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Comparative Study for Optimization of Nutritional Parameters for Enhanced Production of Glucose Oxidase from Wild and Mutants Strains of *A. niger*

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ABSTRACT

Aim: There is a need for bulk production of glucose because of its vital application in various industries.

Material and methods: In the present study for the enhanced production of glucose oxidase from wild and mutant derived strains of *Aspergillus niger*, chemical nutrients were optimized in liquid state shake flask fermentation. Effect of large number of nutritional parameters such as substrates concentration, carbon sources, organic and inorganic nitrogen sources, KH_2PO_4, $CaCO_3$, $ZnSO_4.7H_2O$ and $MgSO_4.7H_2O$ were optimized. Glucose oxidase activity was assayed for all the parameters.

Results: The optimum glucose oxidase activity from the cultivation of wild and mutant derived strains of *Aspergillus niger* was achieved at CSL (3%), glucose (3%), urea (0.4%), diammonium sulphate ($(NH_4)_2SO_4$) (0.4%), potassium dihydrogen phosphate (KH_2PO_4) (0.7%), calcium carbonate ($CaCO_3$) (0.08%), zinc sulphate ($ZnSO_4.7H_2O$) (0%) and magnesium sulphate ($MgSO_4.7H_2O$) (0%) using single factor analysis method.

Impact of study: From the present study it was observed that mutant derived strains have the same nutritional requirement as their parent wild strain *Aspergillus niger* and there is no significant divergence was found in their production trend. In the present investigation, it was also found that calcium carbonate ($CaCO_3$) and potassium dihydrogen phosphate (KH_2PO_4) induced the production of glucose oxidase while zinc sulphate ($ZnSO_4.7H_2O$) and magnesium sulphate ($MgSO_4.7H_2O$) were showed their inhibitory behavior. It was also found that single factor analysis is more suitable for studying the effect of large number of variables/factors rather than other statistical methods..

Keywords: *Aspergillus niger*, Glucose oxidase, Fermentation, Nutritional parameters, Mutant

INTRODUCTION

Glucose oxidase (β-D-glucose: oxygen oxidoreductase; EC 1.1.3.4) also known as glucose aerodehydrogenase is a flavin protein that catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone using oxygen as an electron acceptor and simultaneously producing hydrogen peroxide [1]. Glucose oxidase has been the subject of many research studies due to its numerous applications [2].

Glucose oxidase can be obtained from a number of different sources including red algae, citrus fruits, insects, bacteria and molds but industrially fungal sources are preferred mainly from the genus Aspergillus [3,4] and Penicillium [5], of which *A. niger* is the most commonly utilized for the production of glucose oxidase [6].

It has been found that *Aspergillus niger* is the potential source for the production of glucose oxidase and preferred use of *Aspergillus niger* lies in its easy handling, high production of enzyme, easy recovery of the enzyme and metabolic

versatility of the strain. The ability of *Aspergillus niger* to utilize a wide range of waste products as nutrition source makes it more economical source of the enzyme [7].

In order to increase the production efficiency, it is necessary to optimize production process of glucose oxidase. As common to enzyme production, the most crucial factors in the optimization of process is medium composition, since it affects the production in terms of cost and its productivity [8]. Hence, it is important to consider the optimization of fermentation medium in order to maximize the production efficiency and profits eventually.

In a previous report, the enhanced production of glucose oxidase by *Aspergillus niger* using chemical and physical mutagenesis was described [2,7]. In this study, Single factor analysis method has been employed for rapid screening of large number of medium components in a minimal time and experiments. Therefore, the aim of present work is to investigate the effects of nutritional parameters for the optimum production of glucose oxidase from wild and mutant derived strains of *Aspergillus niger* in ultimate use for vital application in biotechnology and in other various industries.

Materials and Methods

Microorganism

Wild and mutant derived strains of *Aspergillus niger* (*A. niger*) were used in the present study. Stock culture of selected strains of *Aspergillus niger* was maintained on potato dextrose agar media (Table 1) and stored in refrigerator at 4°C, sub-cultured after 15-20 days.

Production of crude glucose oxidase

Glucose oxidase was produced by liquid state fermentation from wild and mutant isolates of *Aspergillus niger*. All growth experiments were carried out in 100 mL conical flasks with 50 ml working volume. The sterilized medium flasks were inoculated with 5 ml of vogal's inoculum (homogenous spore suspension) (Table 1) and incubated at 30°C in a thermal orbital shaker operating at 220 rpm for 36 h in dark [2].

Selection and optimization of chemical nutrients for glucose oxidase production

Nutritional parameters including substrate concentration, carbon sources, nitrogen sources and some other necessary components were investigated by single factor analysis method (SFAM) for the optimum production of glucose oxidase by wild and mutants strains of *Aspergillus niger* [9-12]. This method is useful to determine the effective range of parameters on the production of GOx which can be useful for further optimization study.

In current study, three substrate namely corn steep liquor, molasses and rice polishing was used to get the optimum yield of glucose oxidase. In order to study the effect of these substrates following concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9, 10%) were studied while six carbon sources glucose, fructose, sucrose, starch, galactose and lactose were used for

Table 1: Composition of potato dextrose agar (PDA) and Vogel's medium for *A. niger* growth

S. No.	Components	PDA Quantity (g/100 mL)	Vogel's Medium Quantity (g/100 mL)
1	Glucose	2	2
2	Starch	2	-
3	Agar	2	-
4	Urea	0.3	-
5	Potassium dihydrogen phosphate (KH_2PO_4)	0.008	0.5
6	Potassium chloride (KCl)	0.015	-
7	Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)	0.05	0.02
8	Zinc sulphateheptahydrate ($ZnSO_4.7H_2O$)	0.001	-
9	Yeast Extract	-	0.2
10	Trisodium citrate	-	0.5
11	Diammoniumsulphate [$(NH_4)_2SO_4$]	-	0.4
12	Ammonium nitrate (NH_4NO_3)	-	0.2
13	Peptone	-	0.1
14	$CaCO_3$	-	-
15	Corn steep liquor (CSL)	-	-
16	pH	4	5.5
17	temperature	30°C	30°C

this purpose and followingconcentrations (1, 2, 3, 4, 5, 6, 7, 8, 9, 10%) of these carbon sources were studied for the maximum production of glucose oxidase enzyme.

Both organic and inorganic sources of nitrogen were also studied because of their considerable influence on the production of glucose oxidase enzyme. Following organic [Yeast extract (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1%), Urea (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1%) and Peptone (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1%)] and inorganic [$(NH_4)_2SO_4$(0.2, 0.4, 0.6, 0.8, 1%) NH_4NO_3 (0.2, 0.4, 0.6, 0.8, 1%) and $NaNO_3$(0.2, 0.4, 0.6, 0.8, 1%)] nitrogen sources were studied in order to get optimum production of glucose oxidase.

