CHAPTER 4

EXPERIMENTAL TECHNIQUE FOR BIOSENSING

4.1 BIOSENSING

An increasing demand for real-time monitoring of heavy metal pollution of surface and wastewater is coming up these days. Regarding industrial effluents, where unit process malfunctions or emergencies cause release of heavy metals into the environment, the possibility to gain on-line data is of major importance.

Biosensors responding to the concentration of heavy metal ions in aqueous solution may be the basis for the development of both on-line monitoring systems and alarm sensors.

4.2 LIQUID PHASE SENSING BY POTENTIOMETRIC PROBE BIOMETRY

Biochemical sensing in liquid phase is being practiced especially in biomedical sciences for glucose sensing (Guilbault and Schmidt 1991). Liquid biosensing in the environment, especially in effluents/runoff from industries or their sinks like lakes and pools is being done to assess the quality of water. BOD and COD are estimated with the classical Clarke oxygen probe and are used to assess the water quality (Guilbault 1984). Biosensing and monitoring to assess the xenobiotics in environment is the present challenge. This chapter deals with the experimental methodology for the preparation of biosensor and its laboratory level use for sensing metal ions.
4.3 EXPERIMENTAL METHODOLOGY FOR SENSING

The experimental methodology for sensing, as a laboratory experimental model, is described in this chapter. A two-in-one pH probe was the tool for preparing the biosensor to sense the metal ions. The sensing was carried out in aqueous medium (Fig. 4.1).

4.4 CONSTRUCTION OF WHOLE CELL BIOSENSOR

Microsomes of cytochrome P₄₅₀ induced and degradate induced yeast cells were mixed with sodium alginate in the ratio 1:1 and was made into a paste of working consistency. 100 mg of glucose and 5 mg of glucose oxidase were also added to keep the redox cycle along with 10 mg of activated carbon which eliminates any inhibition in the reaction due to hydrogen peroxide formation (Equations 4.1- 4.4) (Balagopal and Subramanian 1994a; Cho and Bailey 1977 and Cho and Bailey 1979). This paste was used to make the enzyme mat, that is, a thin film of uniform spread with a thickness of 0.25 cm with the help of a glass plate and a membrane casting blade. The area of the paste spreading was optimised at 4 cm² with uniform thickness (Appendix 12). The thin film was kept in a solution of calcium chloride, barium chloride and strontium chloride (5:3:2) and hence, immobilised. The mat formed was cut to pieces of 1 cm².

A single mat piece was used at a time to sense the metal ion in the liquid phase. This mat piece of 1 cm² was put on a pretreated dialysis membrane (Sigma grade) of large size than the sensing mat and was affixed to the sensing probe tip of a pH electrode (two-in-one Pt/Au electrode pH probe with saturated KCl). A sterile thread wound tight around the probe groove along with the membrane held the mat piece in position in situ (Balagopal and Subramanian 1994).
Fig. 4.1 Schematic diagram of biosensing
4.5 SENSING IN MEDIUM

Biosensing of chemical toxins in work environment for health preservation or in any process with an effluent discharge of xenobiotics of different concentration into a medium, especially a liquid membrane is possible. In the case of solid xenobiotics, a liquid membrane is needed to dissolve the same so as to transfer the chemical toxin to the site of biotransformation, for transductive sensing to have signal output of pH and mV depending on the rate of biotransduction. Generally, metal ions are easily soluble in aqueous medium and hence, sensing in aqueous medium is facilitated.

4.5.1 Sensing in aqueous medium

When biocatalytic reduction studies were carried out in organic medium (ethanol), biosensing was carried out in aqueous medium (DDW). This was because of the fact that ethanol as a substrate with cytochrome oxidation system; inspite of specific induction may result in the formation of acetic acid. In biocatalytic reduction studies, the levels of metal ions reduced were accounted by estimating the metals in sophisticated instruments, where any little conversion of ethanol to acetic acid may not interfere. But while sensing, conversions may alter the probable H⁺/OH⁻ ratio and this may affect the sensor output. In biosensing, the duration of sensing was possible only for a maximum of three hours with both the metals and the activity being rejuvenated with ten minutes buffer wash (tris buffer pH 7.4).
4.5.2 Sensing principles

