Review Article

Isolation and purification of Glucose Oxidase from different Fungal sources

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Abstract

Objective: This paper discusses the main aspects of glucose oxidase. Glucose oxidase is an enzyme mainly obtained from filamentous fungi. Its most common source is *A. niger* which is easy to grow and extraction of GOD from it is relatively easy. **Methods:** Main mechanism of glucose oxidase is its property of being a flavor-protein which requires oxygen to form gluconic acid by the oxidation of sugar glucose. The composition of GOD is a dimeric glycoprotein with two similar polypeptide chains covalently linked with each other via di-sulphide linkages. In this review the characteristics and the analysis of glucose activity after its extraction are also discussed. Its extraction from different sources depends upon of few same and few different methods depending upon the organism it is obtained from. **Conclusion:** Glucose oxidase is a wonder enzyme having many functions and uses at the commercial level due to its significant properties. Many new technological advances are made to obtained pure, higher yield of glucose oxidase for various purposes.

Keywords: Glucose oxidase, filamentous fungus, enzyme purification, analysis, biosensors, yield

Introduction

Filamentous fungi are known to grow in different environments like organic waste, soil, plant cell material etc. To obtain nutrients, fungi uses many organic compounds with the help of various enzymes it produce (Gouka et al., 1997). Since 1950's there are a lot of advances in the applications of hydrolytic enzyme, glucose oxidase (Fiedurek and Gromada, 1997). This particular enzyme GOD (EC 1.1.3.4), is purified and developed from a number of sources of fungus like genus Aspergillus (Sukhacheva et al., 2004; Eryomin et al., 2004) and genus penicillium (Hatzinikolaou et al., 1996; Kalisz et al., 1991). Among them *Aspergillus niger* is the mostly used source (Pluschkell et al., 1996). It uses oxygen which accept electrons to produce gluconic acid by carrying out the β-D-glucose oxidation and at the same time produces hydrogen peroxide as well (Hatzinikolaou and Macris 1995). To eliminate glucose from varying sources like dried form of eggs, mayonnaise to prevent rancidity, fruit juices or beverages and to enhance the aroma, expiry, and color of food items, GOD is commercially used. Its application are vast as it is also used in glucose assay kits and as a biosensor for the assessment and the detection of the glucose in fluids of the body like urine, blood etc., and in industrial solutions (Petruccioli et al., 1999). Glucose oxidase is effective against various pathogens especially food-borne ones like *Bacillus cereus, Salmonella infantis, Clostridium perfringens, Taphylococcus aureus, Listeria monocytogens, Campylobacter jejuni* etc., (Kapat et al., 1998). It is also used as a food preservative, to produce gluconic acid on commercial level (Pluschkell et al., 1996) and in toothpaste as an important ingredient (Petruccioli et al., 1999). Glucose oxidase in new preparation with important biotechnological application is of a lot of interest to the commercial world.

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Reaction mechanism of GOD

GOD being a flavo-protein and as mentioned earlier it uses its oxygen molecules to accept electrons to produce gluconic acid which, in fact, is D-glucono-δ-lactone along with the production of hydrogen peroxide. This occurs by the oxidation of β-D-glucose (Hatzinikolaou et al., 1996; Pluschkell et al., 1996). There are two steps to this reaction: reduction and oxidation or redox in other words (Fig. 1). In the first step, GOD oxidises β-D-glucose to D-glucono-δ-lactone, which without an enzyme is forms gluconic acid. In other words it is hydrolyzed to form the acid. Consequently

$FADH_2$ is formed when the ring of GOD (FAD) is reduced (Witt et al., 2000). In other step, the reduced form of GOD is oxidized again to form hydrogen peroxide. This is oxidation. Hydrogen peroxide is cut into smaller units by catalase (CAT) followed by the production of oxygen and water.

GOD composition

If we take ascomycetes, GOD obtained from it is in a form of dimer having two similar subunits or polypeptide chain (Kalisz et al., 1997; Rando et al., 1997) that are joined together covalently by the help of disulfide linkages. Of *P. amagasakiense*, the GOD subunit residues conserved active sites and FAD moiety are shown (figure 2) (Wohlfahrt et al., 1999). Each subunit of the GOD consists of one firmly and non-covalently bound mole as co-factor (Witt et al., 2000; Rando et al., 1997). In figure 2, Glucose oxidase residues having conserved active-sites, from the same specie *P. amagasakiense* are Tyr-73, Asn-518, His-563, Trp-430, Phe-418, Arg-516 and His-520 (Witt et al., 2000). The important amino acid best for the effective binding of β-D-glucose through GOD enzyme is Arg-516 (Witt et al., 2000). Asn-518 also contribute to its binding but to a minor extent. There are some aromatic residues likeTrp-430, Phe-418 and Tyr-73, which are also included for the perfect orientation of substrates and GOD maximal velocity. During reaction, histidine residues like His-520 and His- 563 are important in forming H2 bonds with the OH attached at 1 carbon of glucose molecule (1-OH).

