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*- REWIEV ARTICLE-*

# Glucose Oxidase Applications and Comparison of the Activity Assays

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## Abstract

Glucose Oxidase (GOD) oxidizes glucose to δ-gluconolactone in presence of moleculer oxygen by forming hydrogen peroxide. As a result of the catalysed reaction, GOD is widely used in cases where glucose or molecular oxygen should be removed to extend the shelf life of foods or used in the production of controlled hydrogen peroxide or gluconic acid. One of the most important application areas of GOD is the construction of the glucose biosensors. There are several studies about GOD purification, immobilization, industrial and analytical applications, so, fast and sensitive determination of GOD activity is essential for these studies. In this study, GOD activity determination methods were reviewed mainly four approaches: determination of decrease in glucose or oxygen concentration and determination of increase in hydrogen peroxide or gluconic acid levels.

**Keywords:**
Glucose Oxidase, Activity assays, gluconic acid, hydrogen peroxide

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## Introduction

Glucose Oxidase (EC 1.1.3.4) catalyses the oxidation of β-D-glucose by molecular oxygen to δ-glucono-1,5-lactone and hydrogen peroxide. Gluconolactone can be hydrolyzed simultaneously and non-enzymatically to gluconic acid in presence of water (Figure 1).

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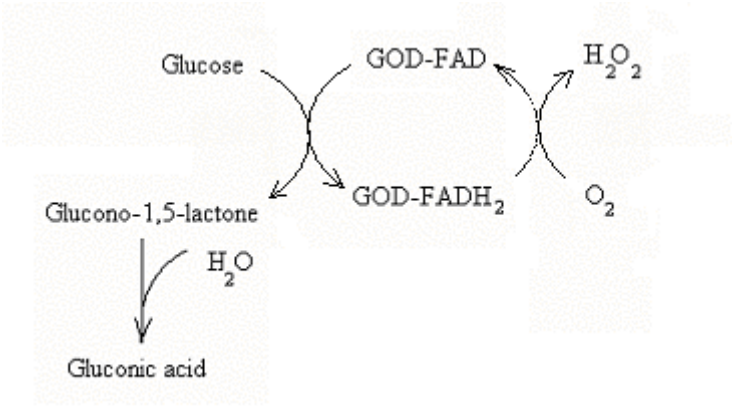


Figure 1. Reaction mechanism of GOD

As seen in Figure 1, glucose and $O_2$ concentrations decrease while products of rection as $H_2O_2$ and gluconic acid concentrations increase. The products cause two undesirable results: Decrease in pH owing to gluconic acid formation and inactivitaion of GOD because of oxidative properties of $H_2O_2$ molecule (Kleppe, 1966). GOD is inactivated from $H_2O_2$ produced in reaction medium, therefore, removing $H_2O_2$ from reaction medium is important to hinder this inactivation. Catalase (CAT) which decomposes the $H_2O_2$ to $H_2O$ and $O_2$ is mostly used for this purpose (Figure 2). GOD is widely used with CAT at its free or co-immobilized form (Ozyilmaz 2007, Parpinello et al. 2002, Isaksen and Adler-Nissen 1997, Pickering et al. 1999, Harborn et al. 1997, Ramanathan et al. 2001, Vrbova et al. 1990, Blandino et al. 2002, Godjevargova et al. 2004a, Godjevargova et al. 2004b, Kang et al. 2003).

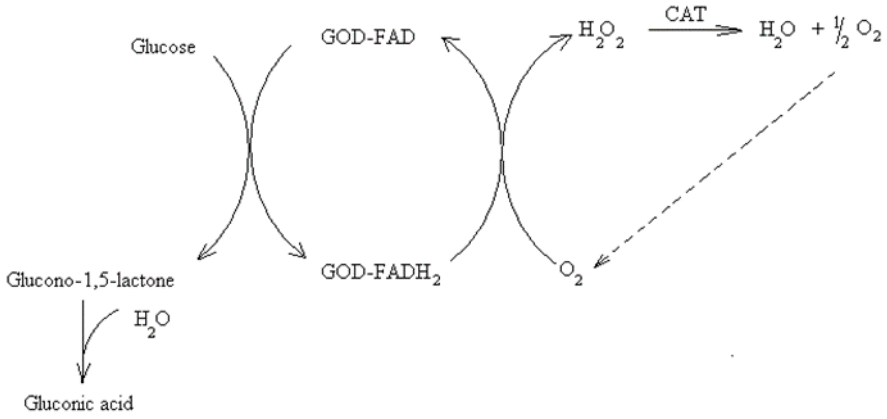


Figure 2. The co-use of GOD-CAT enzyme system

As seen in Figure 2, it is clearly seen that, part of the oxygen depleted by GOD is recovered by the CAT activity.

*Aspergillus niger* origin GOD is a dimeric protein with a molecular weight of 160 kDa, containing one, tightly but noncovalently bound, flavine adenine dinucleotide (FAD) per monomer as cofactor and glycosylated with a carbohydrate content of 16 % (w/w). GOD consists of two

identical polypeptide chain subunits covalently linked by disulfide bonds (O'Malley & Weaver 1972).

