Optimization And Cost-Effective Production Of Fungal Glucose Oxidase Using Palm Jaggery

Uppalapati pavankalyan, Annavarapu Yasaswi, Atchukola Teja, Giridhar Kanuri, Chandrasekhar Chanda

Abstract : Glucose oxidase (GOx) belongs to oxido-reductase class of enzyme produced by few fungal strains and insects. Glucose oxidase has several applications in food, pharmaceutical and biotechnological industries. Glucose oxidase is widely used in diagnostics and biosensors in determining glucose levels. Current study focussed on cost effective production, purification and characterization of glucose oxidase isolated from fungal strain Aspergillus niger grown on palm jaggery. Extracellular fungal proteins were fractionated using cation exchange column chromatography and purified glucose oxidase activity was tested with modified benzoquinone activity assay. Approximately 560 IU/ml of glucose oxidase activity was observed using 1% palm jaggery as primary carbon source in submerged fermentation.

Key words: Aspergillus niger. Benzoquinone assay, Column chromatography, CM Sephadex, Glucose oxidase, Media optimization, Palm Jaggery

1 INTRODUCTION

Glucose oxidase is an intracellular and extracellular enzyme which is produced by several fungal strains like Aspergillus and penicillium on large scale [1]. GOx from Aspergillus niger is a glycoprotein which consists of two 80kDa subunits with FAD as enzyme cofactors [2]. GOx oxidises Beta-D-glucose to D-glucono-1,5- lactone and hydrogen peroxide by using molecular oxygen as an electron acceptor as represented in the Figure 1. This enzyme is also known as Notatin which is an oxidoreductase [3]. The hydrogen peroxide which is formed as one of the product acts as an anti-microbial agent [4],[5],[6].

Figure. 1 *Glucose oxidase catalytic activity*

Glucose oxidase has various applications in food industries it is used as an additive to increase the dough of bakery products [7],[8]. GOx mixed with glucose is used in nasal spray of bag-on-valve devices for the treatment of common cold. Glucose oxidase is used to determine glucose in blood serum and plasma and in the removal of sugars from egg products and oxygen from food and beverages Disposable glucose sensor strips used in the treatment of diabetics to check serum glucose levels [9],[10],[11]. The main principle behind this glucose biosensor strips is the usage of electrode in the place of oxygen to oxidize glucose and produce electric current in response to glucose concentration. There are so many companies producing Glucose oxidase which increases glucose oxidase market worldwide. Purpose of this study is to optimize the glucose oxidase production using cheaper alternative carbon sources like palm jaggery which helps in drastic reduction of production costs.

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2 MATERIALS AND METHODS

2.1 Media and growth Conditions

Aspergillus niger was isolated from soil and was grown on potato dextrose agar (basal media) flasks (100 ml) at 30 °C for 96 h. Inoculum was then transferred on to reese and mandels medium [12]. Composition of the medium (for 1L) is as follows: $(NH_4)_2SO_4$-1.5g, KH_2PO_4-3g, $MgSO_4$-0.3g, $CaCl_2$-0.3g, peptone-2.5g, urea-0.3g, yeast extract-2g, $FeSO_4 \cdot 7H_2O$-0.005g, $ZnSO_4 \cdot 7H_2O$-0.0014g, $MnSO_4 \cdot H_2O$-0.0016g, , and $CoCl_2$-0.0012g(pH 5.0). 1% glucose was supplied after sterilizing the media. The above heat sterilized media was inoculated with Aspergillus niger and incubated for 96h at 30°C. Mycelia was collected from the media after incubation by centrifuging at 6,000rpm for 20 min. The pellet was washed and resuspended in 100 ml of 20mM sterile phosphate buffer, pH 4.8 and stored at 4°C and was used as inoculate fermentation medium.

2.2 Enzyme production by submerged fermentation:

Fermentation media was prepared in 1 litre Erlenmeyer flask by adding Palm jaggery powder (10 g/l) and yeast extract (0.1% w/v), in appropriate proportions and sterilized. 5% (v/v) of inoculum from reese-mandels medium was added to this medium and kept for growth at 30°C for 7 days. Once growth was observed, media was centrifugation at 6000rpm for 10min and mycelium was separated.

