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Optimization of Various Parameters for the Production of Glucose Oxidase from Rice Polishing Using *Aspergillus niger*

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Abstract: The enzyme glucose oxidase was produced by fermentation technology, using *Aspergillus niger* as fermentation organism and rice polishing as substrate. Maximum production of enzyme (3.42 U mL^{-1}) was recovered at substrate level of 2% after 36 h of submerged fermentation. The pH for the optimal production of enzyme was found to be 4. Addition of salts such as urea (0.3%), $CaCO_3$ (0.04%) and KH_2PO_4 (0.6%) into the fermentation medium enhanced enzyme production while $MgSO_4.7H_2O$ was found to inhabit microbial growth and glucose oxidase production by *A. niger*.

Key words: Glucose oxidase, rice polishing, fermentation, *Aspergillus niger*

Introduction

Enzymes can be defined as soluble colloidal organic catalysts which are produced by living cells but are capable of acting independently of the cells (Rao, 1993). Glucose oxidase (EC. 1.1.3.4) belongs to class oxidoreductase and is also called as glucose aerodehydrogenase (Sidney and Northon, 1955). It is a flavo-enzyme that catalyzes the oxidation of β-D-glucose to δ-D-gluconolactone and H_2O_2 is also formed in this reaction. It is highly specific for β-D-glucose while α-anomer is not acted upon. Glucose oxidase has a molecular weight of 160,000 and consists of two identical polypeptide chain subunits linked by disulfide bonds, optimum pH for the enzyme is 5.5 with broad range of 4-7 (Bentley, 1959).

Glucose oxidase is produced from most of the microorganisms such as ***Penicilluim notatum***, ***Penicillium chrysosporium***, ***Aspergillus niger*** and ***Botrytis cinerea*** (Liu ***et al.***, 1998). The glucose oxidase from ***Aspergillus niger*** is an intracellular enzyme present in the mycelium of the organism (Willis, 1966). The present research project was designed for optimization and production of enzyme based glucose oxidase from ***Aspergillus niger*** for ultimate use in glucose estimation kit.

Materials and Methods

Microorganism and fermentation

Pure culture of fungus ***Aspergillus niger*** procured from NIBGE, Faisalabad was raised on potato starch-agar slants, sporulation medium. It was incubated aerobically at pH 4 and 30°C for 72 h.

Conical flasks with 100 mL of rice polishing medium containing different concentrations of micro-nutrients were inoculated with 5 mL of homogenous spore suspension (10^7-10^8 spores mL^{-1}). The flasks were incubated at pH 4 and 30°C on a shaker (120 rpm) for optimum fermentation period (Zubair ***et al.***, 2002). The fermented biomass in each case was filtered and then blended to extract intra-cellular enzyme finally the filtrates were centrifuged. The supernatant was ultra-filtered through filter paper and the filtrate was assayed for glucose oxidase (Zia, 2002).

Optimization of culture conditions

The growth medium of rice polishing was fermented with ***Aspergillus niger*** for different fermentation periods with varying levels of substrate, pH, urea (nitrogen source), $CaCO_3$, $MgSO_4.7H_2O$ and KH_2PO_4 in shake flask. The experiments were carried out in such a way that the parameter optimized in one experiment was maintained in the subsequent investigation.

Enzyme assay

The glucose oxidase activity in crude enzyme extract was determined by a spectrophotometric method at 460 nm wavelength using glucose as substrate and o-dianisidine buffer mixture as coupling reagent (Worthington, 1988).

Results and Discussion

Fermentation period

For the optimization of fermentation period duplicate growth media containing 2% (w/v) rice polishing as substrate were autoclaved, inoculated (5% v/v) and incubated for 24, 36, 48, 60 and 72 h at pH 4 and 30°C. The maximum glucose oxidase activity (3.42 U mL^{-1}) was noted in enzyme solution harvested after 36 h. It was observed that production of glucose oxidase increased with an increase in fermentation period from 12-36 h, reached its maximum after 36 h and decreased, thereafter (Fig. 1).

The results of Willis (1966) are in line of this work, who optimized the fermentation medium for the production of glucose oxidase by ***Aspergillus niger***. He obtained highest glucose oxidase yield after 48 h of fermentation.