GOD has a number of industrial applications due to its ability to remove glucose and $O_2$ or production of gluconic acid and $H_2O_2$. There are several applications to use GOD for removing glucose from medium such as desuggaring of egg-white (Sisak et al. 2006, Quan & Benjakul 2018), production of reduced alcohol wines (Pickering et al. 1999, Biyela et.al. 2009, Valencia et al. 2017, Ruiz et al.. 2018), removal of oxygen and browning control to increase storage time of fruit juices, purees and other stored foods (Parpinello 2002, Isaaksen and Adler-Nissen 1997, Xu et al. 2018, Cruz et al. 2013). GOD use is also an alternative way to production of $H_2O_2$ for textile bleaching (Tzanov et al. 2002, Farooq et al. 2013, Reis et al. 2017, Qui et al. 2017, Madhu & Chakraborty 2019] or milk processing (Zhou &Lim 2009, Cruz et al 2013, Cruz et al 2012, Szweda et al 2013, Batista et al 2015). There are several studies about gluconic acid or gluconate salt production by GOD (Huang et al. 2019, Han et al. 2018, Ramezani et al. 2013, Mariam et al. 2010, Arimatsu et al. 2004, Bao et al. 2003, Shah & Kothari, 1993). GOD has analytical application to determine glucose levels of blood, serum, fruit juice *etc.*, and it is also the most used enzyme in preparation of glucose biosensors (Ozyilmaz et al. 2018, Khun et al. 2012, Guo et al. 2011, Salimi & Noorbahsh, 2011, Mazeiko et al. 2013, Ozyilmaz et al. 2007, Uang & Chou, 2003, Ekiz et al. 2011, Wang et al. 2013). Because of high degree of using areas, there are several studies about GOD.

When researchers study with any enzyme or its modified form such as immobilization, incubation or storage in the certain conditions, *etc*; they usually determine activity to reveal the effects of some parameters or modifications on the enzyme. So, at least one method should be used to determine activity for all enzymes. To determine GOD activity, there are several methods based on mainly four different change in the reaction medium: Consumption of dissolved $O_2$, reducing the glucose concentration, production of gluconic acid and increase in concentration of $H_2O_2$.

***Determination of decrease in dissolved $O_2$ concentration in the reaction medium***

Measuring the $O_2$ level in the reaction mixture was the earliest method for the determination the GOD activity (Kleppe 1966). Usually, $O_2$ depletion is measured with an amperometric oxygen electrode including Clark cell which was discovered by Dr. Clark in 1956.

GOD activity can be measured by oxygen meter basically. For this purpose, an oxygen meter probe is immersed in the stirred glucose solution at certain concentration. After measured $O_2$ concentration become steadily, GOD is added and decrease in $O_2$ concentration is measured for reaction duration. Thus, catalytic activity or specific activity can be calculated.

When GOD activity is measured using oxygen meter, reaction medium must be stirred, that cause $O_2$ diffusion through interface between air and reaction solution. So, while $O_2$ concentration decreases due to reaction, $O_2$ concentration increases slowly at the same time causing $O_2$ diffusion.

There are several studies about GOD at which enzyme activity was determined by measuring $O_2$ concentration (Gulla et al, 2004, Solomon, 1977; Weibel & Brights, 1971; Okuda & Miva, 1970; Okuda & Miva, 1971; Rogers & Brandt, 1971; Haouz et al., 2001, Valentova et al. 1981). As can be seen, GOD activity was determined by using oxygen meter formerly and GOD activity was calculated by using Equation 1.

$$GOD\ Activity = \frac{(Co-C).V}{t} \quad (1)$$

Where
Co: Initial $O_2$ concentration (mM)
C: Last $O_2$ concentration (mM)
V: Reaction volume (mL)
t: Reaction time (min)

According to Equation 1, GOD activity can be calculated as μmol $O_2$. $min^{-1}$

***Determination of reduced glucose concentration***

There are several chemical methods to determine concentrations of reducing sugars including glucose such as Folin –Wu, Somogyi- Nelson, o-Toluidine, Dinitrosalicylic acid method, *etc*. In GOD system, glucose concentration is reduced continuously and GOD activity can be determined by measuring of this decrease by a proper method. In the literature, to measure glucose concentration for determining the GOD activity, usually DNSA reagent is used (Blandino 2001, Ozyilmaz et al. 2005). DNSA reagent was developed by Sumner and co-worker (Sumner & Noback 1924) for the determination of reducing sugar. The determination of the reducing sugars is based on the reduction of 3,5-dinitrosalicylic acid in alkaline medium at 80 °C, forming gluconic acid and 3,5-diaminosalicylic acid which is measured with a spectrophotometer. The stoichiometric ratio between the DNSA and glucose is 1 to 6 and involves 12 interchanged electrons, where 2 nitro groups are reduced to amines due to the exchange of 6 electrons per each nitro group (Canizares-Macias et al. 2001). Reaction of DNSA with glucose was given in Fig.3

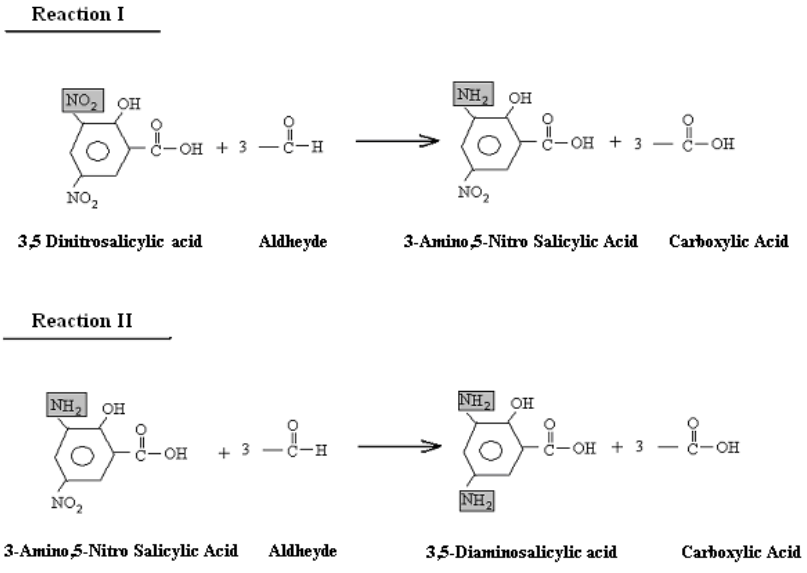


Figure 3. Reaction of DNSA with aldehyde group of glucose

According to Sumner and Noback, the reagents phenol, Rochelle salt (potassium sodium tartrate) and sodium bisulfite are needed to carry out the reaction. The roles of these reagents were analyzed in subsequent works. It was found that, phenol has no reducing effect on the DNSA but

increases the color produced by the glucose (apprx 300 %). Sodium bisulfite is added to prevent instability of the product after heating. The Rochelle salt reduces the oxygen concentration before heating, avoiding degradation of the sugar by the action of the oxygen in alkaline medium; therefore, the intensity of color increases due to higher DNSA reduction. Miller (Miller 1959) adjusted each of these compounds and mainly optimized the Rochelle salt concentration. Components and their compositions of DNSA reagent are 1% of dinitrosalicylic acid, 30 % Rochelle salt (potassium sodium tartrate), 0.5 %phenol, 0.5 % sodium bisulfite and 1 % sodium hydroxide.