Characteristics of GOD

Approximately 130 to 175 kDa is the normal range of GOD molecular weight (Kalisz et al., 1997). Glucose oxidase enzyme shows extreme specificity for β-anomer of D-glucose, whereas, the alpha anomer (α-anomer), seems to be a non-suitable substrate. When using substrates like D-mannose, D-galactose, 2-deoxy-D-glucose, GOD shows less activity. There are certain inhibitors like Cu_2+, Ag+, pchloro-mecuri-benzoate, hydrazine, Hg^2+, dimedone, phenyl hydrazine, sodium bisulphate and hydroxylamine which greatly inhibits the activity of GOD (Kusai et al., 1960).

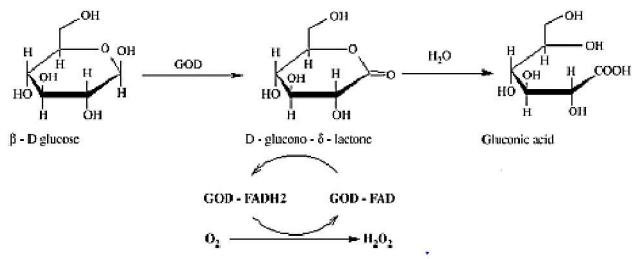

Figure 1. GOD enzyme reaction

In 1968 *P. amagasakiense* and *A. niger* were used to obtain GOD and their molecular weight found was 150 and 152 kDa. GOD obtained from both strains had almost the same carbohydrates composed of hexosamine, glucose and mannose but the GOD from *A. niger* had more hexosamine and mannose and less glucose than the other organism. Even the content of amino acid like more tyrosine, histidine, and arginine and less phenylalanine and lysine was present in *A. niger* than *P.amagasakiense.* GOD optimum ranges for pH were 3.5 to 6.5 and in another case it is 4.0 to 5.5 for *A. niger* and *P. amagasakiense,* respectively. It shows that the better source of GOD is *A. niger* than any other (Nakamura and Fujiki, 1968).

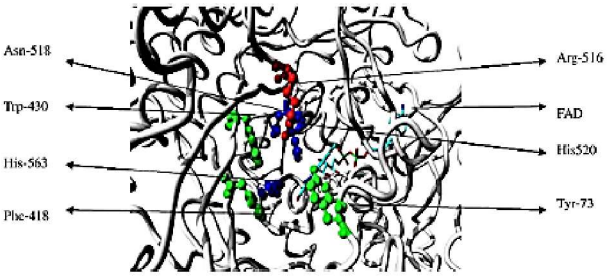

Figure 2. GOD (*P.amagasakiense)* representing FAD moiety and active-site conserved residues

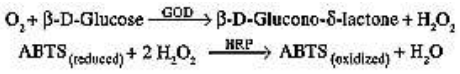

Figure 3. Glucose oxidase reaction with a chromogenic dye, ABTS

Analysis of glucose oxidase activity

Literature shows various glucose activity determining methods. Titrimetric method given by Tongbu et al., (1996) uses enzyme solution in sodium acetate buffer having 2 % β-D-glucose. Solution of sodium hydroxide is used to discontinue the reaction. To measure the amount of added hydrochloric acid and glucose activity, the solution was titrated against standard HCl. Normally analytical method is used which is based on the oxidation of β-D-glucose to β-D-glucono-δ-lactone and H_2O_2 by GOD in the availability of oxygen. In a secondary step of reaction, a chromogenic substrate is then oxidized by the help of H_2O_2 with HRP (horseradish peroxidase) followed by a change in color observed through spectrophotometer. 2, 2′-Azino-di-(3-ethylbenzthiazolin- sulfonate) and Ando-dianisidine (Witt et al., 1998; (Bergmeyer *et al.*, 1974) are the chromogenic substrate required by the GOD reaction. A bluish green oxidized product formulates, as seen at 420 nm through a spectrophotometer, by ABTS. Reactions are carefully explained in Schemes 1 and 2. Quinone-imine dye is formed by the oxidation of o-dianisidine. Spectrophotometer measurement for which is 500 nm.

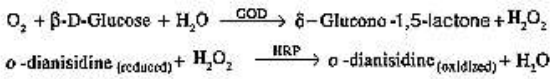

Figure 4. Glucose oxidase reaction with a chromogenic dye, *o*-dianisidine

Properties of Glucose oxidase

The most common source of Glucose oxidase happens to be *A. niger*. Some of the properties of GOD obtained from this particular specie are mentioned at follows. GOD belongs to the oxidoreductase class of enzymes. It is a large group and contain enzymes that can cause both oxidation and reduction. Another name for this enzymes is glucose aero-dehydrogenase (Witteveen et al., 1992; Sidney and Northon, 1955). The Molecular weight of this enzyme is reported to be 160,000 Da. Molecular weight is almost 16 % neutral sugar, 2 % amino sugars, 74 % proteins. All given in w/w measurement (Tsuge et al., 1975). As mentioned before it contains of two subunits of chains of polypeptide each 80,000da bonded covalently by di-sulphide linkages. Each subunits consists of one mole FAD and one mole of iron (Fe) (O'Malley and Wcaver, 1972). FAD can be replaced by FHD without losing the enzymatic activity. The broad range pH is 4-7 while the Optimum pH is 5.5.