Effects of different concentrations of KH_2PO_4 (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1%), $CaCO_3$ (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1%), $ZnSO_4.7H_2O$ (0, 0.01, 0.02, 0.03, 0.04, 0.05%) and $MgSO_4.7H_2O$ (0, 0.01, 0.02, 0.03, 0.04, 0.05%) were also investigated for the optimum production of glucose oxidase.

After each step of optimization, it was clearly indicated that there was a gradual increase in the enzyme activities when it was compared with the enzyme activities of non-optimized conditions. The experiment carried out in such a way that the parameters optimized in each experiment were maintained in the subsequent investigation.

Harvesting of sample

After getting microbial growth in liquid state fermentation, crude product was homogenized for 15 min in cell homogenizer and then subsequent suspension was subjected for high speed centrifugation for the further removal of biomass at 13,000 rpm for 15 min at 4°C. The resultant suspension was clarified using Whatman filter paper [13]. The harvested supernatant was assayed for glucose oxidase activity [9].

Estimation of total proteins content

Total proteins contents in samples were quantitatively analyze by biuret method using biuret reagent and bovine serum albumin as the standard [14].

Glucose oxidase enzyme assay

Glucose oxidase activity was determined quantitatively in clarified suspension by spectrophotometric method [15] at 460 nm for 3-5 min using 18% D-glucose solution as a substrate, peroxidase enzyme and 1% orthodianisidine solution as a coupling reagent. Glucose oxidase activity was defined as quantity of enzyme needed to oxidize one unit of glucose in micromole per minute at 30°C. All the data given in this study is the average of measurements [2].

RESULTS AND DISCUSSION

Nutritional parameters including different substrate sources, carbon sources, organic and inorganic nitrogen sources and other necessary salts ($CaCO_3$, KH_2PO_4, $ZnSO_4.7H_2O$ and $MgSO_4.7H_2O$) were screened and optimized by single factor analysis method for the improved production of glucose oxidase by selected wild and mutants derived strains of *Aspergillus niger* [10,12].

Selection and optimization of substrate

Three substrate namely corn steep liquor (CSL), molasses and rice polishing were used in this study to get the optimum yield of glucose oxidase from all the selected strains of *Aspergillus niger*. Substrate concentration played a vital role on the glucose oxidase production and supported it to a great extent. Selection and screening of substrate for the microbial cultivation is most significant factor for microbial growth.

Pakistan has an economy dependent on agro industries producing large volume of agro-industrial wastes which are mostly suitable for use as substrate. Agro-industrial waste products manufactured as by products in huge amount create problems for the safe removal of desired products. The industrialists and environmentalists faced a trouble in recycling of certain industrial wastes [16] some of such wastes are CSL, molasses and rice polishing. These agro-industrial wastes could be recycled by microbes using these waste products as their nutrient source. In various studies, agro-industrial wastes were commonly and efficaciously used as substrate by microorganisms for the production of glucose oxidase [10]. CSL has also been used by many investigators as cost effective carbon source for the production of numerous laboratory and industrial scale enzyme [17], it is an enrich source of nutrients like carbohydrates, minerals and vitamins while molasses, rice polishing has also been used for glucose oxidase production by Hatziuikolaou and Macris [3], Ramzan and Mehmood [18], respectively. Singh [19] and Semashko et al. [20] also used agro-wastes as a carbon source for the production of glucose oxidase from *Aspergillus niger* and *Penicillium funiculosum*, respectively.

In present investigation, it was clearly showed that the corn steep liquor was a most significant substrate as compared to molasses and rice polishing. Highest glucose oxidase production was achieved with 3% concentration of corn steep liquor from all the selected *Aspergillus niger* strains including wild *Aspergillus niger* (25.64 U/mL), mutant derived strains of *Aspergillus niger* named as TS-UV-200 (46.48 U/mL) and TS-NMU-100 (54.82 U/mL) as shown in Figures 1-3, respectively. In this study it was noticed that with the increase of substrate concentration, the activity of glucose oxidase was also enhanced in all the selected strains and become maximum but up to a certain concentration (3%); then there was found a gradual decreased in enzyme activity with the any further increase in the substrate concentration. Current investigation also revealed that all the selected strains of *Aspergillus niger* was proficient to cultivate in all substrate sources but at a diverse degree and highly significant ($P<0.01$) glucose oxidase production was gained with corn steep liquor.

Present results are in accordance with the finding of literature [10] they found the maximum glucose oxidase activity using CSL as a substrate but at 20 mL/L and 2% concentration of corn steep liquor respectively by wild and mutant derived strains of *Aspergillus niger*.

Selection and optimization of carbon sources

Different carbohydrates as carbon sources comprising glucose, fructose, sucrose, starch, maltose, galactose and lactose were analyzed to determine their influence on glucose oxidase production. When cultivation medium was supplemented with these carbon sources, a sharp increase was seen in enzyme activity produced from all the selected strains. The utmost glucose oxidase production was attained with glucose (3%) followed by sucrose from all fungal strains having the enzyme activity 28.32, 49.23 and 57.48 U/mL as shown in the Figures 4-6 respectively, while galactose, lactose and maltose produced least effect on the enzyme production corresponding to the rest of carbon sources. Fructose and starch also exhibited positive influence on enzyme production but in a lesser extent than glucose and sucrose. Thus, current study evaluated that all *Aspergillus niger* strains was capable to grow in all carbon sources but at a different extent and statistically highly significant ($P<0.01$) glucose oxidase production was obtained with glucose followed by sucrose. This indicates that glucose is a principal inducer for the transcription of the GOD gene [3]. Graphical and statistical analysis showed that the activity of glucose oxidase was improved with the increasing concentration of carbon source in all *Aspergillus niger* strains and become maximum but up to a certain concentration (3%) then there was regular/gradual declined was found in enzyme activity with the increase of the carbon concentration.

Carbon source used in microbial growth medium are major source of energy as they constitute the polysaccharides and also acts as a chief component for cellular building materials during the microbial cultivation. Carbon metabolization rate frequently effect the formation of biomass and higher concentrations of rapidly metabolizing sugars have positive effects on growth-associated products [21]. Different sugars had been used for microbial glucose oxidase production but previous literature cited by many researchers also reported that glucose, sucrose and fructose was the principal inducers for microbial glucose oxidase production [10,12,22,23].