Spectrophotometric measurements were carried out at various wave lengths such as 480 nm (Canizares-Macias et al. 2001), 575 nm (Miller 1959, Marsden et al. 1982, Breuil & Saddler 1985), 546 nm (Yu et al 1998), 540 nm (van Staden & Mulaudzi, 2000, Huang et al. 2019), 550 nm (Blandino et al.,2001).

There are several literature in which GOD activity was determined by measuring decrease in glucose concentration using standard curve prepared by glucose (Ozyilmaz et al. 2005, Huang et al. 2019, Blandino et al.,2001). In DNSA assay, initial and also final glucose concentration are determined by DNSA reagent. Sample is added to the DNSA reagent and left in a boiling water bath for 10 minutes. After cooling in ice-water bath, samples are diluted by deionized water. After 20 minutes for colour stability, absorbance values are read and concentrations are calculated with the help of the standard curve prepared with glucose. GOD activity was calculated as μmol glucose. $min^{-1}$ by using Equation 2 which is given below:

$$GOD\ Activity = \frac{(Co-C).V}{t} \quad (2)$$

Where
Co: Initial glucose concentration (mM)
C: Last glucose concentration (mM)
V: Reaction volume (mL)
t: Reaction time (min)

***Following the gluconic acid production***

Gluconic acid is produced from nonenzymatically hydrolysis of D-glucono-1,5-lactone produced in reaction mixture from glucose by GOD (Figure 4).

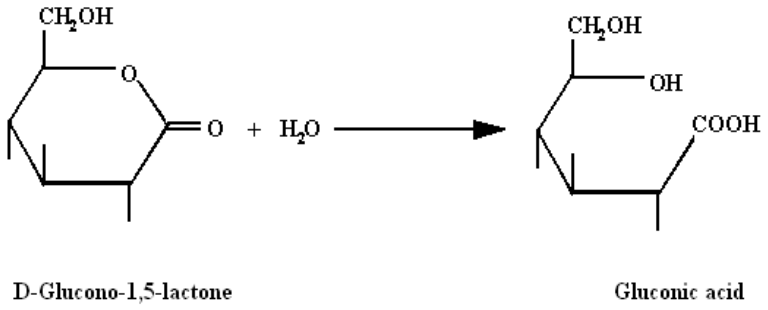


Figure 4. Non-enzymatic hydrolysis of D-glucuno-1,5-lactone to gluconic acid

In this method, during or after stopping GOD activity, produced gluconic acid is neutralized by NaOH. As the volume of NaOH spent is proportional to the gluconic acid concentration, GOD activity is calculated as μmol gluconic acid.min$^{-1}$. There are several studies in which GOD activity was determined by monitoring gluconic acid concentration (Casolaro & Barbuccu, 1991, Bhat et al. 2013, Iturbe et al. 1989, Ramezani et al. 2013, Yang et al. 2004, Cui et al.2019, Zhao et al. 2002).

***Measuring the $H_2O_2$ produced***

Nowadays, measurement of the amount of $H_2O_2$ produced in reaction medium is the most used method to determine the GOD activity. In general, peroxidase based methods were reported in the literature. A proper reagent is used to react with $H_2O_2$ produced by GOD and spectrophotometric measurement is used to calculate glucose oxidase activity. The most used chemicals are o-dianisidine and 4-amino antipyrine for this purpose. Oxidation and colour change of o-dianisidine and 4-amino antipyrine by peroxidase activity in presence of $H_2O_2$ are given in Figure 5 and 6, respectively.

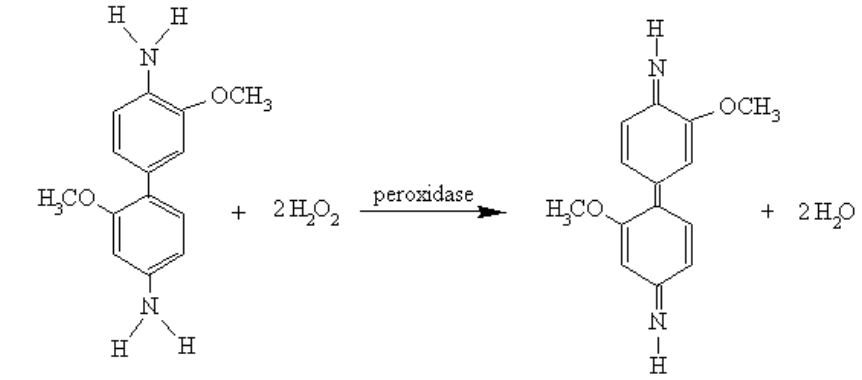


Figure 5. Reaction of o-dianisidine and hydrogen peroxide with peroxidase

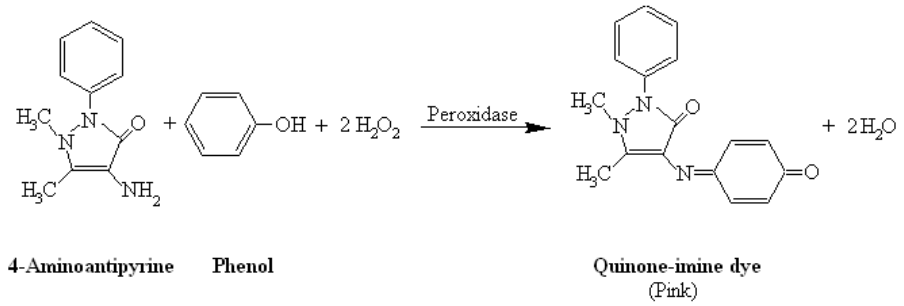


Figure 6. Reaction of 4-aminoantipyrine, phenol and hydrogen peroxide with peroxidase