Sources

There are different sources from where glucose oxidase can be obtained. Some organisms produce intracellular glucose oxidase while others produce extracellular glucose oxidase. List of different sources are given in table 1. In this paper extraction and purification of glucose is discussed from few of the sources. Organisms such as *Aspergillus flavus* and *Penicillium* sp., *Aspergillus niger, Talaromycesflavus, Phanerochaete chrysosporiumt, Penicillium* sp. CBS 120262, *Penicillium pinophilum* and *pencillium amagasakiense* are mainly discussed for the extraction and purification of glucose oxidase. General protocol for the purification of glucose oxidase is given in figure 5 (Sandip et al., 2009). Fungi are important organisms in the field of biotechnology and microbiology due to their various properties, most importantly their capability to yield enzymes. These enzymes are efficient biocatalysts for the fast decomposition and the oxidation of carbohydrates, fats and prot

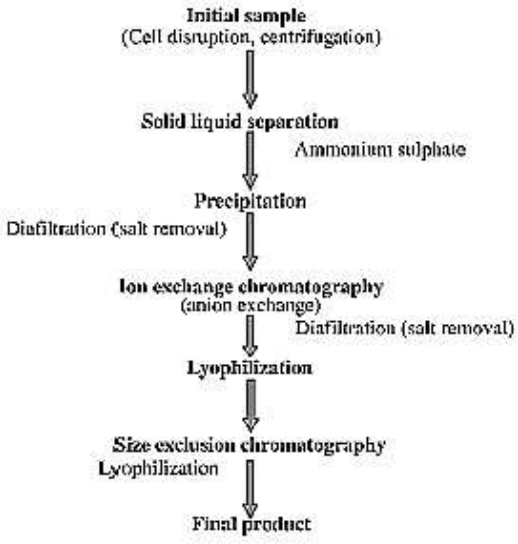

Figure 5. General protocol for GOD purification

Isolation of GOD from *Penicillium Sp. and Aspergillus Flavus*

To isolate these organisms soil and plant samples were taken from Indian soil. In media composition, for the separation of the organisms, PDA (potato Dextrose Agar) was prepared and sterilized. Soil samples were serially diluted and were individually incubated on PDA plates for seven days at room temperature since fungus grows best at 37 ○ C. After incubation, the specie was identified through microscope, on the basis of conidia and morphology using standards information (Ellis, 1971; Barnett and Hunter, 1972). Each successfully obtained specie was then mass cultured on medium; Potato dextrose broth for seven days. After seven days the GOD activity of extracellular material and fungal mass was determined through various techniques like titration and diastix method.

In titration method the 1ml of enzyme solution was poured into 25 ml buffer of 60 mM $C_2H_3NaO_2$ having two percent D-glucose about 2 %. Mixture was shaken well via air and in rotary shaker and the reaction was stopped after adding 20 ml of NaOH about 0.1 M. The mixture was then titrated to obtain dark pink end point using indicator phenolphthalein by adding HCl 0.1M. The GOD activity can be measured by using the formula {(V0-V) x N x 1000}/60 where N in standard HCl solution concentration. Diastix is another method which uses reagent strips for the detection of GOD from intra or extracellular fungal specie fractions. These strips are highly specific and that is why are normally used for vitro diagnosis, at homes and hospitals, to detect glucose in urine and blood. These strips contain 1 % peroxidase, 2.2 % glucose oxidase and 8 % potassium iodide, non-reactive reagents about 18 % and 70 % buffer. At the tip of the strips the peroxidase and GOD are immobilized on a paper pad which is covered in thin membrane of cellulose permeable to small molecules like that of glucose. When these strips are dipped in fractions of extra or intracellular enzymes the reaction takes place, as given in figure 6, and the change in color occurs. More intense the color more concentrated the glucose is (Shweta et al., 2013). Working of the diastix strip is shown below. The enzyme GOD acts on the molecule of glucose and convert it to gluconic acid and H_2O_2

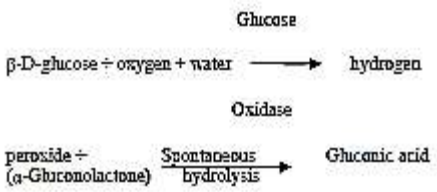

Figure 6. Action of GOD on glucose

Table 1. Different GOD producing microorganisms

Microorganisms	Media composition g/l	Assay method	Yield (Unit)	References
Penicillium variable P16	$FeSO_4.7H_2O$, 0.01; Glucose, 80; $NaNO_3$, 5; KCl, 0.5; Mycological peptone, 1; KH_2PO_4, 1; $CaCO_3$, 35.	Reduction of benzoquinone by hydroquinone measured by the rate of absorbance increase at 290 nm	5.52U ml/1	Petruccioli et al., 1999.
A. niger glucose oxidase gene expressed in *S.cerevisiae*	Yeast peptone dextrose (YPD) medium: Yeast extract, 10; peptone, 20; glucose, 20	Plate assay: with o-dianisidine Coupled coupled O-dianisidine-peroxidase reaction.	125U ml/1	Hodgkins et al., 1993.
Aspergillus niger (BTL)	Sucrose, 70; $(NH_4)2HPO_4$, 0.4; KH_2PO_4, 0.2; $SO_4.7H_2O$, 0.2; peptone, 10; $CaCO_3$, 35.	Coupled o-dianisidine-peroxidase reaction	7.5U ml/1	Hatzinikolaou and Macris, 1996
Recombinant *Saccharomyces cerevisiae*	Yeast extract, 40; hycas, 5; glucose, 20; galactose, 30	Coupled ABTS reaction	3.43U mg/1 dry cell mass	Kapat et al., 2001.
Aspergillus niger AM111	Glucose, 80; peptone, 30; $NaNO_3$, 0.5; KH_2PO_4, 1; $CaCO_3$, 35	Coupled reaction assay	2.5U ml/1	Fiedurek and Gromada
Penicillium pinophilum DSM 11428	Sucrose, 40; $Na_2PO_4.2H_2O$, 4.45; KH_2PO_4, 1.5; $NaNO_3$, 1.9; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.02; malt extract, 10; yeast Extract, 5; Trace element, 10; vitamin, 10.	Coupled ABTS -peroxidase reaction.	1.9U ml/1	Rando et al., 1997.
Aspergillus niger ZBY-7	Glucose, 150; inorganic salts, 0.35; metal caronate, 35	Titrimetric	6U ml/1	Tongbu et al., 1996.