2.3 Glucose oxidase purification:

Supernatant was collected and subjected to ammonium sulphate (75%) precipitation. After precipitation mixture was subjected to centrifugation at 6000rpm for 10 min. Pellet was resuspended in 10 ml of buffer and subjected to dialysis to eliminate salts. Dialysed protein sample was concentrated using sucrose powder. In order to purify Glucose oxidase enzyme, concentrated sample was

fractionated using a cation exchange column. CM Sephadex C-50 matrix soaked in distilled water overnight and column (10 cm X 2 cm) was packed with a bed volume of 15 ml. The column was washed with distilled water, equilibrated with 10mM Potassium phosphate buffer and the sample was loaded on to the column. Flow rate was fixed to 1ml/minute and the fraction size was restricted to 1ml. 20ml of flow through was collected in 1ml fractions followed by elution with NaCl gradient of 0-0.5M. Absorbance of these fractions were measured at 280nm and the protein concentrations were estimated. Chromatogram was plotted against the absorbance values with the fraction number (figure2).

2.4 Glucose oxidase activity assay:

GOx activity was measured spectrophotometrically using modified Ciucu and Patroescu method which is based on reduction of benzoquinone to hydroquinone [13]. Reaction mixture components (0.5 ml 1M glucose, 0.5 ml 0.1% benzoquinone, and 0.5 ml 0.1 M Na-citrate buffer at pH 5) were pre-incubated at 25ºC followed by adding 20 microliter of Glucose oxidase fraction. Absorbance was measured spectrophotometrically at 290 nm (extinction coefficient = 2.31 nM-1 cm-1) using UV Visible spectrophotometer. One-unit of GOx activity is defined as the amount of enzyme required to reduces one micromol benzoquinone ml-1 min-1.

3 RESULTS

3.1 Production of Glucose oxidase using submerged fermentation

Different inoculum sizes of Aspergillus niger i.e., 2%, 5% and 10% were studied for the optimal production of Glucose oxidase. Similarly, pH (4,6&8) and initial substrate concentration of palm jaggery (0.1%, 0.5%, 1%) were used to optimize the fermentation media and to improve the yield of GOx production. Submerged fermentation was carried out for 7 days at different experimental conditions as mentioned above. Media was collected from the flask and separation of extracellular proteins was done using ammonium sulphate precipitation. Crude protein sample were dialysed against 20 mM phosphate buffer and the dialysed samples were concentrated using sucrose powder.

3.2 Purification of Glucose oxidase using ion exchange chromatography

Further purification was achieved by running the crude sample on a cation exchange column chromatography[14][14][13][12][11][10][9][8][7][6][5][4][3][2][1][1](Madhavi, Soosamma, Bincy, & Urmilla, 2011)(Madhavi, Soosamma, Bincy, & Urmilla, 2011)(Madhavi, Soosamma, Bincy, & Urmilla, 2011). Chromatogram obtained after purification was represented in (Figure. 2). Four protein peaks (represented as P I, P II, P III and P IV) were obtained after purification. Proteins present in the peak fraction were selected and tested for Glucose oxidase activity using modified benzoquinone assay. Proteins present in peak III showed Glucose oxidase activity.

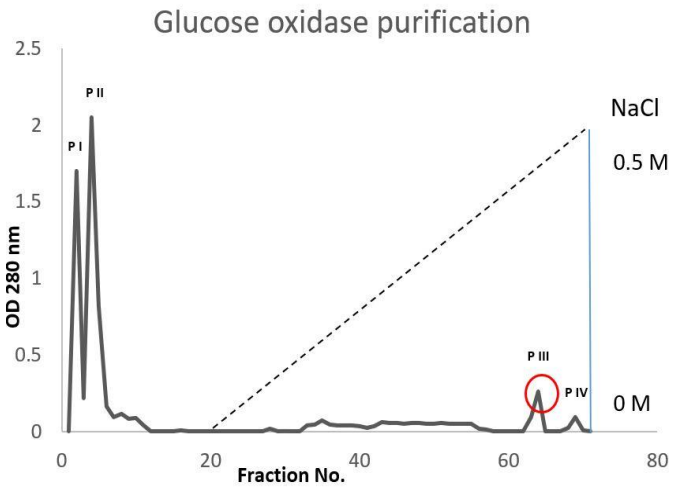

Figure. 2 *Purification of Glucose oxidase from crude protein sample obtained from ammonium sulphate precipitation using CM Sephadex C-50 cation exchange column chromatography. Flow through fractions (1-20) collected eluting with 20mM potassium phosphate buffer. Followed by NaCl gradient elution (0-0.5 M) and collected 50 fractions (21 -70).*

3.3 Effect of inoculum size on glucose oxidase productivity

Optimal production of Glucose oxidase was observed with 5% Aspergillus niger inoculum. With increase in inoculum size from 5% to 10% minimal increase in the production of glucose oxidase was observed. (Figure. 3).