Table 1 gives the literatures about determination of GOD activity by using peroxidase with different choromogenic compounds. In this assay, peroxidase and chromogenic compound are added into GOD medium. Produced $H_2O_2$ is used in peroxidase activity after by GOD activity, so, the color of oxidized chromogenic compound increases during reaction time.

Table 1. Chromogenic compounds are used to determine GOD activity in peroxidase assay

| **Used substrates of peroxidase for the determination of the GOD activity** | **References** |
|---|---|
| o-dianisidine | Ruiz et.al 2018, Atia and Batal, 2005; Godjevargova et al. 2004; Gouda et al., 2003; Ferreira et al., 2005; Godjevargova et al., 2005; Aken et al., 2000; Ying et al., 2002; Arıca and Bayramoğlu, 2004; Kang and Bae, 2003; Elçin et al., 1993; Godjevargova et al., 2000; Bulmuş et al., 1997; Yavuz et al., 2002; Chen et al, 2002; Frederick et al. 1990; Sukhacheva et al., 2004 |
| 4-Aminoantipyrine | Miron et al., 2004; Brahim et al., 2002, Suye et al., 1998; Appleton et al., 1997; Mugo et al. 2019, Zhao et al. 2018 |
| 2,2'-azino-bis(3-ethylbenzathiazoline-6-sulfonic acid) (ABTS) | Mugo et al. 2019; Betancor et al., 2005; Portaccio et al., 2002; Betancor et al., 2004; Ukeda et al., 1998; Witt et al., 2000; Szweda et al. 2013, Mahdizadeh et al. 2014 |
| Dimethylaminobenzoic acid | Brahim et al, 2002 |

***Comparison of GOD activity methods***

GOD as green catalyst is widely used for various reasons such as removing glucose or molecular oxygen or production of hydrogen peroxide and gluconic acid salts. Therefore, the activity of the GOD enzyme, accuracy and sensitivity of the determined activity value are of great importance. There are several methods are used to determine the GOD activity. At the beginning, the first method for determination of the GOD activity was to measure the decrease of dissolved $O_2$ concentration in the reaction medium. But, nowadays, there have been many literatures about GOD in which activites have been determined by using peroxidase and a chromogen system. Activity methods are also used which are based on decrease in glucose concentration or increase in the amount of the produced gluconic acid. GOD activity determination methods have advantages and disadvantages compared to each other in terms of usage area, consumption expenses, convenience and sensitivity. Table 2 summarizes the GOD activity methods with their advantages and disadvantages.

Table 2 is summarized the advantages and disadvantages of the methods used for determination of GOD activity, also suitability for GOD-CAT system.

Table 2. Comparison of activity assays of GOD for 10 min of reaction time.

| | **Determination of depleted $O_2$** | **Glucose determination with DNSA** | **Titration of the produced gluconic acid** | **Peroxidase-chromogen system** |
|---|---|---|---|---|
| **Required equipment** | Oxygenmeter | Spectrophotometer | Burette | Spectrophotometer |
| **Time required** | 10 min | 40 min | 15-20 min | 10 min |
| **Cost of reagents** | Reagentless | Cheap | Very cheap | Expensive due to using of peroxidase enzyme |
| **Easiness** | Required Oxygenmeter stability | Laborious | Medium | Easy |
| **Repeatability** | Low | High | Moderate | High |
| **Suitable for use in GOD+CAT system** | Unsuitable | Suitable | Suitable | Unsuitable |

As seen in Table 2, all methods have some advantages and also disadvantages in terms of required equipment and time, easiness of method and repeatability and also its suitability on GOD-CAT system. Titration method not only the cheapest method but also it can be easily applicable in all laboratories as no device required such as spectrophotometer or oxygenmeter, and also it is suitable for GOD-CAT system. However, in this method, GOD activity must be sufficient to produce enough gluconic acid to be determined by titration. Peroxidase-chromogen system is the most preferred method recently due to its simplicity and repeatability. However it is the most expensive method due to requiring another enzyme system, and also it is unsuitable for GOD-CAT system owing to depletion of $H_2O_2$ by CAT activity. Nevertheless, even very low GOD activity can be measured with high sensitivity by this method. The method depends on oxygen concentration measurement is preferable because it does not require any reagents and no additional processing. In this method, ensuring the stability of the oxygen meter during the measurement of oxygen concentration is very important for the reliability of the results. However, long-term stabilization of the oxygen meter and the fact that oxygen in the air diffuses into the reaction medium during the reaction time significantly affect the sensitivity of this method. Also, The DNSA method is one of the most commonly used methods to determine GOD activity because of its high reproducibility and relatively low cost. It also suitable for GOD-CAT system. The main drawback of this method is that it is time consuming and laborious.

## Conclusion

GOD is widely used for different purposes such as glucose or oxygen removal from the medium or production of controlled peroxide or gluconic acid. It is also widely used in the design of glucose biosensors. For this reason, the determination of GOD activity in many studies varies according to the purpose of the study. In this study, four different GOD activity methods are compared and their usage areas have been reviewed. It is concluded that peroxidase system is widely used in cases where GOD is used alone, whereas DNSA method is frequently used in systems including CAT.