In the second step the H_2O_2 is then catalyzed by peroxidase via potassium iodide which in turn in oxidized to give brown color

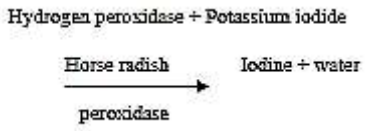

Figure 7. Catalysis of hydrogen peroxidase

Using buffer of o-dianisidie as a coupling reagent and substrate glucose, spectrophotometer was used to check the glucose oxidase activity at wavelength of 460 nm (Worthington, 1988). Residual sugar gives correct estimate of GOD activity, as given by the method of Moneral and Reese (1969) and taking standard bovine serum albumin, proteins were measured through the technique of Lowry et al., (1951), El-Sherbeny et al., (2005). After confirming the activity of GOD, the crude excerpt of enzyme was used to purify the enzyme. Different amount of ammonium sulphate was added to get 20-100 % saturation and was left for a night. The mixture was centrifuged for 15 minutes at 15000rpm to eliminate un-dissolved particles and the remaining fraction was re-dissolved in 0.1 M, 20 ml citrate phosphate buffer (Shweta et al., 2013).

Isolation of glucose Oxidase from *Aspergillus niger*

Media used to grow this specie was again PDA used at 4-60 ○C temperature and was sub-cultured after a time period of every 20 days. Pre-culture of *A.niger* was obtained by growing its spores in Erlenmeyer flask containing suitable media of about 50 ml for maximum GOD production the solution was optimized using BSM (basil salt medium). Centrifugation was carried out at 7000 g for about 20 minutes and mycelia were harvested for intracellular fraction. Supernatant contained extracellular Glucose oxidase. This fraction was suspended in the same buffer of sodium citrate at pH 5.5. Liquid nitrogen was used to freeze the biomass which was then crushed to powder form. Eventually the supernatant obtained after centrifugation, consisted of the intracellular glucose oxidase. The activity was determined via spectrophotometer by reducing benzoquinone to hydroquinone at 290 nm.

Table 2. Data of GOD obtained from two different species of fungi.

Fungi	Activity	Conc. Of glucose	GOD activity
Aspergillus niger	26.2 U/min ml	2+++	4.3 u/ml
Aspergillus flavus	66.3 U/min ml	2+++	6.9 u/ml

Table 3. GOD purification from *Aspergillus niger*

Methods	Units (IU)	Protein (mg)	Specific activity (IU/mg of protein)	Purification Fold	Recovery (%)
Culture filtrate	18	33	0.73	--	100
Ammonium sulphate	17	22.0	0.75	0.1	95
Gel Permeation chromatography	14.4	2.38	6.05	8.6	84.6
Q-Sepharose strong anion exchange chromatography	12.2	0.78	15.6	22.2	71.6
DEAE-Sepharose as weak anion exchange chromatography	11.4	0.51	22.3	30.08	63.3

To precipitate the protein, enzyme obtained was centrifuged at 9000 g for about 10 minutes using ammonium sulphate at 4 ○C. This precipitate was centrifuged again to obtain the pellet for gel filtration to salt out and purify GOD. For further purification ion exchange chromatography was used (Jagdish and Neelum, 2013). Different methods for the purification of GOD are given in table 3.

Isolation of glucose oxidase from *Talaromyces Flavus*

At 260 ○C along with aeration, Erlenmeyer flask having 100 ml of culture medium was used to grow GOD in Submerged culture (Mizuno et al., 1974). The mycelia were harvested through filtration, having 0.45 pm membranes and were washed two to three times in distilled water. 1 g of this wet mycelia was suspended in 10 ml of 0.1 M K_2PO_4 having pH 6. 1 mM EDTA was also used in this mixture. Ice bath was then used to disrupt the mycelium for 15s via Sowall omni-mixer at a setting of 6. Centrifuge followed at 5000 g for ten minutes at 4○C. Both the supernatant and the filtrates were used to determine the glucose oxidase activity. Here the enzyme activity was determined through coupled reaction with o-dianisidine and peroxidase at 250 ○C (Decker, 1977). GOD activity of 1mole is defined as amount of enzyme needed to release 1pm of H_2O_2 per minute at 250 ○C. Concentration of protein was found out using Bradford method with standard bovine serum albumin.