Substrate level

The maximum activity of glucose oxidase (3.42 U mL^{-1}) was observed with 2% rice polishing in continuous shaking culture medium. All other substrates lower are higher than 2% gave lower enzyme productivity (Table 1).

Results are in line with those of Willis (1966) who grew ***Aspergillus niger*** in a submerged culture fermentation in mineral medium containing urea as organic nitrogen source and rice polishing (as carbohydrate source) and observed that growth media containing 2.5% rice polishing produced maximum glucose oxidase.

Table 1: Production of glucose oxidase with varying substrate level

	Glucose oxidase (O.D.)			
Substrate level (%)	A	B	Mean	Activity U mL^{-1}
1	0.055	0.064	0.060	1.77
1.5	0.060	0.070	0.065	1.91
2	0.120	0.111	0.116	3.42
2.5	0.099	0.086	0.093	2.74
3	0.084	0.078	0.081	2.39

LSD value = 0.01757

Table 2: Glucose oxidase production at different pH

	Glucose oxidase (O.D.)			
pH	A	B	Mean	Activity U mL^{-1}
2	0.060	0.047	0.054	1.59
3	0.065	0.121	0.093	2.74
4	0.114	0.120	0.117	3.45
5	0.097	0.100	0.099	2.92
6	0.078	0.081	0.079	2.33

Table 3: Production of glucose oxidase with varying urea

	Glucose oxidase (O.D.)			
Urea (%)	A	B	Mean	Activity U mL^{-1}
Control	0.112	0.121	0.117	3.45
0.1	0.128	0116	0.122	3.59
0.2	0.133	0.154	0.143	4.21
0.3	0.159	0.157	0.158	4.66
0.4	0.147	0.152	0.148	4.35

LSD value = 0.02143

Effect of pH

In this experiment duplicate media of rice polishing (2%) were adjusted at different pH values i.e 2, 3, 4, 5 and 6. The results showed maximum activity of glucose oxidase (3.45 U mL^{-1}) at pH 3 (Table 2).

The results of Rando ***et al.*** (1997) accord with our results when they produced glucose oxidase by ***Penicillium pinophilum***. They determined that the optimum pH for glucose oxidase production was in the range pH 4-4.6.