As a result, it can be said that the choice of GOD activity method will be determined according to the presence of CAT enzyme in the environment, the catalytic efficiency of the GOD, and the sensitivity requirement of the results.

## References

Aken, B.V., Ledent, P., Naveau H., & Agathos, S.N. (2000). Co-immobilization of manganese peroxidase from *Phlebia radiate* and glucose oxidase from *Aspergillus niger* on porous silica beads. *Biotechnology Letters*, 22, 641-646

Appleton, B., Gibson, T.D., & Woodward, J.R. (1997). High temperature stabilization of immobilized glucose oxidase: potential applications in biosensors. *Sensors and Actuators B*, 43, 65-69

Arıca, M.Y., & Bayramoğlu, G. (2004). Polyethyleneimine-grafted poly(hydroxyethyl methacrylate-co-glycidyl methacrylate) membranes for reversible glucose oxidase immobilization. *Biochemical Engineering Journal*, 20, 73-77

Arimatsu, Y., Bao, J., Furumoto, K., Yoshimoto, M., Fukunaga, K., & Nakao, K. (2004). Continuous production of calcium gluconate crystals in an integrated bioreaction-crystallization process using external loop airlift bubble columns with immobilized glucose oxidase gel beads. *Journal of Chemical Engineering of Japan*, 37, 1035-1040

Atia, K.S., & El-Batal, A., (2005). Preparation of glucose oxidase immobilized in different carriers using radiation polymerization. *Journal of Chemical Technology and Biotechnology*, 80, 805-811.

Bao, J., Koumatsu, K., Arimatsu, Y., Furumoto, K., Yoshimoto, M., Fukunaga, K., & Nakao, K., (2003). A kinetic study on crystallization of calcium gluconate in external loop airlift column and stirred tank for an immobilized glucose oxidase reaction with crystallization. *Biochemical Engineering Journal*, 15, 177-184

Batista A.L.D., Silva R., Cappato L.P., Almada CN., Garcia R.K.A., Silva M.C., Raices R.S.L., Arellano D.B., Sant'Ana A., Junior C.A.C., Freitas M.Q., & Cruz A.G. (2015). *Food Research International*, 77, 627-635

Betancor, L., Lopez-Gallego, F., Hidalgo, A., Alonso-Morales, N., Fuentes, M., Fernandez-Lafuente, R., & Guisan, J.M. (2004). Prevention of interfacial inactivation of enzymes by coating the enzyme surface with dextran-aldehyde. *Journal of Biotechnology*, 110, 201-207

Betancor, L., Fuentes, M., Dellamora-Ortiz, G., Lopez-Gallego, F., Hidalgo, A., Alonso-Morales, N., Mateo, C., Guisan, J.M., & Fernandez-Lafuente, R. (2005). Dextran aldehyde coating of glucose oxidase immobilized on magnetic nanoparticles prevents its inactivation by gas bubbles. *Journal of Molecular Catalysis B: Enzymatic*, 32, 97-101

Bhat, S.V., Swathi BR, Rosy M., & Govinappa M., 2013. Isolation and characterization of glucose oxidase (GOD) from Aspergillu flavus and Penicillium sp. International Journal of Mivrobiology and Applied Sciences, 2(6), 153-161

Biyela, B.N.E., du Toit, W.J, Divol, B., Malherbe, D.F., & van Rensburg, P. (2009). The production of reduced-alcohol wines using Gluzyme Mono (R) 10.000 BG-treated grape juice. *South African Journal of Enology and Viticulture*, 30(2), 124-132

Blandino, A., Macias, M., & Cantero, D. (2001). Immobilization of Glucose Oxidase within Calcium Alginate Gel Capsules. *Process Biochemistry*. 36, 601-606

Blandino, A., Macias, M., & Cantero, D. (2002). Modelling and Simulation of a Bienzymatic Reaction System Co-immobilized Within Hydrogel-membrane Liquid-core Capsules. *Enzyme and Microbial Technology*, 31, 556-565

Brahim, S., Narinesingh, D., & Guiseppi-Elie, A. (2002). Kinetics of glucose oxidase immobilized in p(HEMA)-hydrogel microspheres in a packed-bed bioreactor. *Journal of Molecular Catalysis B: Enzymatic*, 715, 1-12

Breuil C., & Saddler J.N., (1985). Comparison of the 3,5-Dinitrosalicylic acid and Nelson-Somogyi methods of assaying for reducing sugars and determining cellulose activity. *Enzyme and Microbial Technology*, 7, 327-332

Bulmuş, V., Ayhan, H., & Pişkin, E. (1997). Modified PMMA monosize microbeads for glucose oxidase immobilization. *The Chemical Engineering Journal*, 65, 71-76

Canizares-Macias, P., Hernandez-Garciadiego, L., & Gomez-Ruiz, H. (2001). An Automated Flow Injection Analysis Procedure for the Determination of Reducing Sugars by DNSA Method. *Journal of Food Science*, 66 (3), 407-411

Casolaro, M., & Barbucci, R. (1991). An insulin-releasing system responsive to glucose: Thermodynamic evaluation of permeability properties. *The International Journal of Artificial Organs*, 14(11), 732-738

Chen, L.Q., Zhang, X.E., Xie, W.H., Zhou, Y.F., Zhang, Z.P., & Cass, A.E.G. (2002). Genetic modification of glucose oxidase for improving performance of an amperometric glucose biosensor. *Biosensors and Bioelectronics*, 17, 851-857

Cruz A.G., Castro W.F., Faria A.F., Bogusz Jr. S., Granato, D., Celeguini R.M.S., Lima-Pallone, J., & Godoy, H.T. (2012). Glucose oxidase: A potential option to decrease the oxidative stress in stirred probiotic yogurt. *LWT-Food Science and Technology*, 47, 512-515

