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OPTIMIZATION OF INOCULUM VOLUME, FERMENTATION MEDIUM AND AERATION RATE FOR THE PRODUCTION OF GLUCOSE OXIDASE BY UV MUTANT STRAIN OF *ASPERGILLUS NIGER* AN-14

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Abstract

In this study the effect of inoculum size, volume of fermentation medium and aeration rate was analyzed for UV mutated strain of *Aspergillus niger* AN-14 regarding enhanced production of glucose oxidase. UV treated 10 variants showing positive variations from parent strain were isolated and analyzed for glucose oxidase production using shake flask fermentation. Mutant strain AN-14 gave maximum production of glucose oxidase (44.61 U/g of cell mass) as compared to wild strain W-47 (21.74 U/g of cell mass). About 100% increase in the production of glucose oxidase was observed by the mutant strain. The experiments were up scaled and carried out in a 7.5 L capacity bioreactor with working volume of 5 L. It was concluded that 65% volume of total bioreactor, 5% inoculum of the total volume of fermentation medium and aeration rate of 0.8vvm were optimum for glucose oxidase production by the mutant strain. The optimized yields of glucose oxidase for wild and mutant strains were recorded as 26.48U/g of cell mass and 50.39 U/g of cell mass, respectively.

Key Words: Inoculum, Bioreactor, Mutant, Fermentation.

Introduction

The enzyme glucose oxidase (β–D-glucose: oxygen 1-oxidoreductases, E.C. 1.1.3.4) is an oxidizing enzyme. It is termed as a flavo-protein since it carries a flavin adenine dinucleotide (FAD) molecule bound non-covalently but tightly to the active site of the homodimer (Horaguchi *et al*., 2012). It is a commercially significant enzyme due to its high stability and turnover, as well as, high specificity for β anomer of glucose. Thus, it has numerous applications in food and beverage preservation, in medical industry as a glucose biosensor, as well as, as a biofuel (Kona *et al*., 2001; Gunny *et al*., 2011).

Glucose oxidase has been isolated from different sources. It is naturally produced from several sources such as bacteria, insects like caterpillars (Musser *et al*., 2005) and fungi. However, mostly the studies of the enzyme from *Aspergillus niger* and *Penicillium amagasakiense* have been carried out. Currently, *Aspergillus niger* is the preferred source for the industrial production of glucose oxidase (Yoon *et al*., 2010). Therefore, the source utilized in the present study is *Aspergillus niger*.

Random mutagenesis can be utilized to improve the yield of the enzyme glucose oxidase. Physical mutagenesis by UV radiation (200-300 nm) results in changes in the DNA. The primary alteration is the formation of pyrimidine dimers which form harmful lesions, resulting in the blockage of transcription and DNA replication (Iftikhar *et al*., 2010). This strategy is used by different researchers for the enhanced production of the desired product.

There are various factors which significantly influence the production of glucose oxidase; these factors are usually studied by the classical method of one factor at a time. Among these factors, inoculum size, volume of fermentation medium and aeration rate of the medium are crucial for the biosynthesis of metabolites by fungi (Odebunmi & Owalude, 2007).

Materials and Methods

Microorganism and UV Mutagenesis: The glucose oxidase producing wild strain of *Aspergillus niger* W-47 was taken from the culture bank of Institute of Industrial Biotechnology, GC University, Lahore. It was maintained on potato dextrose agar slants and sub cultured after every 7 days. For UV mutagenesis, the spore suspension of 48 hrs old *Aspergillus niger* (W-47) culture was prepared. It was then diluted serially up to spore concentration of 1.5 $x10^{-3}$ and about 100 µL of this dilution was inoculated on PDA plates. Each of the plates was kept at a distance of about 8 cm from UV lamp and exposed to the UV rays by varying the time from 5 to 15 min (Azin & Noroozi, 2001). After the exposure, the plates were incubated and protected from light to avoid reversion of the mutation (Fiedurek & Gromada, 1996; Mala *et al*., 2001).

The initial screening of mutant strains was done on the basis of plate assay on agar plates with O-dianisidine and peroxidase. Production of glucose oxidase was indicated by the formation of zone around the colonies (Zia *et al*., 2010). Potentially valuable glucose oxidase producing mutants were analyzed using submerged fermentation.