Crude enzyme GOD was precipitated from the filtrates obtained from the culture using ice-cold acetone (Kim et al., 1988). Extracts of water of acetone precipitates were changed to 50 mM sodium acetate followed by chromatography on HPLC gel-filtration column. This protein was eluted from the column using 0.2 M NaCl at pH 5. The resultant fraction obtained was concentrated and dialyzed by the technique of ultrafiltration. To determine the size of the particles, gel electrophoresis was performed.

Isolation of glucose oxidase from *Phanerochaete Chrysosporium*

Suitable sterile media was prepared and inoculated with conidial suspensions followed by incubation at 37 ○C for about a week without agitation. O-dianisidine and horseradish peroxidase were used to determine the GOD activity. The size of the protein was determined by running the sample on SDS-PAGE followed by the staining of gel with coomassie brilliant blue stain. Protein content was determined via Lowry method. Purification is mainly carried out at 4 ○C. To suspend the protein phosphate buffer was used and the solution was concentrated using ultrafiltration technique. To prepare the extracts, low nitrogen medium was used to grow *P. chrysosporium* at 37 ○C for 6-7 days. Mycelia obtained were washed several times with phosphate buffer and were re-suspended in the same buffer to maintain the enzyme. While adding, glass beads were also included with 1:1 ratio and the mixture was blended for 10-15 minutes. Centrifugation makes it possible to remove glass beads and unbroken mycelia in the end and supernatant was saved/frozen until further use. Pellet was re-suspended in the same buffer.

Frozen extracts of cell were thawed and clarified by centrifugation technique for 15 minutes. The resultant extract was diluted using distilled water, five folds. DEAA-Sephadex was used and this solution of protein was applied on it. Column was then washed and the protein was eluted using high salt concentration solution. Similarly the technique of Sephacryl chromatography and DEAE-Sepharose chromatography can also be used for the purification of enzyme. For the determination of molecular weight, gel filtration chromatography having S-300 Sephacryl column was used.

Table 4. Purification of GOD from culture filtrates of *Talaromyces flavus*

Purification steps	Total protein (mg)	Total units (pmol/min X (10^{-4})	Specific activity (prnol min-1 mg-I)	Yield %
Cultures filtrate	123.7	1.34	108	100
Acetone precipitation	37.4	1.24	332	92
DEAE-5PW	15.8	0.771	488	58

Table 5. GOD Purification data from *P. chrysosporium* ligninolytic cultures

Fraction	Sp act (U/mg)	Total protein (mg)	Total activity (U)	Yield (%)	Purification (fold)
Crude cell extract	0.17	285	48.4	100	1.0
DEAD-Sephadex	0.91	32	29.1	60	5.4
Sephacryl S-300	6.39	3.1	19.8	41	37.8
DEAD-Sepharose	15.1	0.28	4.2	9	89.3

Table 6. GOD purification from *P. pinophilum*

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Mycelium extract	1795	285.0	6.3	1.0	100
S-Sepharose	1355	47.5	28.5	4.5	75
Sephacryl S-300	1331	11.7	113.5	18.0	74

Isolation of Glucose Oxidase from *Penicillium pinophilum*

It was obtained from soil and was grown in agar/malt slants and then grown at 4 ○C. For the production of enzyme it is grown in a bioreactor. After the spores are inoculated and grown the GOD activity was measured by chromogenic assay, using spectrophotometer at 420 nm. Bovine serum albumin was used to determine the concentration of protein (Schmidt et al., 1963). For the purification and the isolation of glucose oxidase, sodium citrate was used to harvest the mycelium. Resultant is suspended in the same buffer and by using blender the solution was homogenized for 10 seconds.

While sonicating the sample, alternate chilling at 4○ C was provided to prevent the sample from denaturing. Cell centrifugation was then performed to remove the debris from the solution. It was also carried out at 4 ○ C. Chromatographic technique such as Sepharose fast flow column was used to apply the sample onto it along with the citrate buffer followed by the elution of GOD using linear gradient of NaCl. The fractions on GOD obtained are concentrated by the help of ultrafiltration. This concentrated protein solution obtained was further applied to S-300 Sephacryl column and gel titration was performed and the fraction obtained were concentrated via ultrafiltration (Rando et al., 1997).

Applications

Commercially glucose oxidase has become a very important enzyme during the last few years mainly because of its vast uses and applications. It is used in almost every field such as microbiology, biotechnology, beverage industry, food, clinical, or pharmaceutical etc. due to its extreme stability and cheapness it is mostly used for the glucose activity determination as an analytical reagent. Other uses may include its ability to preserve food, stabilize color or use as a biosensor of diabetes. With improved GOD methods and techniques higher yield of different products and economic stability can be achieved (Kleppe, 1966). Current important applications of GOD are given as follows.

Glucose biosensor for diabetes monitoring: Diabetes mellitus patients need to regularly check their glucose level in blood in order to check the fluctuations in the glucose level, otherwise which would lead to high or low blood pressure i.e., hyperglycemia or hypoglycemia. There are some monitoring devices in use such as those which use finger-prick blood samples with a digital meter that tells the glucose level automatically, several times in a day. In the modern world, biosensors are in use and glucose oxidase is the best example of one. Other types of GOD sensors work on the principle of sensitive fluorescence measurement which measures the changes in the FAD fluorescence of Glucose oxidase. Some of the biosensors used are given in the table 7.