Cruz, A.G., Castro, W.F., Faria, J.A.F, Bolini, H.M.A., Celeghini, R.M.S., & Raices, R.S.L., Oliviera, C.A.F., Freitas, M.Q., Junior, C.A.C., Marsico, E.T. (2013). Stability of probiotic yogurt added with glucose oxidase in plastic materials with different permeability oxygen rates during the refrigerated storage, *Food Research International*, 51, 723-728

Cui, C., Fang, Y., Chen, B., & Tan, T. (2019). Glucose oxidation performance is improved by the use of a supramolecular selfüassembly of glucose oxidase and catalase. Catalysis Science & Technology, 9, 477-482

Ekiz, F., Oğuzkaya, F., Akın, M., Timur, S., Tanyeli, C., & Toppare, L. (2011). Synthesis and application of poly-SNS-anchored carboxylic acid: a novel functional matrix for biomolecule conjugation, *Journal of Materials Chemistry*, 21, 12337-12343

Elçin, Y.M., & Sungur, S. (1993). Glucose oxidase immobilized on gelatin by various crosslinkers. *Macromolecular Reports*, A30, 137-147

Farooq A., Ali S., Abbas N., Fatima G.A., & Ashraf M.A. (2013). Comparative performance evaluation of conventinal bleaching and enzymatic bleaching with glucose on knitted cotton fabric. *Journal of Cleaner Production,* 42, 157-171

Ferreira, L.F.P., Taqueda, M.E., Vitolo, M., Converti A., & Pessoa Jr., A. (2005). Liquid-liquid extraction of commercial glucose oxidase by reversed micelles. *Journal of Biotechnology*, 116, 411-416

Frederick, K.R., Tung, J., Emerick, R.S., Masiarz, F.R., Chamberlian, S.H., Vasadava, A., & Rosenberg, S. (1990). Glucose oxidase from *Aspergillus niger*. *The Journal of Biological Chemistry*, 265, 3793-3802

Godjevargova, T., Dayal, R., & Marinov, I. (2004). Simultaneous Covalent Immobilization of Glucose Oxidase and Catalase onto Chemically modified Acrylonitrile Copolymer Membranes. *Journal of Applied Polymer Science,* 91, 4057-4063

Godjevargova, T., Konsulov, V., Dimov, A., & Vasileva, N. (2000). Behavior of glucose oxidase immobilized on ultrafiltration membranes obtained by copolymerizing acrylonitrile and *N*-vinylimidazol. *Journal of Membrane Science*, 172, 279-285

Godjevargova, T., Nenkova, R., & Dimova, N. (2005). Covalent immobilization of glucose oxidase onto new modified acrylonitrile copolymer/silica gel hybride supports. *Macromolecular Bioscience*, 5, 760-766

Godjevargova, T., Dayal, R., & Turmanova, S. (2004a). Gluconic acid production in bioreactor with immobilized glucose oxidase plus catalase on polymer membrane adjacent to anion-exchange membrane. *Macromolecular Bioscience*, 4, 950-956

Godjevargova, T., Dayal, R., & Marinov, I. (2004b). Simultaneous Covalent Immobilization of Glucose oxidase and catalase onto chemically modified acrylonitrile copolymer membranes. *Journal of Applied Polymer Science*, 91, 4057-4063

Gouda M.D., Singh, S.A., Appu Rao, A.G., Thakur M.S., & Karanth, N.G. (2003). Thermal inactivation of Glucose Oxidase Mechanism and stabilization using additivies. *The Journal of Biological Chemistry,* 278, 24324-24333.

Gulla, K.C., Gouda, M.D., Thakur, M.S., & Karanth, N.G. (2004). Enhancement of stability of immobilized glucose oxidase by modification of free thiols generated by reducing disulfide bonds and using additives. *Biosensors and Bioelectronics*, 19, 621-625

Guo, M., Fang, H., Wang, R., Yang, Z., & Xu, X. (2011). Electrodeposition of chitosan-glucose oxidase biocomposite onto Pt-Pb nanoparticles modified stainless steel needle electrode for amperometric glucose biosensor, *Journal of Material Science: Mater. Med*, 22, 1958-1992

Han X., Liu G., Pan Y., Song W., & Qu Y. (2018). Consolidated bioprocessing for sodium gluconate production from cellulose using Penicillium oxalicum. *Bioresource Technology*, 251, 407-410

Haouz, A., Glandieres, J.M., & Alpert, B. (2001). Involvement of protein dynamics in enzyme stability The case of glucose oxidase. *FEBS Letters*, 506, 216-220

Harborn, U., Xie, B., Venkatesh, R., & Danielsson, B. (1997). Evaluation of a Miniaturized Thermal Biosensor for the Determination of Glucose in Whole Blood. *Clinica Chimica Acta*, 267, 225-237

Huang J., Zhuang W., Ge L., Wang K., Wang Z., Niu H., Wu J., Zhu C., Chen Y., & Ying H. (2019). Improving biocatalytic microenvironment with biocompatible ε-poly-L-lysine for one step gluconic acid production in low pH enzymatic systems. *Process Biochemistry,* 76, 118-127

Isaaksen, A., & Adler-Nissen, J. (1997). Antioxidative Effect of Glucose Oxidase and Catalase in Mayonnaises of Different Oxidative Susceptibility.I. *Product Trials. Lebensm. Wiss.u.Technology.* 30, 841-846

Iturbe, F., Ortega, E., & Lopez-Munguia, A.C. (1989). A pH-stat method to measure glucose oxidase. *Biotechnology Techniques*, 3(1), 19-22

Kang, S.I., & Bae, Y.H. (2003). A Sulfonamide based glucose-responsive hydrogel with covalently immobilized glucose oxidase and catalase. *Journal of Controlled Release*, 86, 115-121

Khun, K., Ibupoto, Z.H., Lu, J. Al Salhi, M.S. Atif, M., Ansari, A.A., & Willander, M. (2012). Potentiometric glucose sensor based on the glucose oxidase immobilized iron ferrite magnetic particle/chitosan composite modified gold coated glass electrode, *Sensors and Actuators B* 173, 698-703.