Inoculum preparation: A slant of mutant strain of *A. niger* (AN-14) was used to prepare spore suspension. The homogenized spore suspension (2ml) was transferred to the flask containing 50mL of nutrient broth and then incubated at 30°C for 24 hrs in order to get vegetative inoculum.

Submerged Fermentation: The fermentation experiments were carried out in 7.5 liters bioreactor containing 5 L of fermentation medium which consisted of (g/L) glucose, 80; peptone, 3.0; $(NH_4)_2HPO_4$, 0.388, KH_2PO_4, 0.188; $MgSO_4.7H_2O$, 0.156 and $CaCO_3$, 35 (Fiedurek & Szczodrak, 1995). All the experiments were

carried out under constant conditions of medium pH (7.0), incubation temperature (30°C) and Shaking (250 rpm). Various inoculum sizes, from 1-10% of the fermentation medium were checked for production of glucose oxidase. Similarly, the volume of the fermentation medium was varied from 50 to 80% of the total volume of the bioreactor to determine optimum volume of fermentation medium. The effect of aeration was also analyzed in a range of 0.2 to 1 vvm.

Down streaming of Glucose Oxidase: The fungal cell mass was filtered and homogenized in 0.1 M citrate phosphate buffer. It was subjected to centrifugation and crude extract obtained was further used for glucose oxidase assay. The protein content was determined by Bradford method (Bradford, 1976).

Glucose Oxidase Assay: The activity of glucose oxidase was determined by O-dianisidine method. The enzyme catalyzes the conversion of glucose to gluconic acid. The basic principle is the detection of hydrogen peroxide in the presence of peroxidase, indirectly indicated by a color change which is analyzed by spectrophotometry. One unit of glucose oxidase is defined as the amount of enzyme catalyzing one micromole of glucose per minute.

Statistical Analysis: Computer software Costat, cs6204W.exe was used for the statistical analysis. Significance difference among replicates has been presented as Duncan's multiple range tests in the form of probability (p) values.

Results and Discussion

Mutant strain: UV-mutant strain AN-14 gave the maximum output of glucose oxidase (44.61 U/g of cell mass) as compared to parent strain (21.74 U/g of cell mass) as shown in Fig 1. Thus, this strain was selected for the optimization of said parameters.

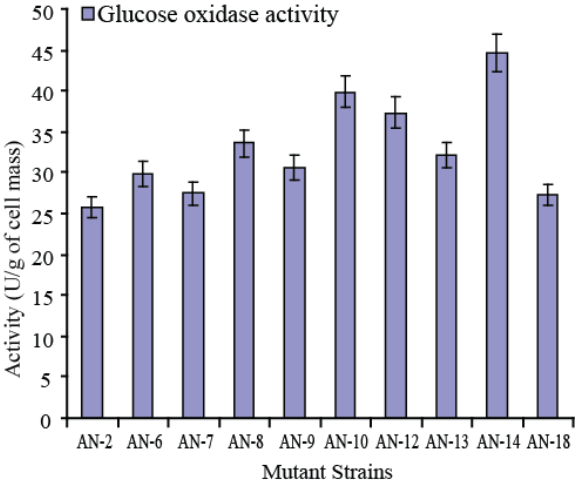

Fig. 1. Glucose oxidase activity of positive variants of parent strain W-47. The standard deviation (SD ≤ ± 0.05) between the three replicates is represented by Y- error bars

Effect of volume of fermentation medium: It was observed that rate of production was enhanced as volume of the medium was gradually increased above 55%. The maximum production of glucose oxidase was observed at 65% of total volume of the bioreactor for both mutant (47.98 U/g of cell mass) and wild strains (24.37 U/g of cell mass). The rate of biomass and enzyme production was reduced as the volume of the fermentation medium was increased above 65% (Figs. 2 & 3).

Effect of inoculum size: The maximum production of the enzyme was observed at 5% of inoculum size for both the mutant (50.09 U/g of cell mass) and wild strains (26.47 U/g of cell mass) (Figs. 4 & 5). Above 5% of inoculums cell mass and enzyme production started to decrease. However cells mass production was less up to the use of 4% inoculums size. Same trend was observed for the product yield.