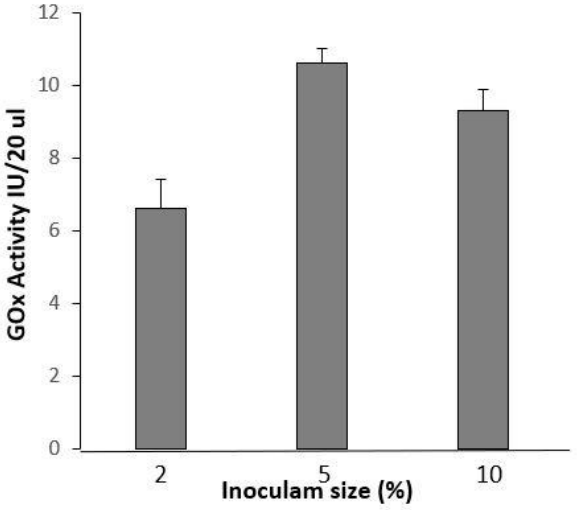

Figure. 3 *Effects of inoculum size on GOx production*

3.4 Effect of pH on glucose oxidase productivity

Maintaining a pH-6 during submerged fermentation was observed to yield better productivity of Glucose oxidase enzyme (Figure 4).

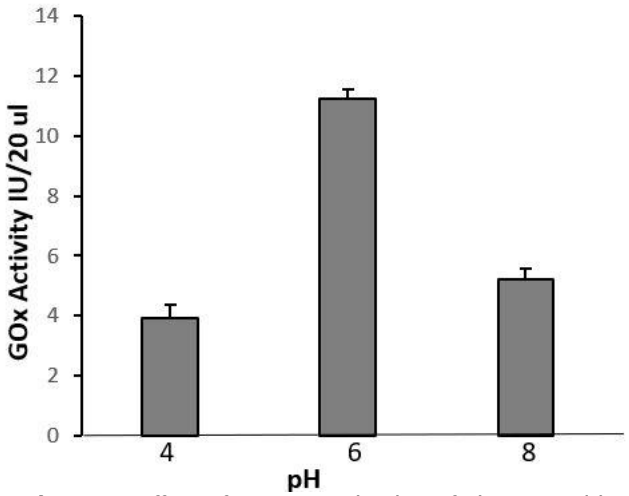

Figure. 4 *Effect of pH on production of glucose oxidase*

3.5 Effect of initial substrate concentration (palm jaggery) on glucose oxidase productivity

With increase in palm jaggery initial concentration from 0.1 % to 1% there is a substantial increase in the production of glucose oxidase by submerged fermentation. We observed optimal production of glucose oxidase with 560 IU/ml of GOx activity at 1% initial concentration of palm jaggery.

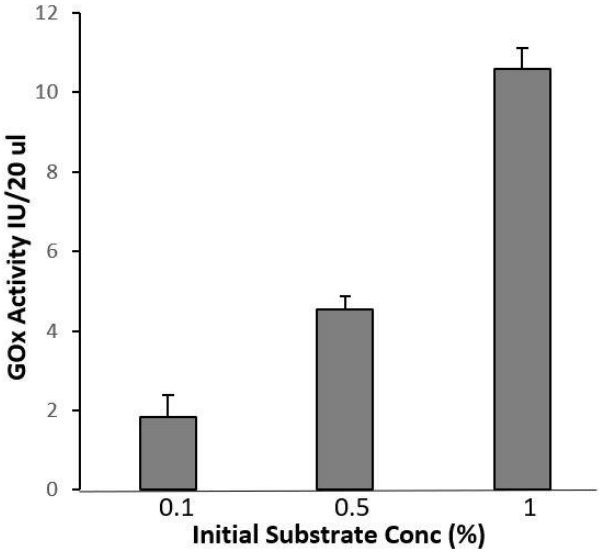

Figure. 5 *Effect of initial substrate concentration on production of glucose oxidase*

4 CONCLUSION

Glucose oxidase has wide variety of application in food, pharma and biotechnological industries. Its use in diagnostics and biosensors created interest in several companies and researchers to produce this enzyme in bulk quantities and minimize the cost of production. In this study we focussed on purification, media optimization and production of glucose oxidase using cheap alternative carbon sources i.e., palm jaggery. Plam jaggery not only acts as a good carbon source it is also rich in vital minerals like calcium, Iron, phosphorous and vitamins which can help the growth of organism. With 5% Aspergillus niger inoculum size and by maintain a pH 6 we were able to produce optimal concentrations of glucose oxidase by submerged fermentation. Although not a prior study objective, informal cost estimates in our laboratory indicate that production of glucose oxidase with palm jaggery is cheaper than conventional carbon source.

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