Kleppe K. (1966). The Effect of Hydrogen Peroxide on Glucose Oxidase from Aspergillus niger *Biochemistry*, *5* (1), 139–143

Madhu, A., & Chakraborty, J.N., (2019). Bio-bleaching of cotton with H2O2 generated from native and immobilized glucose oxidase. *AATCC Journal of Research,* 6, 7-17

Mahdizadeh, F., & Eskandarian, M. (2014). Glucose oxidase and catalase co-immobilization on biosynthesized nanoporous SiO2 for removal of dissolved oxygen in water: Corrosion controlling of boilers. *Journal of Industrial and Engineering Chemistry*, 20, 2378-2383

Marsden, W.L, Gray P.P, Nippard G.J., & Quinlan M.R., (1982). Evaluation of the DNS method for Analysing Lignocellulosic Hydrolysates. *J. Chem. Tech. Biotechnol*. 32, 1016-1022

Mazeiko V., Kausaite-Minkstimiene A., Ramanaviciene A., Balevicius Z., & Ramanavicius A. (2013). Gold nanoparticle and conducting polymer-polyaniline-based nanocomposites for glucose biosensor design. *Sensors and Actuators B: Chemical,* 189, 187-193

Miller, L.G., (1959). Use of dinitrosalicylic acid reagent for determination reducing sugar. *Analytical Chemistry*, 31, 426-428

Miron, J., Gonzalez, M.P., Vazquez, J.A., Pastrana, L., & Murado, M.A. (2004). A mathematical model for glucose oxidase kinetics, including inhibitory, deactivant and diffusional effects, and their interactions. *Enzyme and Microbial Technology*, 34, 513-522

Mugo, S.M., Berg, D., & Bharath, G. (2019). Integrated microcentrifuge carbon entrapped glucose oxidase poly(N-isopropylacrylamide) (pNIPAm) microgels for glucose amperometric detection. Analytical Letters, 52, 825-838

Okuda, J., & Miva, I. (1970). Action of pure β-D-glucose oxidase on higher animals. *Biochemical Pharmacology*, 19, 1777-1782

Okuda J., & Miva, I. (1971). Mutarotase effect on micro determinations of D-glucose and its anomers with β-D-glucose oxidase. *Analytical Biochemistry*, 43(1), 312-315.

O'Malley, J., & Weaver, J. (1972). Subunit Structure of Glucose oxidase from *Aspergillus niger*, *Biochemistry,* 11, 3527

Ozyilmaz G., Tukel S.S., & Alptekin O. (2005). Activity and storage stability of immobilized glucose oxidase onto magnesium silicate. *Journal of Molecular Catalysis B: Enzymatic*, 35, 154-160

Ozyilmaz G., & Tukel S.S. (2007). Simultaneous Co-immobilization of Glucose Oxidase and Catalase in Their Substrates. *Applied Biochemistry and Microbiology*, 43, 29-35

Ozyilmaz G., & Ozyilmaz A.T, Ağçam S. (2018). Using Response Surface Methodology for Amperometric Glucose Biosensor Construction, Natural and Engineering Sciences, 3(1), 1-15

Parpinello, G.P., Chinnici, F., Versari, A., & Ripont, C. (2002). Preliminary Study on Glucose Oxidase-Catalase Enzyme System to Control the Browning of Apple and Pear Purees. *Lebensm.-Wiss. U.-Technol*. 35, 239-243

Pickering, G.J., Heatherbell, D.A., & Barnes, M.F. (1999). Optimising Glucose Conversion in the Production of Reduced Alcohol Wİne Using Glucose Oxidase. *Food research International*. 31 (10), 685-692

Portaccio, M., El-Masry, M., Rossi Diano, N., De Maio, A., Grano, V., Lepore, M., Travascio, P., Bencivenga, U., Pagliuca, N., & Mita, D.G. (2002). An amperometric sensor employing glucose oxidase immobilized on nylon membranes with different pore diameter and grafted with different monomers. *Journal of Molecular Catalysis B: Enzymatic*, 714, 1-19

Quan T.H., & Benjakul S. (2018). Impacts of desugarization and drying methods on physicochemical and functional properties of duck albumen powder. *Drying Technology*, 37, 864-875

Qui, F., Li F.Y., & Yang, Q.Y. (2017). Prepararion of Magnetic immobilized Glucose Oxidase and Bleaching of Cotton Fabrics, *Textile Bioengineering and Informatics Symposium Proceedings*, 3, 829-835

Ramanathan, K., Jönsson, B.R., & Danielsson, B. (2001). Sol-gel Based Thermal Biosensor, for Glucose. *Analytica Chimica Acta*. 427, 1-10

Ramezani M., Mostoufi, N., & Mehrnia, M.R. (2013). Effect of hydrodynamics on kinetics of gluconic acid enzymatic production in bubble column reactor. Chemical Industry &Chemical Engineering Quarterly, 19, 411-422

Reis C.Z., Fogolari O., Oliveira D., de Souza S.M.A.G.U., & de Souza A.A.U. (2017). Bioscouring and bleaching of knitted cotton fabrics in one-step process using enzymatically generated hydrogen peroxide. The Canadian Journal of Chemical Engineering, 95, 2048-2055.