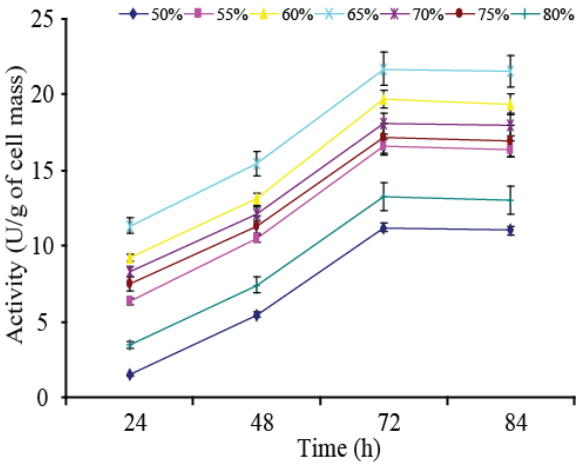

Fig. 2. Effect of volume of fermentation medium on the production of Glucose oxidase by wild strain W-47. The standard deviation (SD ≤ ± 0.05) between the three replicates is represented by Y- error bars.

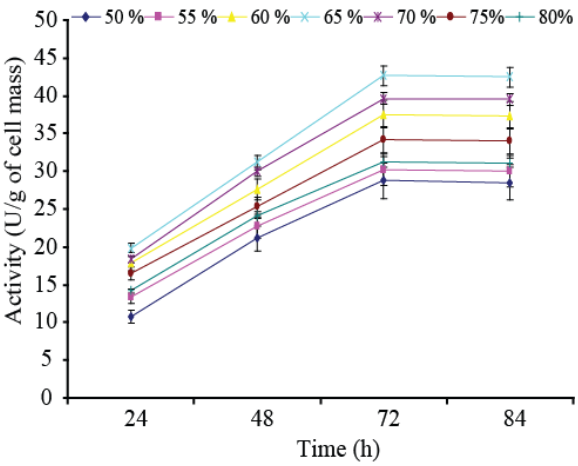

Fig. 3. Effect of volume of fermentation medium on the production of Glucose oxidase by mutant strain AN-14. The standard deviation (SD ≤ ± 0.05) between the three replicates is represented by Y- error bars.

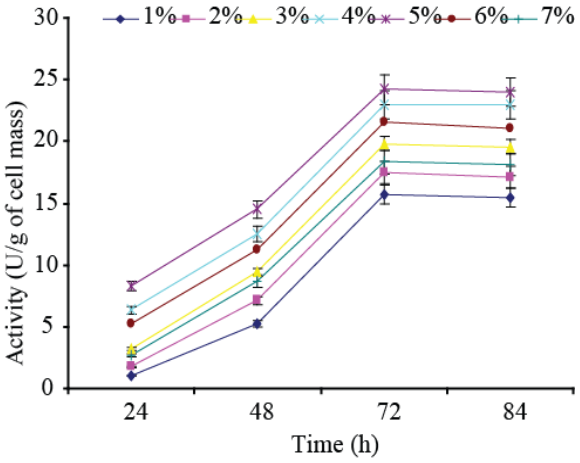

Fig. 4. Effect of inoculum size on the production of Glucose oxidase using wild strain W-47. The standard deviation (SD ≤ ± 0.05) between the three replicates is represented by Y- error bars.

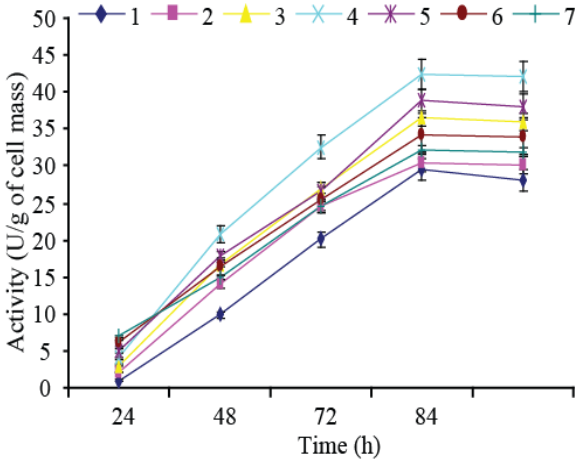

Fig. 5. Effect of inoculum size on the production of Glucose oxidase using mutant strain AN-14. The standard deviation (SD ≤ ± 0.05) between the three replicates is represented by Y- error bars