Table 7. Types of biosensors

Type of biosensors	References
On-line glucose monitoring for fermentations	Vodopivec et al., 2000
Fiber optic biosensor for analyzing glucose concentrations in soft drinks	Chudobova et al., 1996
Disposable strip-type biosensor for blood and serum monitoring	Cui et al., 2001
Strip type biosensor for blood (GOD-HPR-dye)	Kim et al., 2001
Miniaturized thermal biosensor for whole blood	Harborn et al., 1997
Glucose sensor for whole blood	Santoni et al., 1997
Glucose biosensor for serum from human blood	Zhu et al., 2002

Low alcohol wine production: Glucose oxidase has been used for the production of wine over so many years. It can lower the amount of alcohol content by removing some of the glucose. Production of reduced alcohol with steps are given in figure 8 (Pickering et al., 1998).

Oral hygiene: Glucose oxidase has antimicrobial properties and can be used for the care of oral health (Afseth and Rolla, 1983). In a healthy human, a mouth contains several microbe species such as streptococcus mutans. This is mainly responsible for tooth decay and bad breath. Glucose oxidase is effective against it because it produces hydrogen peroxide to fight away the microbes.

Gluconic acid: Production of gluconic acid is perhaps the most important and the most common commercial use of glucose oxidase. It acts as an acidity regulator used in food additives, used in sterilization solution and for bleaching in food industries. In medical industry it is used as a salt for the production of medicines. It is also used in the leather and metal industries as an acidulant and used as an additive to enhance stability of cement in the cement industry. Naturally one can find it in fruits, honey etc., (Klein et al., 2002).

Textile industry: GOD has many uses in the textile industry especially for the production of hydrogen peroxide for bleaching purposes (Tzanov et al., 2002). No stabilizers were needed for this process because the gluconic acid produces behaves like a stabilizing agent.

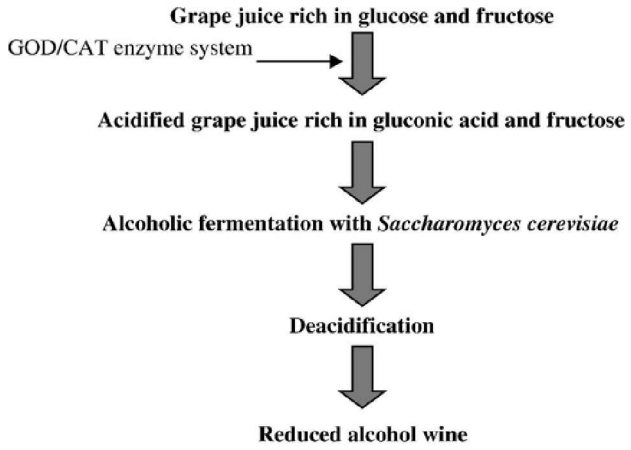

Figure 8. Low alcoholic wine preparation from GOD treated grape juice

Biofuel cells: Electric devices require a lot of energy to perform their functions. To reduce the energy consumption, bio-electric devices are produced which require only a small source to stabilize the operations. These devices work by converting the bio-chemical energy into useful electrical energy with a help of a biocatalyst. The cell (biofuels) consists of 2 set of electrodes, of any suitable stable energy conducting material to reduce or oxidize the substances using bio-catalytic enzymes. Energy demanding bio-electric devices.

Food and beverage additive: To remove oxygen and the residual glucose from food substances to increase their shelf life, glucose oxidase is used. As mentioned earlier the glucose oxidase produces hydrogen peroxidase as a useful bactericide and can be easily removed using another enzyme known as CAT, which ends up breaking down hydrogen peroxide into water and oxygen. Some of the food items in which GOD is used are; production of egg powder, dehydration via Millard reaction causes browning to the puree storage and processing fruits, GOD is used instead which cause non-enzymatic browning of the food items, it has stabilizing properties to various food items, it also prevents the loss of color and flavor in tined foods, fish, soft drinks, beer etc., by removing O_2 from them (Crueger and Crueger, 1990). Also used in wine making and mayonnaise production to prevent the browning mechanism. There are countless benefits of GOD but care should be taken since the excess amount of the enzyme can cause adverse effects.

Conclusion

As discussed above, GOD is the future of the world. It is the most important enzyme used in industries such as pharmaceuticals, textile, beverages, food and biotechnology etc. there is a huge need to convert the commercially available procedures to large scale production fermenters. However, there is not much work done on the large scale production of this enzyme. It is still commonly obtained from microorganism sources easily gained. These sources at commercial level are limited to only few strains that produce GOD. Therefore, there is high demand of large scale production or increase in production of GOD from the existing sources. This can be achieved by conducting chemical modifications, through recombinant technology in the existing enzyme producing protein. This is quite famous in the biopharmaceutical industries.

References

Afseth J, Rolla G. 1983. Clinical experiments with a toothpaste containing amyloglucosidase and glucose oxidase. Caries Research, 17(5):472–5.

Barnett HL, Hunter BB. 1972. Illustrated genera of imperfect fungi. Burgess Publishing Company Minnesota, 2(1): 2-4

Bergmeyer H, Gawehn K, Grassl M. 1974. Methods of Enzymatic Analysis Bergmeyer, HU. New York: Academic Press Inc., 2(1):457–8.

Chudobova I, Vrbova E, Kodicek M, Janovcova J, Kas J. 1996. Fiber optic biosensor for the determination of D-glucose based on absorption changes of immobilized glucose oxidase. Analytica Chimica Acta, 319(1):103–10.