Current study was in agreement with Traeger et al. [24], Schomburg and Stephan [25], Semashko et al. [20], Bankar et al. [11], Bodade et al. [12] they reported that glucose and sucrose were most significant and effective carbon sources as

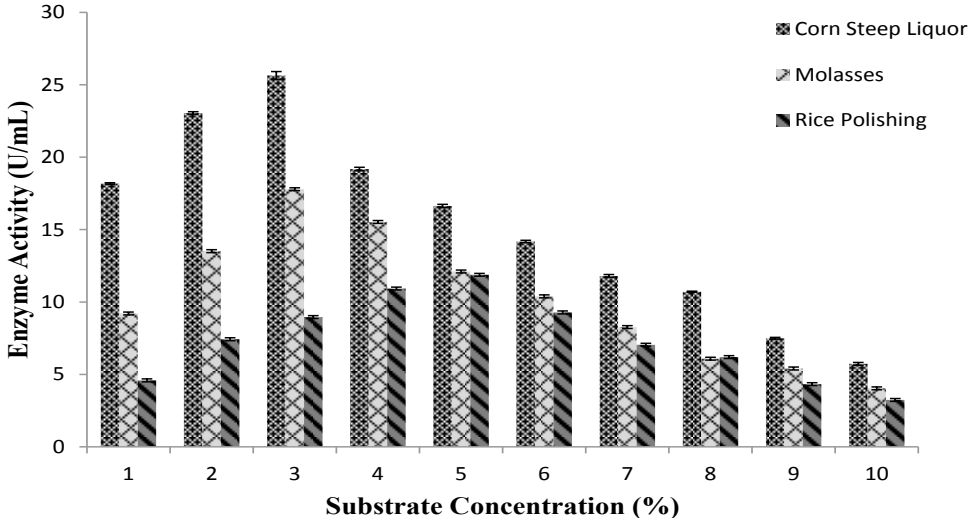

Figure 1: Effect of substrate concentration on the production of glucose oxidase from wild *Aspergillus niger*

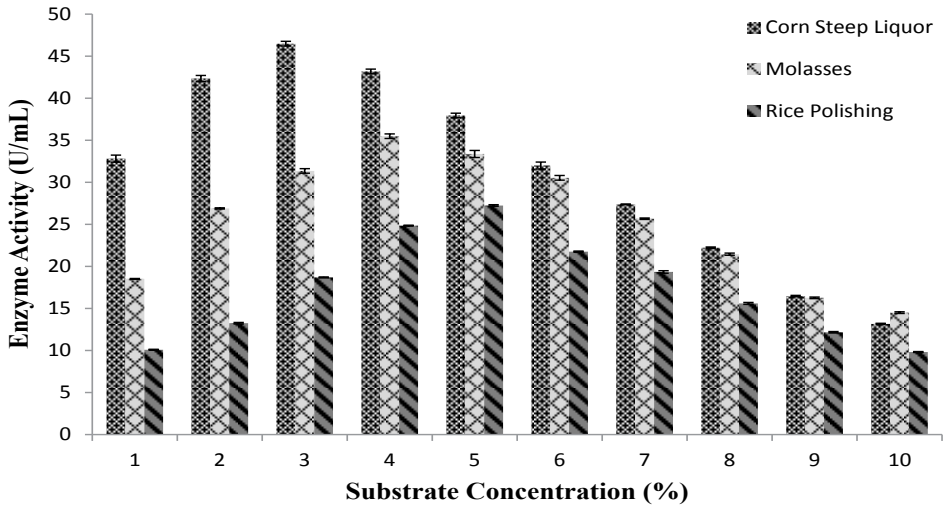

Figure 2: Effect of substrate concentration on the production of glucose oxidase from mutant derived TS-UV-200 *Aspergillus niger* strain

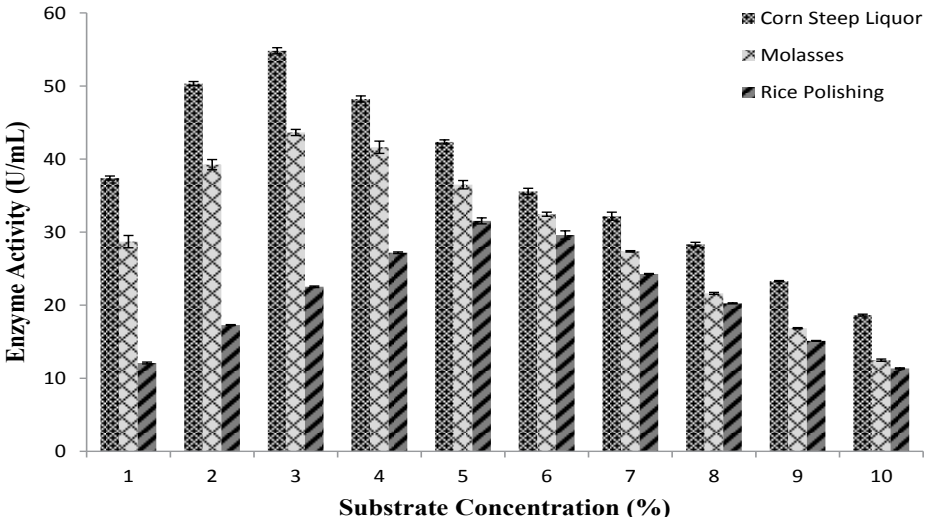

Figure 3: Effect of substrate concentration on the production of glucose oxidase from mutant derived TS-NMU-100 *Aspergillus niger* strain

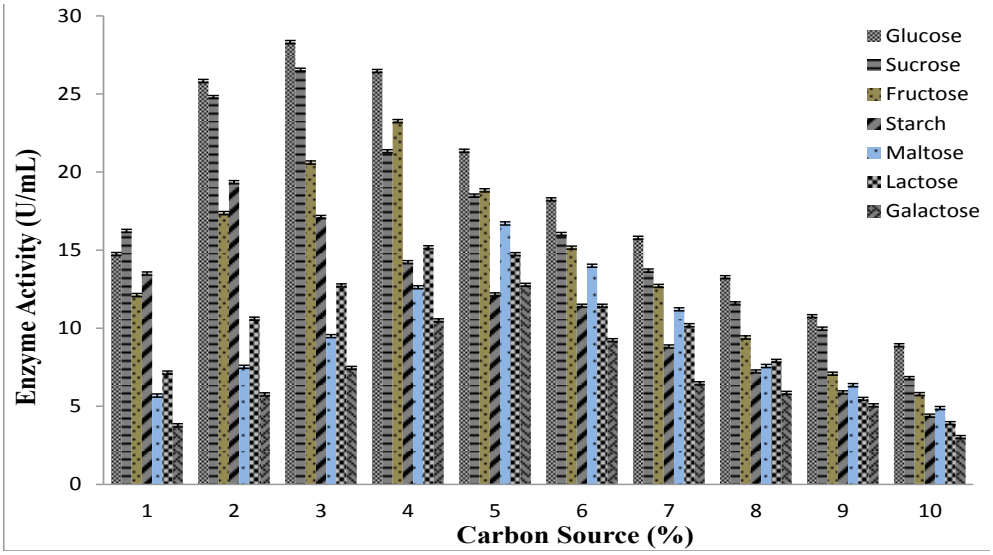

Figure 4: Effect of different carbon sources on the production of glucose oxidase from wild *Aspergillus niger*