Rogers, M.J., & Brandt, K.G. (1971). Multiple inhibition analysis of *Aspergillus niger* glucose oxidase by D-glucal and halide ions. *Biochemistry*, 10, 4636-4641

Ruiz E., Busto M.D:, Ramos-Gomez S., Palacios D., Pilar-Izquierdo M., & Ortega N. (2018). Encapsulation of glucose oxidase in alginate hollow beads to reduce the fermentable sugars in simulated musts, *Food Bioscience*, 24, 67-72

Salimi, A., & Noorbakhsh, A. (2011). Layer by layer assembly of glucose oxidase and thiourea onto glassy carbon electrode: fabrication of glucose biosensor, *Electrochimica Acta* 56, 6097-6105

Shah, D.N., & Kothari, R.M., (1993). Glucose oxidase-rich *Aspergillus niger* strain, cost-effective protocol and an economical substrate for the preparation of tablet grade calcium gluconate. *Biotechnology Letters*, 15, 35-40

Sisak, C., Csanadi, Z., Ronay, E., & Szajani, B. (2006). Elimination of glucose in egg white using immobilized glucose oxidase. *Enzyme and Microbial Technology*, 37, 1002-1007

Solomon, B. (1977). Enzymic activity and conformational properties of native and crosslinked glucose oxidase. *Biopolymers,* 16, 1837-1852

Sukhacheva M.V., Davydova M.E., & Netrusov A.I. (2004). Production of *Penicillium funiculosum* 433 glucose oxidase and its properties. *Applied Biotechnology and Microbiology*, 40, 25-29

Sumner, J.B., & Noback, C.V.I. (1924). The estimation of sugar in diabetic urine, using dinitrosalcylic acid. *Journal of Biological Chemistry,* 62: 287-290

Suye, S., Kumon Y., & Ishigaki, A. (1998). Immobilization of glucose oxidase on poly(L-Lysine)-modified polycarbonate membrane. *Biotechnology Applied Biochemistry*, 27, 245-248

Szweda R.T., Schmidt K., & Zorn H. (2013). Bleaching of colored whey and milk by a multiple-enzyme system. *Eurapian Food Research Technology,* 237, 377-384

Tzanov, T., Costa, S.A., Gübitz, G.M., & Cavaco-Paulo, A. (2002). Hydrogen peroxide generation with immobilized glucose oxidase for textile bleaching. *Journal of Biotechnology,* 93, 87-94

Uang, Y.M., & Chou, T.C. (2003). Fabrication of glucose oxidase/polypyrrole biosensor by galvanostatic method in various pH aqueous solutions, Biosensors and Bioelectronics 19, 141-147.

Ukeda, H., Ohira, M., Sarker, A.K., & Sawamura, M. (1998). Deposition of glucose oxidase on the surface of microtiter plate: its application to determination of glucose in foods. *Food Research International*, 31, 297-302

Valencia P., Espinoza K., Ramirez C., Franco W., & Urtubia A. (2017). Technical Feasibility of Glucose Oxidase as a Prefermentation Treatment for Lowering the Alcoholic Degree of Red Wine. *American Journal of Enology and Viticulture*. 68(3), 386-389

Valentova O., Marek M., Svec F., & Stamberg J. (1981). Comparison of different methods of glucose oxidase immobilization. Biotechnology and Bioengineering, 23(9), 2093-2014

Vrbova, E., & Marek, M. (1990). Application of the Ugi Reaction for the Preparation of Enzyme Electrodes. *Analytica Chimica Acta*. 239, 263-268

Wang, L., Gao, X., Jin, L., Wu, Q., Chen, Z., & Lin, X. (2013). Amperometric glucose biosensor based on silver nanowires and glucose oxidase, *Sensors and Actuators B,* 176, 9-14

Weibel, M.K., & Brights, H.J. (1971), The glucose oxidase mechanism interpretation of the pH dependence. *The Journal of Biological Chemistry*, 246, 2734-2744

Witt, S., Wohlfahrt, G., Schomburg, D., Hecht, H.J., & Kalisz H.M. (2000). Conserved arginine-516 of *Penicillum amagasakiense* glucose oxidase is essential for the efficient binding of β-D-glucose. *Biochemical Journal*, 347, 553-559

Xu D., Sun L., Li C., Wang Y., & Ye R. (2018). Inhibitory effect of glucose oxidase from *Bacillus* sp. CAMT22370 on the quality deterioration of Pacific white shrimp during cold storage. *LWT*, 92, 339-346

van Staden, J.F., & Mulaudzi L.V., (2000). Flow injection spectrophometric assay of α-amylase activity. *Analytica Chimica Acta*, 421, 19-25

Yang, Y.M., Wang, J.W., & Tan, R.X. (2004). Immobilization of glucose oxidase on chitosan-$SiO_2$ gel. *Enzyme and Microbial Technology*, 34, 126-131

Yavuz, H., Bayramoğlu, G., Kaçar, Y., Denizli, A., & Arıca, M.Y. (2002). Congo Red attached monosize poly(HEMA-co-MMA) microspheres for use in reversible enzyme immobilization. *Biochemical Engineering Journal*, 10, 1-8

Ying L., Kang, E.T., & Neoh, K.G. (2002). Covalent immobilization of glucose oxidase on microporous membranes prepared from poly(vinylidene fluoride) with grafted poly(acrylic acid) side chains. *Journal of Membrane Sience*, 208, 361-374

Yu S., Olsen C.E., & Marcussen J., (1998). Methods for the assay of 1,5-anhydro-D-fructose and α-1,4-glucan lyase. *Carbohydrate Research*, 305, 73-82

Zhao, H., Zhang, X., Zhou, X., & Li, Z. (2002). Use of the glucose oxidase system for estimation of oxygen transfer rate in a solid-state bioreactor. *Enzyme and Microbial Technology*, 30, 843-846

Zhao B., Zhou L., Ma L., He Y., Gao J., Li D., & Jiang Y. (2018). Co-immobilization of glucose oxidase and catalase in silica inverse opals for glucose removal from commercial isomaltooligosaccharide. *International Journal of Biological Macromolecules*, 107, 2034-2043

Zhou Y., & Lim L.T. (2009). Activation of lactoperoxidase system in milk by glucose oxidase immobilized in electrospun polylactide microfibers. *Journal of Food Science*, 74(2), C170-C176