating the presence of disulphide bridges in the endoglucanase enzyme[11]. Usharani and Muthuraj[12] worked on protease production by *Bacillus laterosporus* and reported that 2-mercaptoethanol with concentration of 1mM slightly inhibit the enzyme activity. Murthy and Naidu[13] produced protease from *Aspergillus oryzae* in solid state fermentation and reported that the protease activity was inhibited by 2-mercaptoethanol.

Avicelase production was also checked by adding various concentrations of 2-mercaptoethanol to the fermentation medium. Results (Figure 3) indicated that addition of 20µl of 2-mercaptoethanol to the fermentation medium supported the enzyme production (1.6 ± 0.09 IU) as compared to control (0.8±0.04IU). But as the concentration of 2-mercaptoethanol was increased there was decline in enzyme production was observed. Addition of 100µl of 2-mercaptoethanol totally blocked the enzyme production which was 0.001 IU.

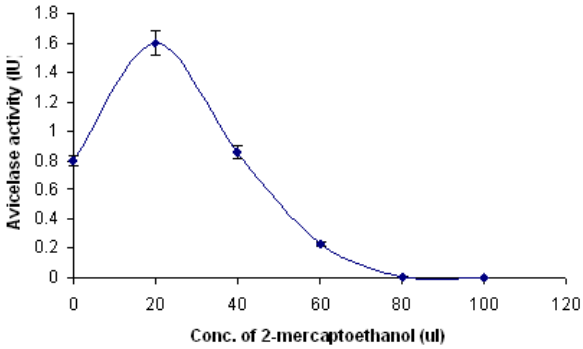

Figure 3: Effect of different concentrations of 2-mercaptoethanol on avicelase production by *Aspergillus niger* in submerged fermentation at 30°C.

Figures 4, 5 and 6 illustrated the same pattern showing that 20µl of 2-mercaptoethanol significantly favored enzyme production by *Aspergillus niger* in submerged fermentation. When CMCase production (Figure 4) was observed, it was found that addition of 20 µl of 2-mercaptoethanol gave 2.2 ± 0.03 IU as compared to 0 µl (control) 1.7±0.02IU. FPase production (Figure 5) is also greatly improved by the addition of 20 µl of 2-mercaptoethanol to the fermentation medium. FPase activity of 3.9±0.05IU was achieved as compared 0 µl (control) 1.2±0.03IU. Maximum total protein secretion of 1263.5±2.65µg/ml was also observed at the addition of 20µl of 2-mercaptoethanol to the fermentation medium as shown in the figure 6. Further increase in concentration of 2-mercaptoethanol resulted in decline in CMCase, FPase and total protein secretion by the tested fungi. Damaso et al[14] worked on xylanase production *Thermomyces lanuginosus* Ioc-4145 in submerged fermentation and reported that 2-mercaptoethanol at the concentration of 5mM has stimulatory effect on xylanase activity. Saurbh et al[15] studied on protease production by *Bacillus* sp. and reported that 2-mercaptoethanol at the concentration of 1mM strongly stimulate the protease activity.

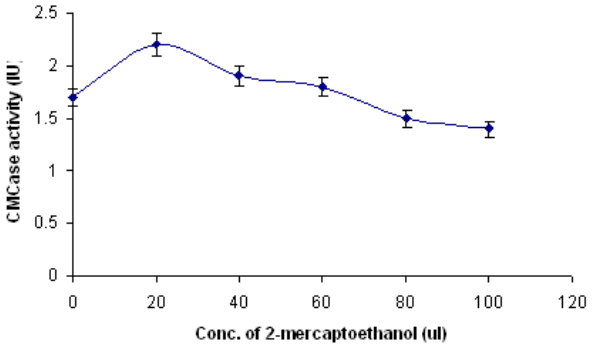

Figure 4: CMCase production at different concentrations of 2-mercaptoethanol by *Aspergillus niger* in submerged fermentation.

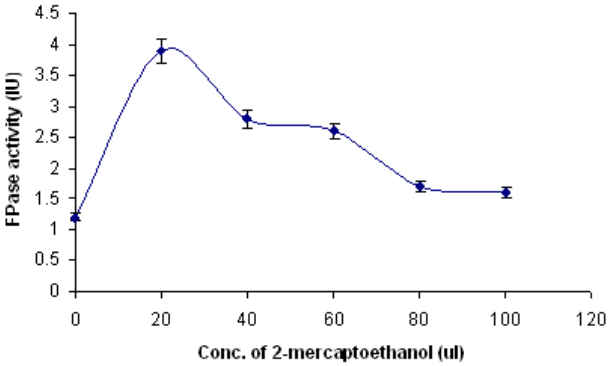

Figure 5: Production of FPase at different concentrations of 2-mercaptoethanol by *Aspergillus niger* in submerged fermentation at 30°C for three days.

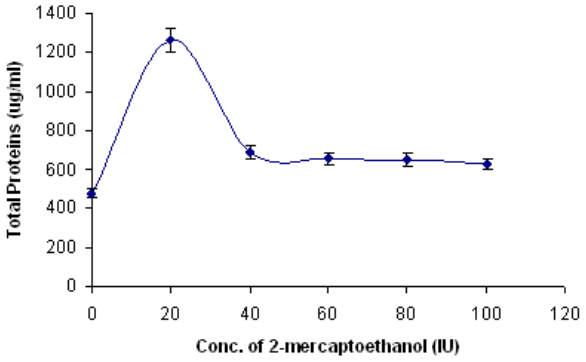

Figure 6: Total protein secretion at different concentrations of 2-mercaptoethanol by *Aspergillus niger* in submerged fermentation.

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