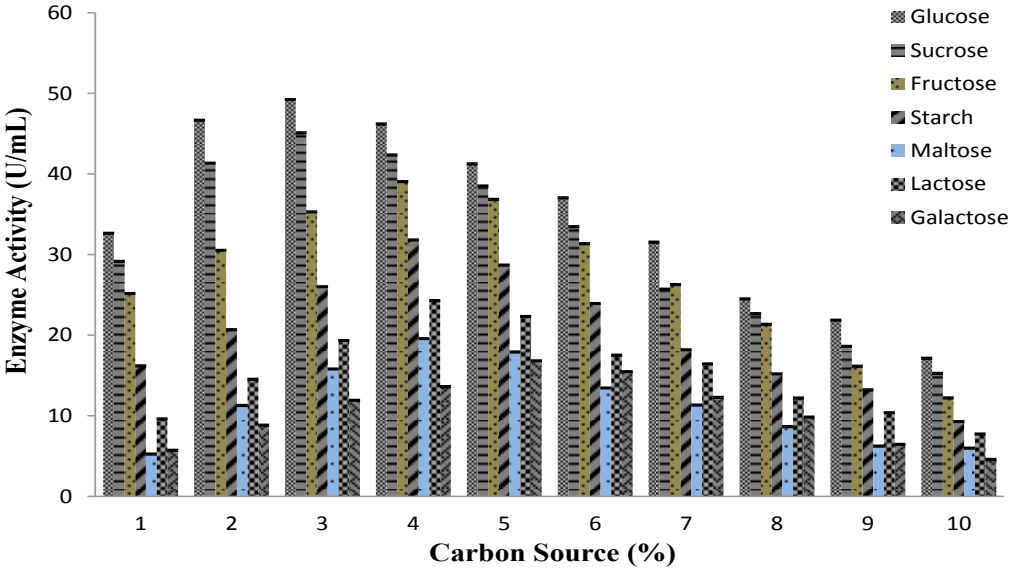

Figure 5: Effect of different carbon sources on the production of glucose oxidase from mutant derived TS-UV-200 *Aspergillus niger* strain

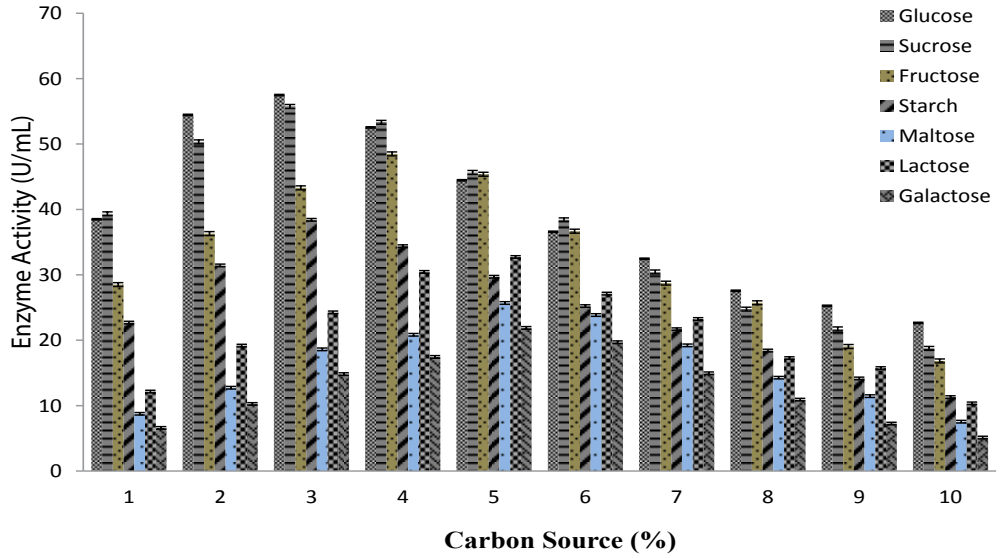

Figure 6: Effect of different carbon sources on the production of glucose oxidase from mutant derived TS-NMU-100 *Aspergillus niger* strain

compared to other carbohydrate moieties for glucose oxidase production in *Penicillium* sp. and *Aspergillus* sp. Present findings are also in line with the results of Semashko et al. [20], where they reported that the optimum glucose oxidase was yielded using glucose, sucrose and fructose as a carbon source while least activity was found using lactose as carbon source from all mutant derived strains of *Penicillium funiculosum*. Kona et al. [10] found that 6% sucrose resulted in highest *A. niger* glucose oxidase enzyme activity (500 U/mL) followed by same concentration of glucose (450 U/mL) while Javed et al. [23] investigated that optimum synthesis of glucose oxidase (0.325 U/mL) was gained with sucrose followed by fructose from thermophilic *Penicillium* sp. and observed the optimum glucose oxidase activity (2.66 U/mL) with the maltose as compared to the rest of carbon sources including starch, sucrose, lactose and fructose by *Aspergillus niger*. Hence, current results differ with the previous findings in term of concentration of carbon source which might be due to different microbial strains and environmental condition, etc.

Selection and optimization of nitrogen sources

Nitrogen source has been used to control the pH of microbial medium, nitrogen substances are also a building block of various amino acids and nitrogenous basis. Various inorganic and organic sources are used for gaining the improved production of glucose oxidase like urea, peptone, yeast extract, sodium and nitrogen salts.

Both organic and inorganic sources of nitrogen were studied because of their considerable influence on the production

of glucose oxidase enzyme. Various types of organic nitrogen sources namely yeast extract, urea and peptone were investigated for the effective production of glucose oxidase enzyme. Urea was found to be most effective organic nitrogen source at 0.4% concentration for glucose oxidase production when compared to rest of the organic nitrogen sources. Detailed results shown in Figures 7-9 displayed highest enzyme activity 30.8 U/mL, 53.65 U/mL and 61.32 U/mL at optimum concentration of urea (0.4%) from wild strain and mutant derived strains of *Aspergillus niger* namely TS-UV-200 and TS-NMU-100, respectively. Enzyme activity increased with the increase of urea concentration and become optimum at its 0.4% concentration as shown in the above mentioned figures and then decrease was seen in enzyme activity thereafter with any increase of urea concentration. Similar trend was found in all selected *Aspergillus niger* strains. Present findings were also statistically supported.

Inorganic nitrogen sources including diammonium sulphate ($(NH_4)_2SO_4$), ammonium nitrate (NH_4NO_3) and sodium nitrate ($NaNO_3$) were scrutinized for the improved production of glucose oxidase. Their existence in growth medium has a significant influence on the enzyme production. Highest glucose oxidase activity was obtained with diammonium sulphate (0.4%) followed by sodium nitrate in all selected fungal strains. Maximum enzyme activity was noted as 31.63 U/mL, 55.55 U/mL and 64.08 U/mL for wild and mutant derived strains of *Aspergillus niger* named as TS-UV-200 and TS- NMU-100 respectively as displayed in Figures 10-12. These results are highly significant when compared and tested statistically. Similar trend in enzyme activity was noted as found with organic nitrogen sources with the increase of nitrogen source.