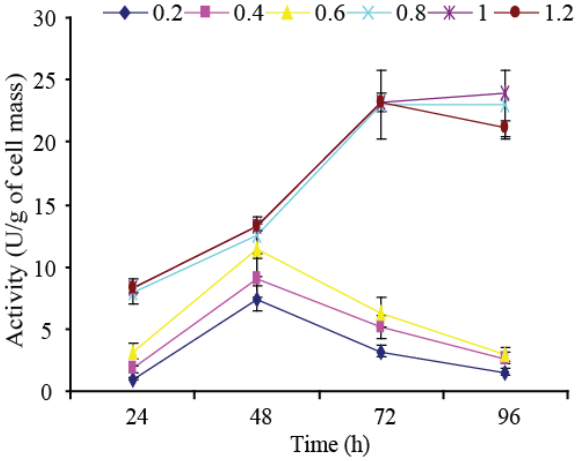

Fig. 6. Effect of aeration rate (%) using wild strain W-47. The standard deviation (SD ≤ ± 0.05) between the three replicates is represented by Y- error bars.

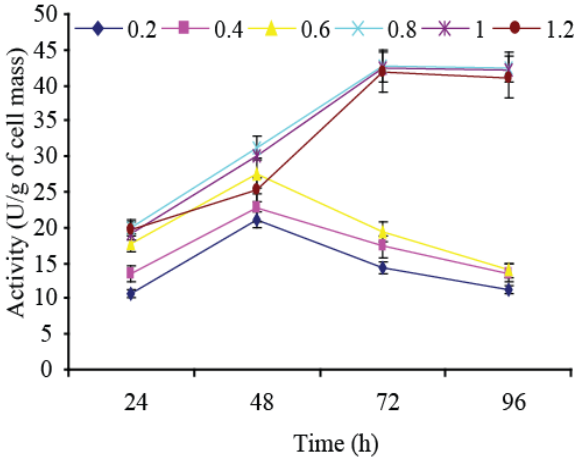

Fig. 7. Effect of aeration rate (%) using mutant strain W-47. The standard deviation (SD ≤ ± 0.05) between the three replicates is represented by Y- error bars.

Effect of aeration rate: Variation in the aeration level of the culture medium has specific effect on the growth of microorganism and product formation during a submerged fermentation. On increasing the aeration rate from 0.2 to 1.2vvm, increase in activity was recorded initially up to 0.6vvm. The enzyme activity however remained constant from 0.8 to 1.2vvm. Both wild (26.48 U/g of cell mass) and mutant strains (50.39 U/g of cell mass) showed the same pattern of change in the activity as that of change in the aeration rate indicated in Fig. 6 & Fig.7.

Discussion

The rate of maximum cell mass product formation observed using 65 % of fermentation medium was due to the optimum supply of oxygen in the medium and might be due to efficient agitation which can be done using this volume in bioreactor that allows equal distribution of nutrients to the microorganisms. The further increase in fermentation medium volume resulted decrease in product yield which might be due to the decreased rate of oxygen supply to the organism due to less available space in fermenter as reported by Mukhtar & Haq (2007). Our results are in correspondence with Bankar *et al*., (2009) and contrary to Irfan *et al*., (2011) who reported optimum inoculum percentage as 3% (V/V). This might be due to the equal distribution of nutrients available in media to the amount of spores present in particular percentage of inoculum which leads to the rapid and enhanced growth of cell mass as well as product formation. As the inoculum percentage increased the nutrients available in the medium mightn't not are enough to support their growth in such high amount. Maximum enzyme production using 0.8 vvm might be due to the increase in cell mass with increase in oxygen supply which is sufficient for the cell mass to grow completely to produce maximum amount of glucose oxidase. Similar types of results are reported by Mukhtar & Haq (2007) with same pattern of increase in activity with shift in aeration rate.

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