UNIVERSITI PUTRA MALAYSIA

IMMOBILIZATION OF URICASE ONTO GRAPHENE OXIDE

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk " Immobilization of Uricase onto Graphene Oxide" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Nur Amirah binti Sidek (164673) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

IMMOBILIZATION OF URICASE ONTO GRAPHENE OXIDE

Immobilization of uricase onto graphene oxide was done by using two different methods, physical adsorption and also chemical modification of the surface of graphene oxide by using *N*'-(3-(dimethylamino)propyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as cross-linkers. Graphene oxide is proved to be an excellent carrier for uricase immobilization due to it having a large surface area and abundant functional groups. The enzyme immobilization on graphene oxide can be done readily without using any cross-linking reagents or other additional surface modification. It can also be done by modifying the surface of graphene oxide for an enhanced performance. In this study, apart from using different methods, other paramaters such as the concentration of graphene oxide and also enzyme unit used are also varied to determine the best conditions for an optimum performance of the immobilized enzyme. The results obtained indicate that the immobilized enzyme perform better by using the chemical modification method, and the best parameters of uricase immobilization by this method were found as 1 mg/mL graphene oxide concentration and 1 u/ml uricase.

ABSTRAK

PENGIMOBILISASIAN URICASE PADA GRAFEN OKSIDA

Pengimobilisasian urikase pada grafen oksida telah dijalankan dengan menggunakan kaedah yang berbeza, iaitu penjerapan fizikal dan pengubahsuaian kimia permukaan grafen oksida dengan menggunakan (NHS) dan (EDC) sebagai reagen silang pautan. Grafen oksida terbukti menjadi pilihan material yang terbaik untuk pengimobilisasian urikase kerana ia mempunyai luas permukaan yang besar dan kumpulan berfungsi yang banyak. Pengimobilisasian enzim pada grafen oksida boleh dilakukan tanpa menggunakan sebarang reagen silang pautan atau pengubahsuaian pada permukaan grafen. Ia juga boleh dilakukan dengan mengubah permukaan grafen oksida untuk memperoleh prestasi yang lebih baik. Dalam kajian ini, selain daripada menggunakan kaedah yang berbeza, parameter lain seperti kepekatan grafen oksida dan unit enzim yang digunakan juga berbeza-beza untuk memastikan syarat-syarat yang terbaik untuk prestasi enzim yang optimum. Keputusan yang telah diperolehi menunjukkan bahawa pengimobilisasian enzim melalui teknik pengubahsuaian kimia pada permukaan grafen oksida adalah lebih baik dari penjerapan dan paramater pengimobilisasian urikase yang terbaik untuk kaedah ini adalah 1 mg/mL grafen oksida dan 1 u/ml urikase.

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LIST OF ABBREVIATIONS

%	Percentage
^{o}C	Degree Celsius
µl	Microlitre
µg/mL	Microgram per millilitre
H_2O_2	Hydrogen peroxide
GO	Graphene oxide
mg	Milligram
mg/mL	Milligram per millilitre
mg/100g	Milligram per hundred gram
nm	Nanometer
ppm	Parts per million
u/ml	Units per ml

CHAPTER 1

INTRODUCTION

Biosensors are integral appliances that have been used for biomolecule detection in several fields including environmental monitoring, food industry, and also clinical analysis. A biosensor is a device consists of two components that are correlated to each other, a bioreceptor and also a transducer (Sassolas et al., 2012). A bioreceptor is made up of a biological receptor element with high selectivity and sensitivity, while the transducer acts as an element that convert the biological recognition mechanism that occurs into a signal that can be measured (Le Goff et al., 2011). At the present time, there are many types of biosensors that have been developed such as DNA biosensors, microbial biosensors, and also enzyme biosensors.

Enzyme biosensor is an analytical device that functions by means of the combination of an enzyme with a transducer to generate a signal that corresponds with the target analyte concentration (Li et al., 2009). Enzyme biosensors are often used in several areas, mainly applied in health care. Such applications revolve around monitoring blood glucose levels, detection of uric acid, and also the detection of cardiovascular diseases. Anyhow, without an additional altering or recondition of the enzyme itself, the enzyme in its natural state will not be suitable for the processes. Immobilization of enzyme has been used as one of the means of solving this problem.

Enzyme immobilization technology is developed in order to enhance the stability and also the reusability of the enzymes that are used as biosensors. Nowadays,

nanostructured materials have been used as a matrix for enzyme immobilization. In enzymatic device, the main thing that has to be considered is the control over enzyme activity that is reliant on the integration between the nanomaterial and the enzyme (Crespilho et al., 2009).

Enzyme immobilization is a procedure where the enzyme has to be confined to a matrix that differs from its substrates and also products. Matrices that are commonly used for carrying the enzyme are inert polymers and inorganic materials (Datta et al., 2012). There are various methods that can be used to immobilize an enzyme on the nanocarriers. The methods used are such as covalent binding, entrapment, encapsulation, adsorption, ionic binding, affinity binding, and metal ion immobilization (Nisha et al., 2012).

The main aim of this study is focused on the best method to immobilize the enzyme uricase onto graphene oxide and also to investigate the parameters that could improve the efficiency and stability of the enzyme after immobilization.

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