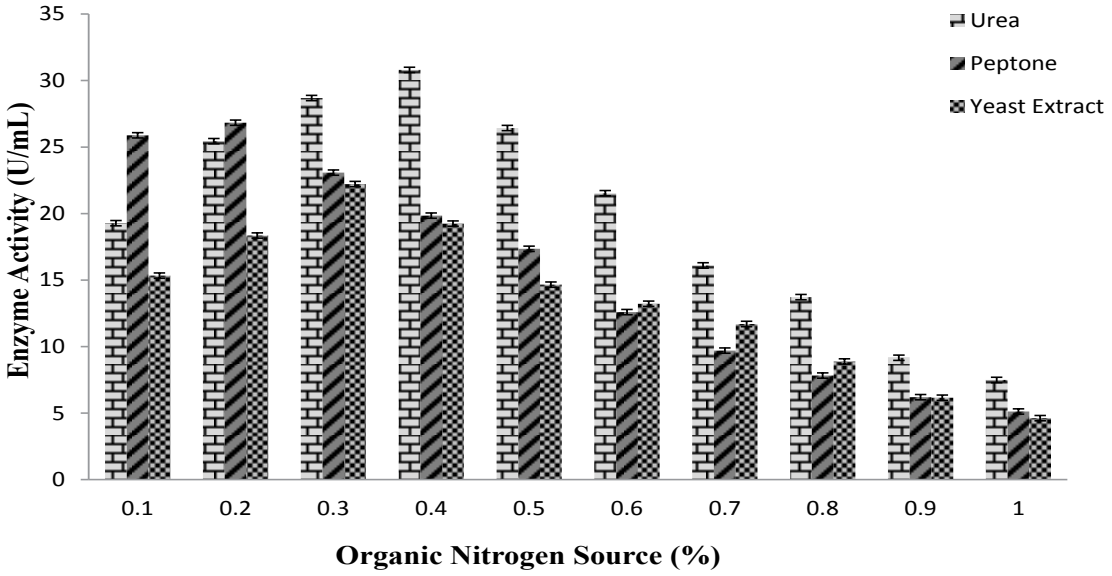

Figure 7: Effect of different organic nitrogen sources on the production of glucose oxidase from wild *Aspergillus niger*

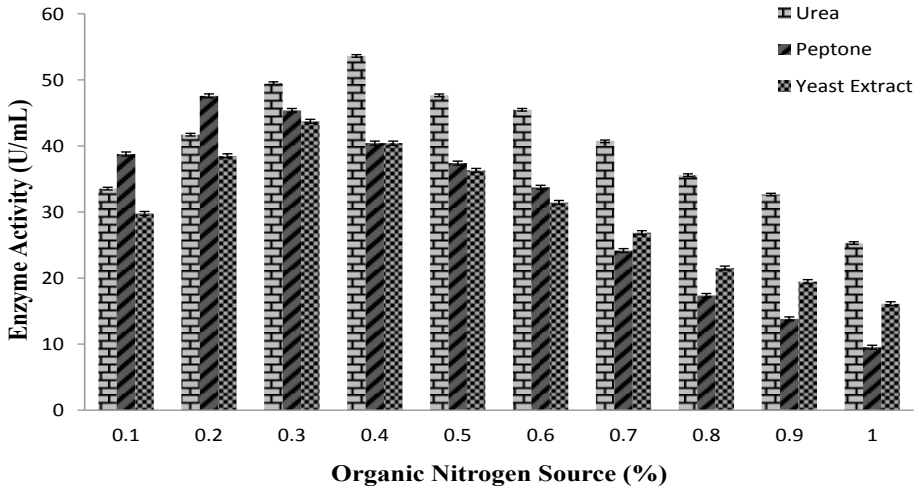

Figure 8: Effect of different organic nitrogen sources on the production of glucose oxidase from mutant derived TS-UV-200 *Aspergillus niger* strain

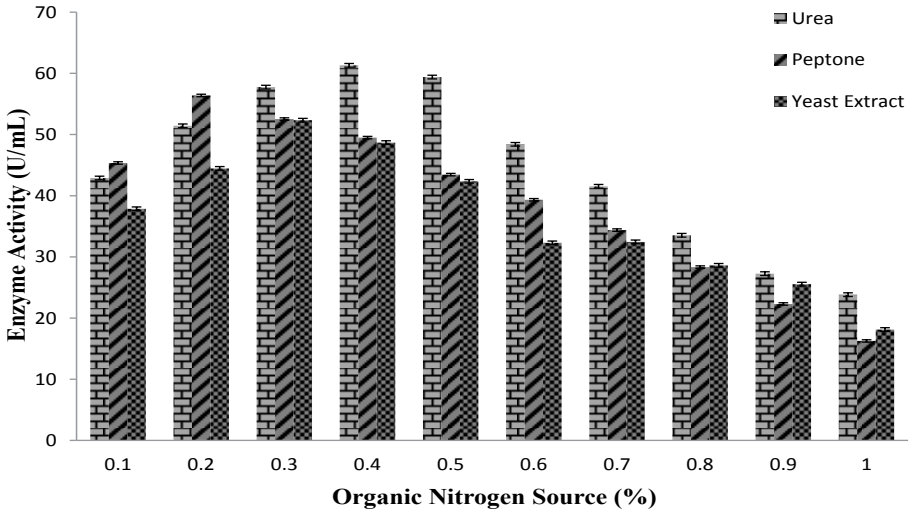

Figure 9: Effect of different organic nitrogen sources on the production of glucose oxidase from mutant derived TS-NMU-100 *Aspergillus niger* strain

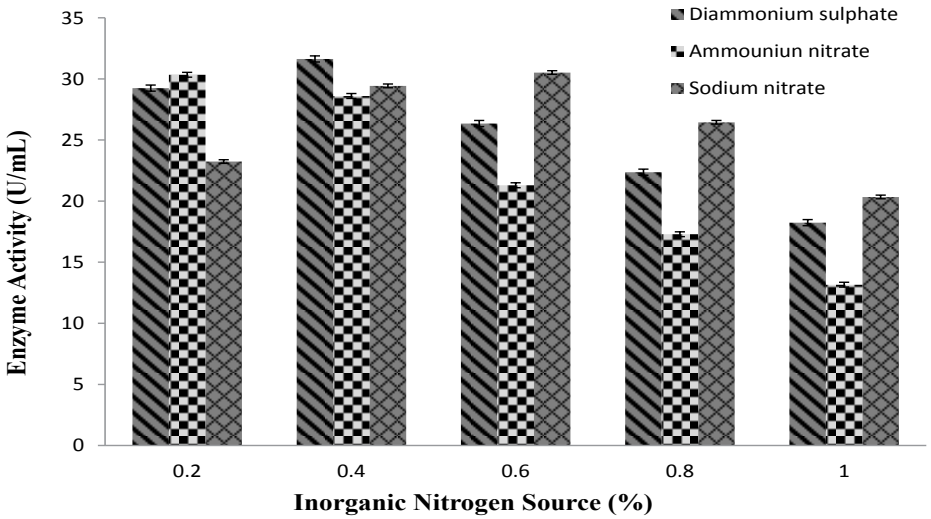

Figure 10: Effect of different inorganic nitrogen sources on the production of glucose oxidase from wild *Aspergillus niger*

