ABSTRACT

Hydrocarbons and their derivatives play key roles in the overall metabolism of living systems as reaction intermediates and a constituent of biomolecules. The wide range of extrinsic uses may range from toothpaste to medicines, cosmetics to motor vehicle fuels. Their ubiquity and vast availability for human use led to serious environmental problems to the habitats of living organisms in land, air and water. Oil spill as well as their continuous combustion results in the disposal of toxic compounds and affects carbon footprint and life on the planet adversely. It has been estimated that during the last 45 years, the crude oil spillage only due to transportation is around 20 million tons and around 142 million tons due to accidents. Such quantity of formidable discharge of petroleum hydrocarbons needs effective, regular in-situ treatment and easy monitoring of hydrocarbon concentration in the areas of spillage/operation. Aliphatic, as well as polycyclic aromatic hydrocarbons (PAHs) are usually present in such an environment having harmful effects on human and other life forms. The biological remediation of these compounds needs the engagement of mostly microorganism and their enzymes in a controlled environment. Out of these enzymes, Cytochrome P450 monooxygenase (CYP450) plays a primary role in the CH activation of aliphatic and aromatic hydrocarbons for their further degradation. This property of CYP450 has been well exploited for pollution mitigation and monitoring. The redox reaction catalysed by cytochrome P450 monooxygenases can be used as a sensing tool for hydrocarbon detection. Such a device could be operational at room temperature in a cost-efficient and easy to use manner. In this connection, this study was carried out

to develop a biosensor for the detection of hydrocarbons at room temperature using cytochrome P450 as the bioreceptor.

Hydrocarbon-degrading bacterial strains were isolated from oil contaminated soil samples and assayed for the CYP450 content. Based on CYP450 content, out of the 72 isolates, two were identified using 16s rDNA sequencing and identified to be *Bacillus pumilus* and an extremophile *Bacillus stratosphericus.* Due to higher CYP450 content, *Bacillus stratosphericus* was optimised for the maximal CYP450 content and was selected for purification of CYP450. For purification, the lysozyme treated spheroplast of *Bacillus stratosphericus* is gone through ion exchange chromatography followed by the gel filtration chromatography, and after SDS two Bands were obtained one between 68 and 53kDa and the other between 10 and 25kDa. Further, the size of the bands was verified using mass spectroscopy and determined to be 68.183 kDa and 13.681 kDa respectively. After trypsin digestion, for the bigger band, the sequence predicted showed a top score of 80, hitting a cytochrome P450 of *Bacillus megaterium.*

A Schottky-based ISFET was fabricated in collaboration with the Department of Electronics and Communication Engineering. The ISFET 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 an electronic response in the presence of n-hexadecane and reduced nicotinamide adenine dinucleotide phosphate. The variation of gate and source potential difference with respect to the concentration of the measurand at constant current has 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 54.34 mV/molar for the enzyme field-effect transistor (ENFET). However, the fabricated ENFET have produced repeatable output only for 48 hours.

For improvement of the stability of the device, the method of immobilisation of the enzyme has been changed. This study on enzyme immobilisation reports entrapment of a partially purified cytochrome P450 component**,** isolated from the extremophile *Bacillus stratosphericus* in a novel polyaphrons matrix prepared from a mixture of bacterial rhamnolopid and olive oil. The resulting enzymepolyaphron layer was then used as the biological recognition element in an ENFET for detections of alkane hydrocarbon n-hexadecane. The enzyme-polyaphron layer was immobilised over a Si_3N_4 gate for its electronic response in the presence of substrate n-hexadecane and co-factor nicotinamide adenine dinucleotide phosphate (NADPH). The variation of potential difference across the gate to a source with respect to the concentration of the substrate n-hexadecane was recorded at constant currents to observe its effects with the change in pH, temperature, storage, hysteresis and concentration of the cofactor. The output showed good repeatability of the device with a sensitivity of 67 mV/molar with stability up to a week.