



# Biosensors based on $\beta$ -galactosidase enzyme: Recent advances and perspectives



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## ABSTRACT

Many industries are striving for the development of more reliable and robust  $\beta$ -galactosidase biosensors that exhibit high response rate, increased detection limit and enriched useful lifetime. In a newfangled technological atmosphere, a trivial advantage or disadvantage of the developed biosensor may escort to the survival and extinction of the industry. Several alternative strategies to immobilize  $\beta$ -galactosidase enzyme for their utilization in biosensors have been developed in recent years in the quest of maximum utility by controlling the defects seen in the previous biosensors. The overwhelming call for on-line measurement of different sample constituents has directed science and industry to search for best practical solutions and biosensors are witnessed as the best prospect. The main objective of this paper is to serve as a narrow footbridge by comparing the literary works on the  $\beta$ -galactosidase biosensors, critically analyze their use in the construction of best biosensor by showing the pros and cons of the predicted methods for the practical use of biosensors.

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## Contents

Introduction .....	1
Significance of use of enzymes as biological element .....	3
Immobilization of $\beta$ -galactosidase enzyme in biosensors .....	4
Immobilization of $\beta$ -galactosidase by simple adsorption .....	5
Immobilization of $\beta$ -galactosidase by physical entrapment .....	5
Immobilization of $\beta$ -galactosidase by covalent attachment .....	5
Immobilization of $\beta$ -galactosidase by encapsulation .....	6
Immobilization by the solid-phase method .....	6
Nanostructure immobilization of $\beta$ -galactosidase .....	6
$\beta$ -Galactosidase immobilized by layer-by-layer (LBL) and Langmuir-Blodgett films .....	6
Use of $\beta$ -galactosidase in allosteric biosensors .....	7
Multi enzyme biosensor .....	7
Other $\beta$ -galactosidase biosensors .....	8
Conclusion and future perspectives .....	9
Conflict of interest .....	9
Acknowledgements .....	9
References .....	10

## Introduction

Biosensors are the analytical hybrid devices which correspond to the substance being measured by transforming information to an analytically useful signal through a biochemical mechanism in

*Abbreviations:* GaO, Galactose Oxidase; GO, Glucose Oxidase; P3HT, Poly(3-hexyl thiophene); FIA, Flow Injection Analysis; GCE, Glassy Carbon Electrode.

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which the sensing element is biological in nature [1]. In other words, biosensors are devices that include a biological sensing element connected to a transducer [2]. The history of biosensors starts from simple laboratory level and has emerged to field testing and commercialization in almost every part of the world. There has been significant development in this area in which even gene expression patterns, genome mapping and detection of genetic mutations is feasible due to the DNA microchip technology. The first used biosensors were called the enzyme electrodes [3]. Since then biosensors have been studied and developed continuously till this day. A patient can extract one drop of blood and readout the glucose concentration in less than one minute. The biosensors are not only used in identifying and quantifying the analytes, but also used in screening of specific molecules present in complex compounds even in a very low concentration. This property has gained their utility in analytical research, food and chemical industries [4], pollution checking [5], process monitoring and in clinical diagnosis [6]. Biosensors can be considered as a complementary tool due to their simplicity, rapid response, relative low cost, continuous monitoring and ability to be miniaturized [7]. (see Table 1)

The evolution in  $\beta$ -galactosidase biosensors can be categorized in three divergent generations that describes the stages of integration, and differentiate between modes of signal convey in a redox enzyme and electrode. Fig. 1 shows schematic representation and features of three different generations of biosensors. The signal transfer between natural secondary substrates, products, and an electrode is regarded as the first generation. Whereas, second generation biosensors consist of artificial electron mediators instead of natural co-substrates and third generation biosensors involve direct electron transfer among redox-active biomolecule and the electrode surface [8]. The main drawback of first generation biosensors was associated with high applied potentials. This problem was controlled by the use of mediators (second generation). However, use of mediators facilitated various interfering reactions, so direct electron transfer (DET) in third generation biosensor opens the avenue to highly selective biosensors virtually free of interference.