Crueger A, Crueger W. 1990. Glucose transforming

enzymes. In: Fogarty WM, Kelly CT, editors. Microbial enzymes and biotechnology. New York: Elsevier, p: 177–226.

Cui G, Yoo JH, Woo BW, Kim SS, Cha GS, Nam H. 2001. Disposable amperometric glucose sensor electrode with enzyme-immobilized nitrocellulose strip. Talanta, 54:1105–11.

Deckeral A. (Editor) 1977. Worthington enzyme manual. Worthington Biochemical Corporation, 37-39.

El-Rashidy LA, Bahlol HEM, El-Desoky AA. 2015. Improving quality of pan bread by using glucose oxidase and lipase enzymes. Middle East Journal of Applied Sciences, 5:1035-1043.

El-Sherbeny GA, Sshindia AA, Sheriff. 2005. Optimization of various factors affecting glucose oxidase activity produced by *Aspergillus niger*. International Journal of Agriculture and Biology, 7(6): 953-958.

Eryomin AN, Droshdenyuk AP, Zhavnerko GK, Semashko TV, Mikhailova RV. 2004. Sand as an adsorbent for purification of extracellular glucose oxidase from *Penicillium funiculosum.* Applied Biochemistry and Microbiology, 40(2): 178–85.

Fiedurek J, Gromada A, Jamroz J. 1996. Effect of medium components and metabolic inhibitors on beta-galactosidase production and secretion by *Penicillium notatum* 1. Journal of Basic Microbiology, 36(1):27–32.

Fiedurek J, Gromada A. 1997. Screening of mutagenesis of moulds for improvement of the simultaneous production of catalase and glucose oxidase. Enzyme and Microbial Technology, 20:344–7.

Fiedurek J, Gromada A. 2000. Production of catalase and glucose oxidase by *Aspergillus niger* using unconventional oxygenation of culture. Journal of Applied Microbiology, 89(1):85–9.

Gerritsen M, Kros A, Lutterman J, Nolte R, Jansen J. A percutaneous device as model to study the in vivo performance of implantable amperometric glucose sensors. Journal of Materials Science: Materials in Medicine, 12(2):129–34.

Gouka RJ, Punt PJ, Van den CA. 1997. Efficient production of secreted proteins by *Aspergillus*: progress, limitations and prospects. Applied Microbiology and Biotechnology, 47:1–11.

Gunasundari S. 2014. Production of glucose oxidase from *Aspergillus oryzae* by liquid state fermentation for the preservation of food. International Journal of Ethnomedicine and Pharmacological Research, 2:51-57

Harborn U, Xie B, Venkatesh R, Danielsson B. 1997. Evaluation of aminiaturized thermal biosensor for the determination of glucose in whole blood. Clinica Chimica Acta, 267:225–37.

Hatzinikolaou DG, Hansen OC, Macris BJ, Tingey A, Kekos A, Goodenough P. 1996. A new glucose oxidase from *Aspergillus niger* characterization and regulation studies of enzyme and gene. Applied Microbiology and Biotechnology, 46:371–81.

Hodgkins M, Mead D, Balance DJ, Goodey A, Sudbery P. 1993. Expression of the glucose oxidase gene from *Aspergillus niger* in Hansenula polymorpha and its use as a reporter gene to isolate regulatory mutation. Yeast, 9:625–35.

Jagdish S, Neelam V. 2013. Glucose oxidase from *Aspergillus niger:* Production, characterization and immobilization for glucose oxidation. Advances in Applied Science Research, 4(3):250-257

Kalisz H, Hendle J, Schmid R. 1997. Structural and biochemical properties of glycosylated and deglycosylated glucose oxidase from *Penicillium amagasakiense*. Applied Microbiology and Biotechnology, 47:502–7.

Kapat A, Jung J, Park YH. 1998. Improvement of extracellular recombinant glucose oxidase production in fed-batch culture of Saccharomyces cerevisiae: effect of different feeding strategies. Biotechnology Letters, 20(3):319–23.

Kayk K, Deborah F, George P. 1989. Production, purification, and properties of glucose oxidase from the biocontrol fungus *Talaromycesflavus*. Canadian Journal of Microbiology, 36: 199-205.

Kelley RL, Adinarayana R. 1986. Purification and Characterization of Glucose Oxidase from Ligninolytic Cultures of *Phanerochaete chrysosporiumt*. Journal of Bacteriology, 166(1):269-274.

Kim K, Fraveld K, Papavizags C. 1988. Identification of a metabolite produced by *Talaromyces flavus* as glucose oxidase and its role in the biocontrol of Verticillium dahlia. Phytopathology, 78: 488-492.

Kim SH, Lee SM, Kim DU, Cui JZ, Kang SW. 2001. Enzyme-based glucose biosensor using a dye couple system. Dyes and Pigments, 49:103–8.

Klein J, Rosenberg M, Markos J, Dolgos O, Kroslak M, Kristofikova L. 2002. Biotransformation of glucose to gluconic acid by *Aspergillus niger* — study of mass transfer in an airlift bioreactor. Biochemical Engineering Journal, 3568:1–9.

Kleppe K. 1966. The Effect of hydrogen peroxide on glucose oxidase from *Aspergillus niger*. Biochemistry,

5(1):139–43.