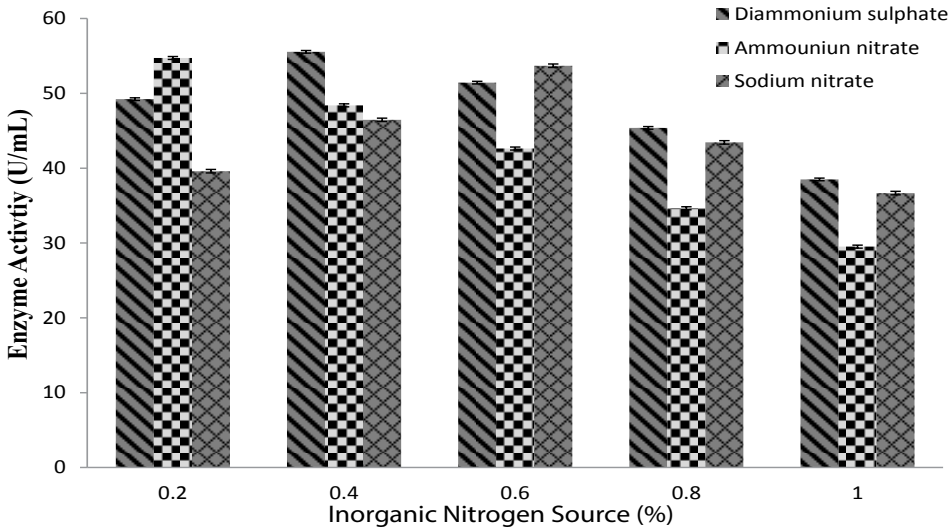

Figure 11: Effect of different inorganic nitrogen sources on the production of glucose oxidase from mutant derived TS-UV-200 *Aspergillus niger* strain

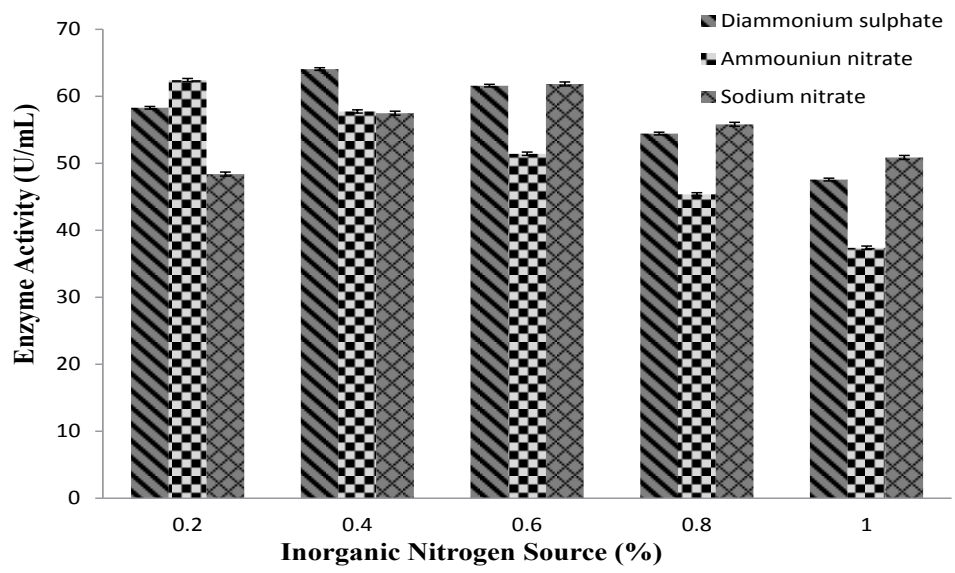

Figure 12: Effect of different inorganic nitrogen sources on the production of glucose oxidase from mutant derived TS-NMU-100 *Aspergillus niger* strain

Scientists studied the combined effect of nitrogen and phosphorous and revealed that inorganic sources of nitrogen (nitrate) were more suitable for Gox production and gluconic acid than the organic (peptone) sources. They reported that 4% concentration of $NaNO_3$ have a significantly positive effect on glucose oxidase production from *Aspergillus terreus*. Present study displayed a good coincidence with the previous findings. while Hatziuikolaou and Macris [3] and Bankar et al. [11] claimed that peptone was the most effective organic nitrogen source for microbial glucose oxidase production from *Aspergilus* sp. and *Penicillium* sp. Bankar et al. [11] produced a significant amount of glucose oxidase from *Aspergillus niger* with sodium nitrate ($NaNO_3$) as the inorganic nitrogen source and peptone as the organic nitrogen source. Kona et al. [10] examined the different nitrogen sources like nitrates of calcium, potassium, ammonium and sodium, yeast extract, malt extract and peptone and found that none of these was a beneficial nitrogen source for the glucose oxidase biosynthesis. Researchers scrutinized the influence of various nitrogen sources such as yeast extract, $NaNO_3$, peptone, nutrient broth and potassium nitrate and reported that potassium nitrate showed higher production of glucose oxidase with 3.5 U/ml enzyme activities by *Aspergillus niger*.

Effect of other necessary salts on glucose oxidase production

Effects of different concentrations of KH_2PO_4, $CaCO_3$, $ZnSO_4.7H_2O$ and $MgSO_4.7H_2O$ were also examined for the optimum production of glucose oxidase in the present study.

It was found that glucose oxidase production was remarkably enhanced with the addition of calcium carbonate ($CaCO_3$) and potassium dihydrogen phosphate (KH_2PO_4) up to a certain extent then there was gradual decrease was noticed with any further addition of these components as displayed in Figures 13 and 14. Optimum glucose oxidase activity was acquired at 0.7% concentration of potassium dihydrogen phosphate (KH_2PO_4) in wild and mutant derived strains of *Aspergillus niger* (TS-UV-200 and TS-NMU-100) with (32.45 U/mL), (56.84 U/mL) and (65.72 U/mL) value of enzyme activity respectively while maximum enzyme activity was evaluated at 0.08% calcium carbonate ($CaCO_3$) concentration in fermentation medium in all three strains namely wild *Aspergillus niger*, TS-UV-200 *Aspergillus niger* and TS-NMU-100 *Aspergillus niger* with enzyme activity (36.58 U/mL), (62.98 U/mL) and (74.67 U/mL), respectively. Any further addition of these constituents above these levels resulted in decline in enzyme activity. Statistical data through ANOVA tables elucidated that these results are highly significant.

Gradual decline was seen in glucose oxidase production with the gradual addition of zinc sulphate ($ZnSO_4.7H_2O$) and magnesium sulphate ($MgSO_4.7H_2O$) in microbial culture medium representing in the Figures 15 and 16, respectively. Figures 15 and 16 showed that at zero percent concentration of zinc sulphate ($ZnSO_4.7H_2O$) and magnesium sulphate ($MgSO_4.7H_2O$), there was optimum activity of glucose oxidase was attained while with the addition of zinc sulphate ($ZnSO_4.7H_2O$) and magnesium sulphate ($MgSO_4.7H_2O$) sudden declined was observed in enzyme activity in all selected *Aspergillus niger*.