$\beta$ -Galactosidase (E.C. 3.2.1.23) is a crucial enzyme that hydrolyzes D-galactosyl residues like lactose or  $\beta$ -galactose comprising chromogenic or fluorogenic substrates from oligosaccharides and

**Table 1**  
Selected list of  $\beta$ -galactosidase biosensor showing its source and specific properties.

Biosensor/Transducer	Method of immobilization	Immobilizing agents	Source of $\beta$ -galactosidase	Linear response rate and other properties	Reference
Ingold potentiometric	Physical entrapment	Diethylaminoethyl cellulose (DE-52) and agar	<i>Enterobacter agglomerans</i>	0.0146–0.2920 mM, Stability: 2 months	[41]
Chronoamperometric	Coated with mercury thin film	Gelatin and glutaraldehyde onto mercury thin film GCE	<i>Aspergillus oryzae</i>	Linear range: $1 \times 10^{-4}$ – $3.5 \times 10^{-3}$ M.	[42]
Chemiluminescence	Physical Adsorption	Dioxetane substrate/porous silicon	<i>E. Coli</i>	Lower Detection limit: 10–100 CFU of <i>E. coli</i>	[45]
Amperometric	Immobilization in LB films of P3HT/SA	P3HT/stearic acid (SA)	Sigma Chemicals, USA ( <i>E. coli</i> )	0.029–0.175 mM, Half -life: above 120 days	[47]
Conductometric	Deposition on electrodes	Phosphate buffer, glycerol, glutaraldehyde	Sigma Chemicals, USA ( <i>E. coli</i> )	30 and 600 $\mu$ M	[46]
Amperometric	Adsorption	Perfluorosulfonated polymer	Unknown	Detection limit: $1 \times 10^{-5}$ – $1 \times 10^{-2}$ M, Analysis time: 2–3 min	[57]
Amperometric	Adsorption	Thin-layer plexi-cells on natural protein membranes	Unknown	Analytical range: $1 \times 10^{-5}$ – $1 \times 10^{-2}$ M, Detection limit: $1 \times 10^{-3}$ M	[58]
Amperometric	Immobilized in layer-by-layer (LbL) films	PEI and PVS on an ITO electrode modified with a layer of Prussian Blue (PB)	<i>Aspergillus oryzae</i>	Sensitivity: 0.31 $\mu$ A mmol <sup>-1</sup> cm <sup>-2</sup>	[71]
Amperometric	Adsorption	MPA, Mediator: (TTF) coimmobilized by a dialysis membrane	<i>E. coli</i>	Detection limit: 1.13 mM L <sup>-1</sup> , $1.5 \times 10^{-6}$ to $1.2 \times 10^{-4}$ M, Detection limit: $4.6 \times 10^{-7}$ M	[72]
Amperometric	Adsorption (bi-enzyme)	Gelatin	<i>E. Coli</i>	0.02 and 3.00 mmol dm <sup>-3</sup> of lactose	[83]
Unknown	Adsorption	Cellulose triacetate, glutaraldehyde	Unknown	58.5–181.3 mM	[84]
Unknown	Not specified	pH sensitive gates of an eight-channel field transistor(FET)	<i>E. Coli</i>	0.0–1.5 mM	[85]
Amperometric	Thin-film technology	Glutaraldehyde	<i>E. Coli</i>	0.0–4.7 mM	[86]
Bio-FET sensor	Crosslinking	Glutaraldehyde	<i>E. Coli</i>	Response rate: 120 s, Stability: 20 days	[87]
Unknown	Crosslinking and co-immobilization	Gelatin crosslinked with glutardialdehyde	<i>Kluyveromyces marxianus</i> ,	0–0.41 mM	[88]
Amperometric	Cross-linking with Glutaraldehyde and $\beta$ -cyclodextrin polymer	Ferrocene and $\beta$ -cyclodextrin	Sigma Chemicals, USA ( <i>E. coli</i> )	$50 \times 10^{-6}$ to $13.5 \times 10^{-3}$ M	[89]
Amperometric	Deposition/adsorption	Derivatized polyethersulphone membrane	<i>Aspergillus oryzae</i>	Sensitivity: 6.81 nAM <sup>-1</sup> ,	[90]
Amperometric	Deposition/adsorption	Ferrocene and DEAE-dextran as stabilizer	<i>E. Coli</i>	Detection Limit: 44–339 mM	[91]
Amperometric	AC -Electrodeposition	Not needed	<i>Aspergillus oryzae</i>	Sensitivity: 111 nA/mM mm <sup>2</sup> , Linear range: 14 mM lactose	[92]
Amperometric	Electrochemical deposition/cross linking with glutaraldehyde	PEI + glutaraldehyde	<i>E. Coli</i>	Linear range: $2.9 \times 10^{-5}$ to $9.9 \times 10^{-4}$ M, Response Rate: 2–4 s, Stability: 140 days	[93]
Amperometric: Microdialysis-coupled flow injection	Enzyme injection and electrochemical	0.1 M phosphate buffer at pH 7.0, containing 10 mM NaCl	<i>E. Coli</i>	Response rate: 0.05–20 mM	[94]

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