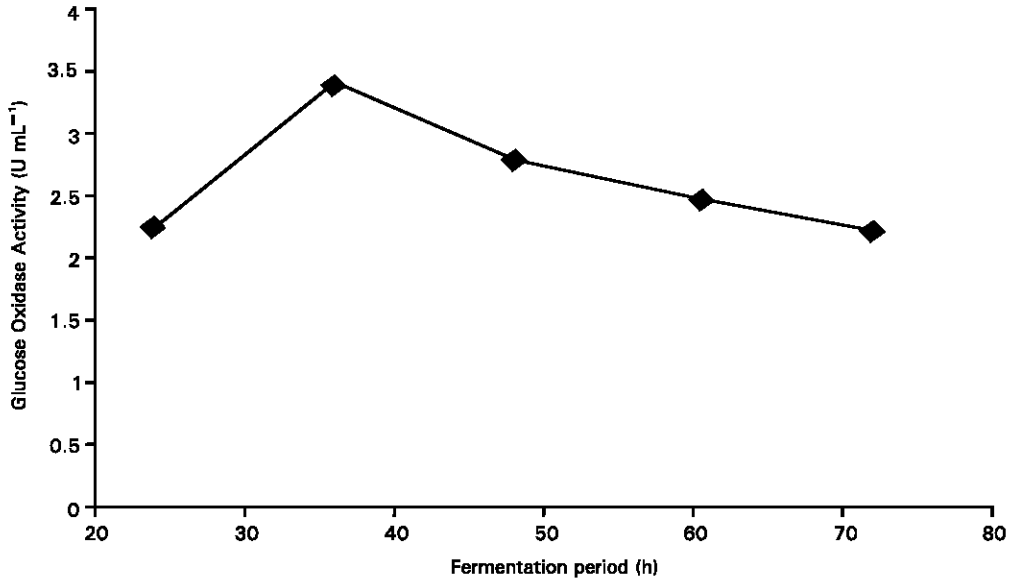

Fig. 1: Effect of fermentation period on glucose oxides production

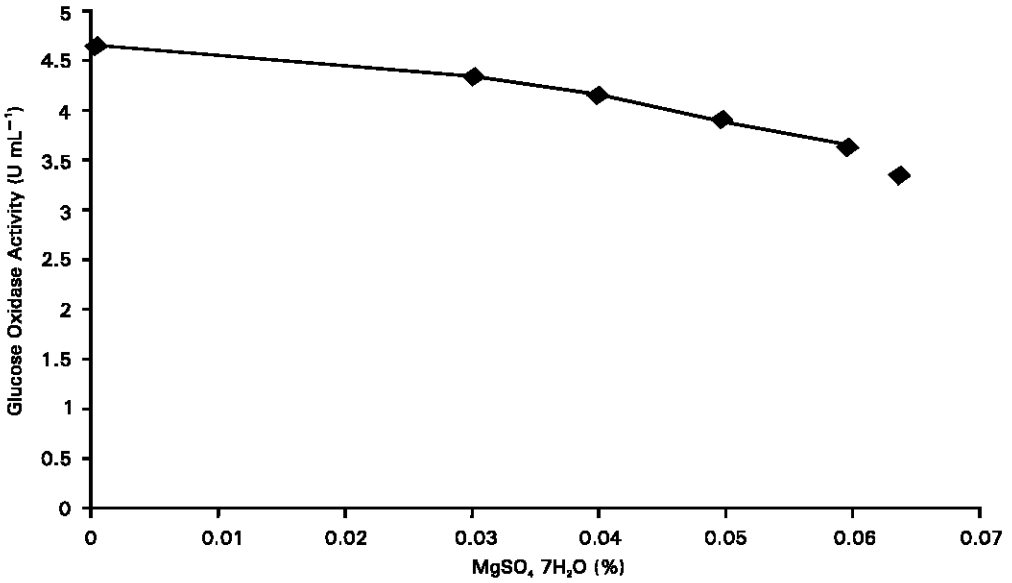

Fig. 2: Effect of different concentrations of $MgSO_4$. $7H_2O$ on glucose oxidase production

Effect of urea

The concentration of nitrogen source in the growth media has a considerable influence on enzyme production. The results showed maximum activity of glucose oxidase (4.66 U mL^{-1}) with

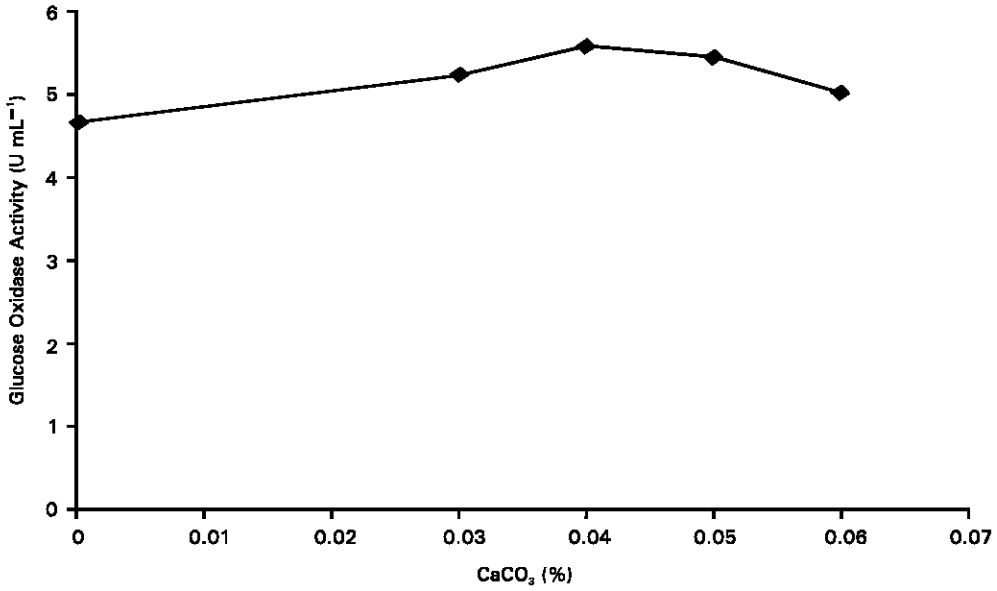

Fig. 3: Effect of different concentrations of $CaCO_3$ on glucose oxidase production