Thus, in the present investigation calcium carbonate ($CaCO_3$) and potassium dihydrogen phosphate (KH_2PO_4) was found as inducer of glucose oxidase production while of zinc sulphate ($ZnSO_4.7H_2O$) and magnesium sulphate

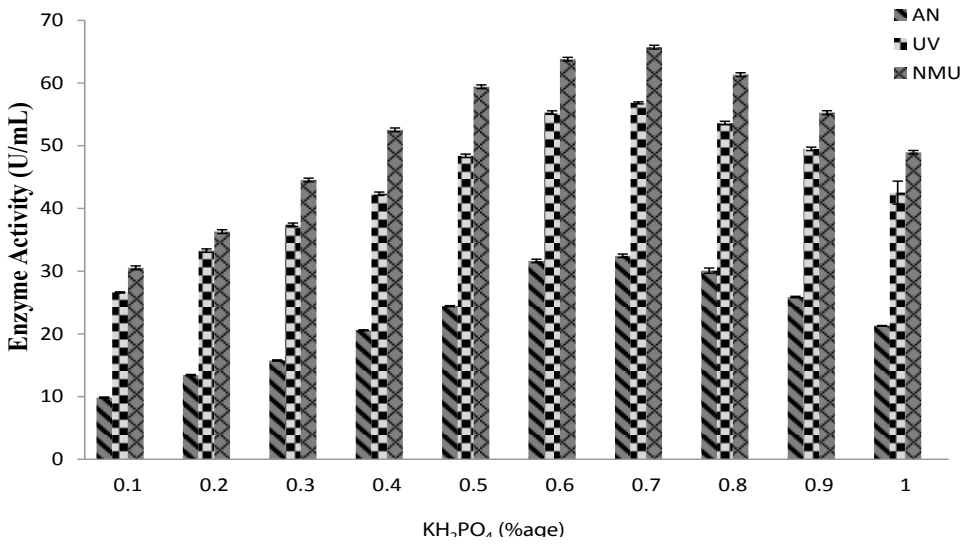

Figure 13: Effect of different KH_2PO_4 concentrations on the production of glucose oxidase from wild and mutant derived strains of *Aspergillus niger*

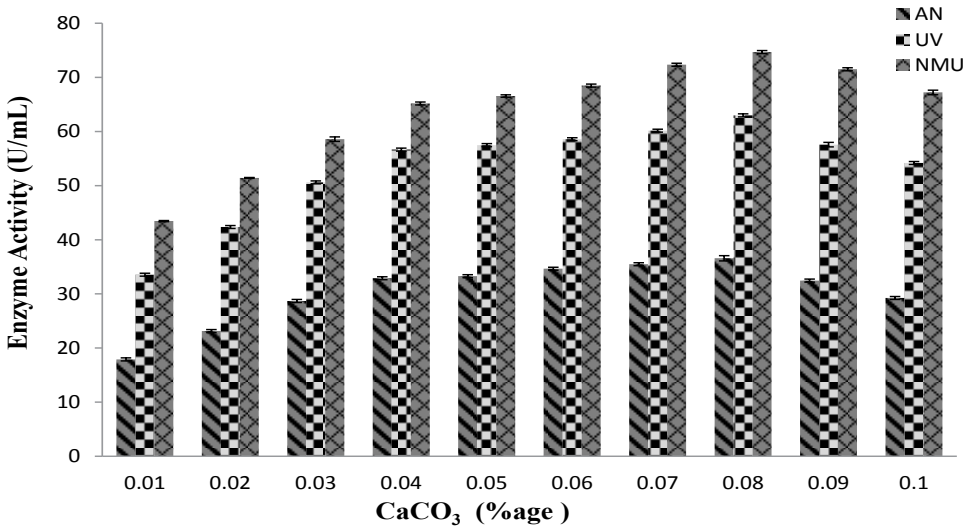

Figure 14: Effect of different $CaCO_3$ concentrations on the production of glucose oxidase from wild and mutant derived strains of *Aspergillus niger*

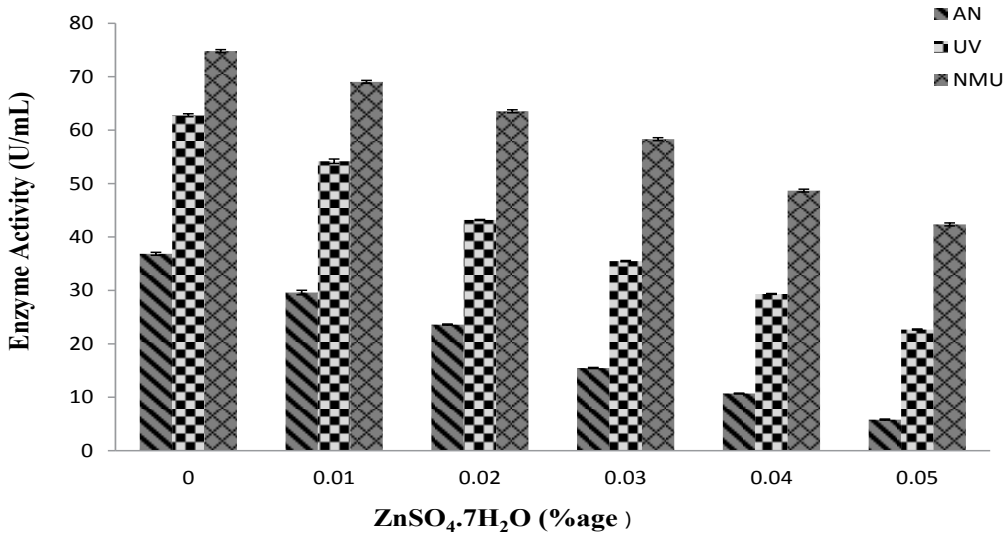

Figure 15: Effect of different $ZnSO_4.7H_2O$ concentrations on the production of glucose oxidase from wild and mutant derived strains of *Aspergillus niger*

