Published Journals:

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Use of Cytochrome P450 Enzyme Isolated From *Bacillus Stratosphericus sp.* as Recognition Element in Designing Schottky-Based ISFET Biosensor for Hydrocarbon Detection

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***Abstract*— Cytochrome P450 enzyme isolated from an extremophile *Bacillus Stratosphericus sp.* was used as the recognition element for an ion-sensitive field effect transistor (ISFET)-based biosensor. The fabricated ISFET is Schottky-based and has silicon dioxide as a sensing layer. The cytochrome P450 component in its present purified form (pH-7.2) in 5% agarose was laid over the SiO_2 gate to examine its capability of generating electronic response in the presence of n-hexadecane and reduced nicotinamide adenine dinucleotide phosphate. The partially purified preparation was obtained from the lysozyme treated spheroplast via ion exchange chromatography followed by the gel filtration chromatography. The variation of potential difference between the gate and the source with respect to pH for the bare ISFET and the variation of gate and source potential difference with respect to the concentration of the measurand at constant current have been recorded for analyses. The observed correlation showed good repeatability of the sensor output suggesting a potential functional sensing device for hydrocarbon detection. The sensitivity parameters for the device have been determined to be 50.65 mV/pH and 54.34 mV/molar for the bare ISFET and enzyme field-effect transistor, respectively.**

***Index Terms*— Biosensor, cytochrome P450, enzyme field effect transistor, hydrocarbon degradation, protein purification.**

I. Introduction

HYDROCARBON sensing is considered very important in the oil industry for monitoring crude oil contamination and oil exploration. The existing method of detection of hydrocarbon suffers from inconveniences of poor sensitivity, processing time and cost of analysis. In nature activation energy of hydrocarbon oxidation is very high and performed mostly by Cytochrome P450 (CYP450) monooxygenases. This catalytic phenomenon could be machine appropriated for their in-situ detection with minimum intervention. Biochemically cytochrome P450 enzymes contain heme group as functional moiety capable of catalyzing oxidation of variety of drugs, carcinogens, pesticides, hydrocarbons and lots of other chemicals by means of single oxygen atom incorporation [1], epoxidation of double bonds, oxidation of heteroatoms, dealkylationetc [2], [3]. The iron proto porphyrin IX moiety with cysteine as the fifth ligand and a free sixth co-ordination site facilitates binding and activation of molecular oxygen by two successive one-electron transfer reactions leading to their hydroxylation [4]. This property of hydroxylation of hydrocarbon by Cytochrome P450 enzymes can be used for the development of biosensor of superior redox properties for instant detection of hydrocarbon.

Biosensors based on semiconductor structures such as ENFET having ISFET as the base structures have received considerable attention due to several advantages such as batch production, miniaturized form, reliability, etc. The ISFET device which is a successor of pH electrode was introduced in the semiconductor industry in the year 1970s [5]–[8]. It is analogous to MOSFET, wherein the former is devoid of metal gate, and the sensing insulator layer is in direct contact with the electrolyte. SiO_2 was the insulating layer used by Bergveld for experimentation of ions in and around a nerve. Further, Si_3N_4 as sensing layer was introduced by Matsuo and Wise during the same time [9], [10]. Over last four decades, numerous works have been reported by several researchers on ISFET. Besides, related devices such as ENFET's (enzyme FET's), ChemFET, ImmunoFET, etc. were also introduced as different biosensors [11]–[13]. Enzyme modified field effect transistor is a biosensor where an enzyme layer is deposited over the sensing layer, and the electrolyte is in contact with the immobilized enzymatic layer. Hydrogen ions (H^+) are generated or consumed at the interfacial layer due to the biocatalytic transformations by the enzyme immobilized over the sensor surface. The charges affect the surface potential of the ISFET device which results in the change in the drain current. Thus, ENFET responds specifically to a catalytic event corresponding to immobilized counterpart and transforms it into an electrical signal [14]. The concept of ENFET was introduced after a decade elapsed since the inception of ISFET.

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Characterisation of a Schottky ISFET as Hg-MOSFET and as cytochrome P450-ENFET

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ABSTRACT

A cost-effective and simple method is proposed wherein a Schottky ion sensitive field effect transistor (Schottky ISFET)-based sensor is characterised as metal oxide semiconductor and enzyme field effect transistor (ENFET). This technique involves deposition of mercury (Hg) as gate material over the sensing layer mitigating the complexity of fabrication process, thereby eliminating the need of refabricating an identical device. A Schottky-based ISFET simplifies the fabrication process as the requisite for doping of source and drain regions becomes redundant. Steps involved in lithography process for fabricating metal oxide semiconductor field effect transistor (MOSFET) are reduced with the use of liquid metal Hg as gate over layer. Such a device can be transformed back to an ISFET without any additional etching process. Furthermore, the same ISFET device can be utilised as an ENFET when the former is used in conjunction with a biological element. In this work, a Schottky-based ISFET has been characterised as Hg-MOSFET and as cytochrome P450-ENFET. Multiple tests on the device exhibit that the same ISFET sensor can be used both as a MOSFET and an ENFET with good repeatability and versatility without losing its sensitivity.

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KEYWORDS
ISFET; MOSFET; ENFET; lithography; etching

1. Introduction

Field effect transistor (FET) devices came into being in the semiconductor industry in the early decades of the twentieth century. In 1926, the first FET concept was patented (Edgar, 1930). Metal oxide semiconductor FET (MOSFET) came into existence in 1959 (Kahng, 1960). After the passage of a decade, ion sensitive FET (ISFET) was introduced as pH sensor in 1970 (Bergveld, 1970, 1972). The ISFETs were the first FETs to be used for pH measurement. Since then, numerous forms of FET devices have flooded the semiconductor industry such as enzyme FET (ENFET), CHemFET etc. (Bergveld, 2003; Bistolas, Wollenberger, Jung, & Scheller, 2005; Larramendy, Mathieu, Charlot, Nicu, & Temple-Boyer, 2013).

MOSFET when devoid of the gate metallisation transforms into an ISFET device where the gate insulator in the form of sensing layer is in direct contact with the electrolyte. Further, ISFET when used in conjunction with a biological membrane results into ISFET-based biosensor such as ENFET. The history of biosensors dates back to early sixties with the development of enzyme electrodes (Clarke, Cox, Gonder-Frederick, Carter, & Pohl, 1987). Depending on the transducing mechanisms used, biosensors are of many types out of which ISFET-based biosensors are one of its kinds. ENFETs are the type of biosensors which are constructed by immobilising a polymer layer over the

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List of conferences:

1. Dhruba Jyoti Sarma, Amarendra kumar, Tapas Medhi, Production Optimisation of n-hexaneinduced cytochrome P450 production in hydrocarbon degrading bacteria Bacillus pumilus sp. 58th Annual conference of Association of Microbiologist India 2017.

2. Dhruba Jyoti Sarma, Tapas Medhi, Production of biodiesel by immobilisation of partially purified lipase isolated from Bacillus sp. 57th Annual conference "Microbes and Biosphere 2016"

3. Bhagyashree Roy, Tapas Medhi, Dhruba Jyoti Sarma SantanuSharma, Development of a Capacitive Sensor for Glucose Concentration Detection (ICECS 2016)

4. Bhagyashree Roy, Santanu Sharma, Tapas medhi, Dhruba Jyoti Sarma, Modeling the transient and steady state behaviour of a Glucose Oxidase based Enzyme Field Effect Transistor International Conference on Electrical, Electronics, Signal, Communication and Optimisation-2015