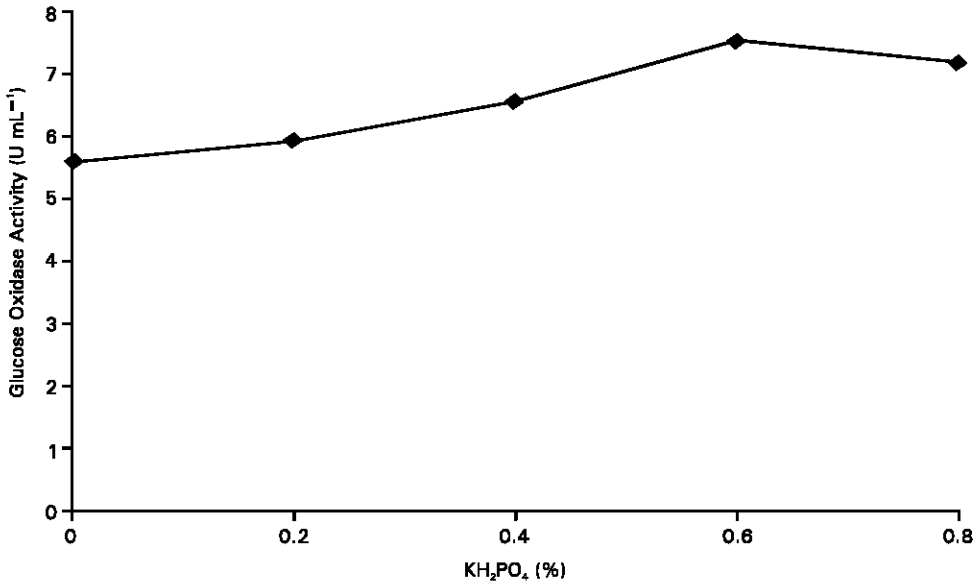

Fig. 4: Effect of varying concentrations of KH_2PO_4 on glucose oxidase production

0.3% urea (Table 3) as additional nitrogen source. Further increase in nitrogen concentration resulted in a decrease in enzyme yields.

The results of Pazlarova and Votruba (1996) accord with this work. They used synthetic and natural ammonium-sorbing zeolite to control the ammonium (NH_4) level in the medium to growth *Bacillus amyloliqueficiens*.

Effect of $MgSO_4.7H_2O$

Four different concentrations of $MgSO_4.7H_2O$ were tested for glucose oxidase production in the growth medium containing optimum level of substrate (2%) and urea (0.3%) at pH 4 and 37°C temperature. It was observed that addition of $MgSO_4.7H_2O$ into the medium glucose oxidase production decreased gradually (Fig. 2). So it was recommended that $MgSO_4.7H_2O$ should not be added in the medium.

The results of present study are in line with the work of Yang ***et al.*** (1996) who studied the production of glucose oxidase from waste mycelium of ***Aspergillus niger*** and effect of metal ions on the activity of glucose oxidase. The results showed that addition of Mg^{2+} in the medium strongly inhibited the production of glucose oxidase.

Effect of $CaCO_3$

To enhance the rate of fermentation and glucose oxidase production by ***Aspergillus niger*** $CaCO_3$ was adduced into the optimum rice polishing medium. The results regarding the effect of $CaCO_3$ on glucose oxidase production in rice polishing (2%) containing urea (0.3%) showed that the addition of $CaCO_3$ into the growth medium enhanced enzyme production and showed maximum glucose oxidase activity (5.57 U mL^{-1}) with 0.04% level of this salt.

These results accord with Hatzinikolaou and Macris (1995) who reported factors regulating production of glucose oxidase by ***Aspergillus niger***. They identified $CaCO_3$ as a particularly strong inducer of glucose oxidase activity.

Effect of KH_2PO_4

Effect of different levels of KH_2PO_4 was studied on the production of glucose oxidase in growth medium containing optimum concentrations of rice polishing, urea and $CaCO_3$ at pH 4.0.

Results indicated maximum glucose oxidase activity (7.49 U mL^{-1}) with 0.6% KH_2PO_4 in the medium under pre-optimized culture conditions. Glucose oxidase production was found to be enhanced by the addition of KH_2PO_4 upto 0.6% and decreased by its further addition (Fig. 4).

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