Kusai K, Sekuzu I, Hagihara B, Okunuki K, Yamauchi S, Nakai M. 1960. Crystallization of glucose oxidase from *Penicillium amagasakiense*. Biochimica et Biophysica Acta, 40:555–7.

Mizuno A, Takadam M, Yamaguchi A. 1974. A new antibiotic, talaron. The Journal of Antibiotics, 27:560-563.

Monreal J, Reese ET. 1969. The chitinase of Serratia marcescens. In Canadian. The Journal of Microbiology, 15: 689 93.

Nakamura S, Fujiki S. 1968. Comparative studies on the glucose oxidases of *Aspergillus niger* and *Penicillium amagasakiense.* The Journal of Biochemistry, 63(1):51–8.

O' Malley JJ, Weaver JL. 1972. Subunit Structure of glucose oxidase from *Aspergillus niger.* Biochemistry, 2: 3527.

Petruccioli M, Federici F, Bucke C, Keshavarz T. 1999. Enhancement of glucose oxidase production by *Penicillium variabile* P16. Enzyme and Microbial Technology, 24:397–401.

Pickering GJ, Heatherbell DA, Barnes ME. 1998. Optimizing glucose conversion in the production of reduced alcohol wine using glucose oxidase. Food Research International, 31(10):685–92.

Pluschkell S, Hellmuth K, Rinas U. 1996. Kinetics of glucose oxidase excretion by recombinant *Aspergillus niger*. Biotechnology and Bioengineering, 51:215–20.

Rando D, Kohring G, Giffhorn F. 1997. Production, purification and characterization of glucose oxidase from a newly isolated strain of *Penicillium pinophilum*. Applied Microbiology and Biotechnology, 48:34–40.

Santoni T, Santianni D, Manzoni A, Zanardi S, Mascini M. 1997. Enzyme electrode for glucose determination in whole blood. Talanta, 44:1573–80.

Schmidt K, Liaaen JS, Schlegel HG. 1963. Die Carotinoide der Thiorhodaceae. I. Okenon als Hauptcarotinoid von Chromatium okenii Perty. Archives of Microbiology, 46: 117±126.

ShwetavVB, Swathi BR, Maria R, Govindappa M. 2013. Isolation and charecterization of Glucose Oxidase (GOD) from *Aspergillus flavus* and *Penicillium sp*. International Journal of Current Microbiology and Applied Sciences, 2(6): 153-161.

Sidney PC, Northon OK. 1955. Methods in Enzymology. Academic Press. Inc. pub. New York, 1:340-345.

Sukhacheva MV, Davydova ME, Netrusov AI. 2004. Production of *Penicillium funiculosum* 433 glucose oxidase and its properties. Applied Biochemistry and Microbiology, 40(1):25–9.

Sundip BB, Mahesh VB, Rekha SS, Laxmi A. 2009. Glucose oxidase — An overview. Biotechnology Advances, Elsevier, 27: 489–501.

Tongbu L, Peng X, Yang H, Liangnian J. 1996. The production of glucose oxidase using the waste myceliums of *Aspergillus niger* and the effects of metal ions on the activity of glucose oxidase. Enzyme and Microbial Technology, 19:339–42.

Tsuge H, Natusaki O, Ohashi K. 1975. Purification, properties and molecular features of Glucose Oxidase from *Aspergillus niger*. The Journal of Biochemistry (Tokyo), 78: 835-838.

Tzanov T, Silgia, A, Gubitz GM, Cavaco-Paulo A. 2002. Hydrogen peroxide generation with immobilized glucose oxidase for textile bleaching. Journal of Biotechnology, 93:87–94.

Vodopivec M, Berovic M, Jancar J, HRPgornik A, Strancar A. 2000. Application of convective interaction media disks with immobilized glucose oxidase for on-line glucose measurements. Analytica Chimica Acta, 407:105–10.

Witt S, Wohlfahrt G, Schomburg D, Hecht H, Kalisz H. 2000. Conserved arginine-516 of *Penicillium amagasakiense* glucose oxidase is essential for the efficient binding of β-D-glucose. The Journal of Biochemistry, 347:553–9.

Witt S, Singh M, Kalisz H. 1998. Structural and kinetic properties of nonglycosylated recombinant *Penicillium amagasakiense* glucose oxidase expressed in *Escherichia coli*. Applied and Environmental Microbiology, 64(4):1405–11.

Witteveen C, Veenhuis M, Visser J. 1992. Localization of glucose oxidase and catalase activities in *Aspergillus niger*. Applied and Environmental Microbiology, 58(4):1190–4.

Worthington CC. 1988. Worthington Enzyme Manual: Enzyme and Related Biochemical. Worthington Biochemical Co, USA, p: 155-158.

Zhanjun Q, Yuanfang G, Xiaoming B, Jianrong H, Gaoying S, Bingyin P, Wenxiang B. 2016. Expressions of *Aspergillus niger* glucose oxidase in yeast *Pichia pastoris* SMD1168. Biotechnology & Biotechnological Equipment, 30:998-1005

Zhu J, Zhu Z, Lai Z, Wang R, Guo X, Wu X. 2002. Planar amperometric glucose sensor based on glucose oxidase immobilized by chitosan film on Prussian blue layer. Sensors, 2:127–36.