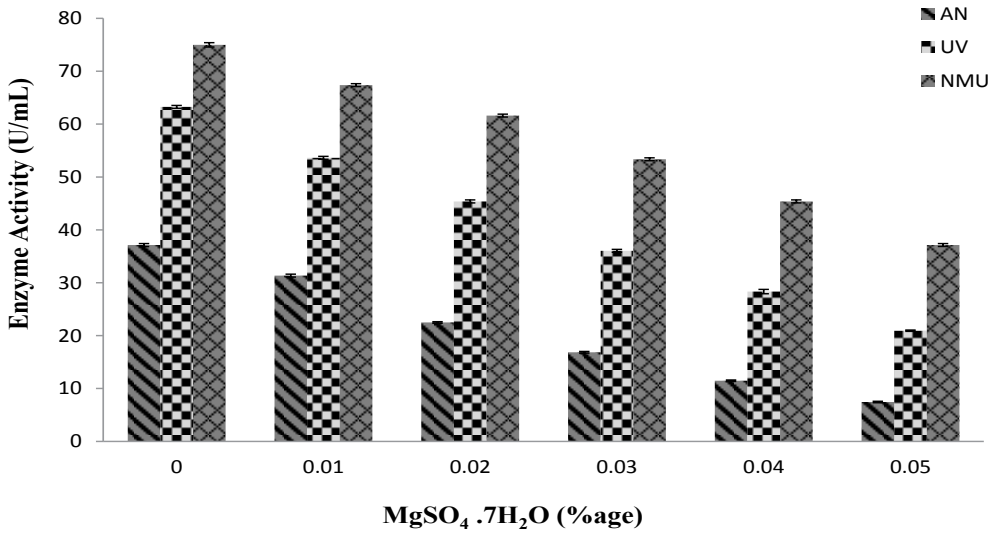

Figure 16: Effect of different $MgSO_4.7H_2O$ concentrations on the production of glucose oxidase from wild and mutant derived strains of *Aspergillus niger*

Table 2: Optimized physical and nutritional parameters for the improved production of glucose oxidase from selected wild and mutant derived strains of *Aspergillus niger*

S. No.	Optimized parameters	Quantity
	Corn steep liquor (Substrate)	3 g/100 mL
	Glucose (C-source)	3 g/100 mL
	Urea (organic N_2 source)	0.4 g/100 mL
	$(NH_4)_2SO_4$ (inorganic N_2 source)	0.4 g/100 mL
	KH_2PO_4	0.7 g/100 mL
	$CaCO_3$	0.08 g/100 mL
	$ZnSO_4.7H_2O$	0 g/100 mL
	$MgSO_4.7H_2O$	0 g/100 mL

($MgSO_4.7H_2O$) were showed their inhibitory behavior. Significance of these results was checked by ANOVA showed that with the addition of zinc sulphate ($ZnSO_4.7H_2O$) and magnesium sulphate ($MgSO_4.7H_2O$) in fermentation medium, yield of enzyme dropped.

Phosphate and potassium salts induce the biosynthesis of microbial enzymes while calcium carbonate (insoluble salt) prevent the decline in pH of microbial medium (engaged as buffering agent) which is dropped due to the concomitant production of gluconic acid during glucose oxidase catalyzed reactions in fermentation process and is frequently used as the mechanical support for the fungal growth. Hatzinikolaou and Macris [3] testified that calcium carbonate ($CaCO_3$) is strong inducer for glucose oxidase production that accompanied by a metabolic shift from glycolysis to the pentose phosphate pathway. While Fiedurek et al. [13] described that calcium carbonate ($CaCO_3$) is vital for the stimulation of enzyme production and its (calcium ions) higher concentration might be reduced the enzyme production. Fiedurek et al. [12], Petruccioli et al. [13] and Bodade et al. [22] also reported that there was gradual increase in glucose oxidase activity with the increase of the concentration of calcium carbonate ($CaCO_3$) and potassium dihydrogen phosphate (KH_2PO_4) while declined in enzyme activity was found with the addition of magnesium sulphate ($MgSO_4.7H_2O$) and zinc sulphate ($ZnSO_4.7H_2O$) in microbial culture medium. Scientist studied the effect of numerous metal ions ($CaCO_3$, $MgCO_3$, $MnCO_3$, $K2CO_3$, $CaCl_2$, $NaHCO_3$, $ZnCO_3$ and Ca3 $(PO_4)_2$ at 35 g/L) on glucose oxidase and catalase concurrent production by *Aspergillus niger* and was found that $CaCO_3$ have a remarkable effect on enzyme synthesis and it act as a strong inducer while other metal ions inhibits the microbial growth resultant no yield of glucose oxidase and catalase.

Salt concentrations play an important role on the enzyme activity. Thus, it is recommended that zinc sulphate ($ZnSO_4.7H_2O$) and magnesium sulphate ($MgSO_4.7H_2O$) should not be added/supplement in microbial culture medium for glucose oxidase production from *Aspergillus niger* because Mg^{+2} addition in microbial medium intensely inhibited the glucose oxidase production. Higher concentration of these salts produces the metal ions which compete with enzyme substrate for binding on enzyme's active sites that partially inhibited the Gox activity. As shown in figures, where AN represents wild *Aspergillus niger*, UV represents mutant derived TS-UV-200 *Aspergillus niger* strain and NMU represents TS-NMU-100 *Aspergillus niger* strain. The difference in findings in terms of concentrations as

reported earlier with current study findings may be due difference in microorganism and their sources, cultivation conditions and medium and other environmental factors, etc.

Nutritional requirement of wild and mutant derived strains of *Aspergillus niger* was appeared to be precisely similar as found under the current investigation. Similar findings were also described by Kona et al. [4], Zia [10], Bankar et al. [11] and Bodade et al. [12].

CONCLUSION

The use of economically cheaper and optimized suitable medium nutrient components is paving the way for cost effective glucose oxidase production process on commercial scale at industrial level. Optimized nutritional parameters for improved production of glucose oxidase from the cultivation of wild and mutant derived strains of *Aspergillus niger* achieved during this study was CSL (3%), glucose (3%), urea (0.4%), diammoniumsulphate ($(NH_4)_2SO_4$) (0.4%), potassium dihydrogen phosphate (KH_2PO_4) (0.7%), calcium carbonate ($CaCO_3$) (0.08%), zinc sulphate ($ZnSO_4.7H_2O$) (0%) and magnesium sulphate ($MgSO_4.7H_2O$) (0%) using single factor analysis method. Overall optimized cultivation conditions and nutritional parameters are given in the Table 2. After each step of optimization, it was clearly indicated that there was a gradual increase in the enzyme activity as compared to enzyme activity of non-optimized conditions. It was observed that mutant derived strains have the same environmental and nutritional requirement as parent wild strain of *Aspergillus niger* and there is no significant divergence was found in their production trend. It was also found that single factor analysis is more suitable for studying the effect of large number of variables/factors rather than other statistical methods.

From the current study, it is concluded that selected and optimized fermentation medium nutrients have a noteworthy capability for enhanced glucose oxidase production as compared to its un-optimized fermentation medium nutrients and this information about fermentation medium nutrients provided in the present study could be valuable for further advance research investigations and commercial scale production of glucose oxidase at industrial level.

FUTURE PROSPECTS

This study discusses selection and optimization of low cost fermentation medium nutrient components and renewable raw substrates sources in glucose oxidase production processes and their role in reducing the production cost. In future, our research on fermentation medium nutrients components for optimized glucose oxidase production must be targeted on the economics of the fermentation processes of glucose oxidase production, predominantly carried out through the practice of alternative low-cost effective production media and recovery processes.

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