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\begin{align*}
\text{NADP} & \quad \text{CYT P}_{450}^{\text{(red)}} \\
\text{NADPH} & \quad \text{CYT P}_{450}^{\text{(oxd)}} \\
2\text{MCl}_2 + 2\text{H}_2\text{O} + \frac{1}{2}\text{O}_2 & \quad \rightarrow \quad 2\text{M}^{2+} + 4\text{HCl} + 2\text{O}_2 \\
\text{GOD (FAD)} + \text{Glucose} & \quad \rightarrow \quad \text{GOD (FADH}_2) + \text{Gluconolactone} \\
\text{GOD (FADH}_2) + \text{O}_2 & \quad \rightarrow \quad \text{GOD (FAD)} + \text{H}_2\text{O}_2
\end{align*}
\]

4.5.3 Cofactor equation

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\begin{align*}
\text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2 & \quad \rightarrow \quad \text{Gluconic acid} + \cdot\text{H}_2\text{O}_2 \\
\text{A/C} & \quad \rightarrow \quad \text{H}_2\text{O} + \frac{1}{2}\text{O}_2
\end{align*}
\]
4.6 SENSING METHODOLOGY

The metal salts were dissolved in double distilled water to get metal solutions. The experiments were conducted using a magnetic stirrer which stirred an aqueous medium of 50ml with known concentration of metal salts i.e., 1µg/ml, 5µg/ml and 10µg/ml which was within the workable range of the sensor. The biosensor made out of pH probe was introduced into the stirring aqueous medium containing the metal salt. Care was taken, so that the tip of the biosensor was completely immersed in the medium. Homogeneous stirring was also maintained without any liquid throw up on the sides. The process was continuously monitored and the potentiometric output due to biotranformation and biotransduction in interms of pH and mV were noted regularly at every 15-minute interval continuously.

Throughout the experiment, pH was considered as the scale of biosensing, inspite of biotransduction estimated as dmV, which was correspondingly proportional to change in dpH (figures 4.2 & 4.3). The range of pH scale was always higher when compared with the range of ΔmV scale, that is, for every pH change of 1 unit which is divided into 100 parts (in 0.01 units); the change in mV ranged only from 50 to 60 (in single units), hence, pH was considered a sensitive scale for a better precision than mV.

Samples for metal analysis in the medium were also collected at an interval of 15 minutes and were analysed using AAS.

Sensing was done for nearly 6 to 8 hours every day with a single sensor for any of the two metals. The lowest concentration that is 1µg/ml was sensed and the readings were recorded, over time with samples drawn at regular intervals. The process was stopped when the ongoing reaction ended that is given by an indication of nil change in pH or mV. The biosensor was then given
Fig 4.2 Cadmium chloride biosensor output
Fig. 4.3 Copper chloride biosensor output
a buffer wash (50 mmol/l Tris HCl buffer, pH 7.4) in a stirred condition for 10 minutes. The same biosensors were used to sense the next higher concentration level that is 5μg/ml which was again buffer washed after the reaction and thus 10μg/ml was also sensed in a day. The lower concentrations were sensed initially everyday because of the fact that lower toxin concentration would have lower inhibitory effect if any, so that biotransduction can be sustained.

4.6.1 Short range sensing

The sensing was done for each concentration described above (section 4.6) in one day and this was continued for a number of days depending on the chemical toxin biosensed, sensing being done for 6 to 8 hours per day, till the biosensor response was maintained. This everyday sensing methodology is called short range sensing. In places of work and in other important area of environmental monitoring, a continuous day to day sensing is required at frequent intervals throughout the day and hence, the short range sensing was carried out.

4.6.1.1 Data comparison - Probe biometry with instrumental methods

A standard graph was prepared to calibrate the biosensor probe with known concentration of metal salts against ΔpH. This was applied in the standard equation derived using the standard graphs (Figures 4.4 & 4.5). This biosensed concentration of the metals over time was compared with the estimation of the concentration of the same samples collected over varied time intervals in AAS.
Fig. 4.4 Biosensor standard graph for Cadmium chloride
Fig. 4.5 Biosensor standard graph for Copper chloride
4.6.2 Long range sensing

Biosensing of metals may be necessitated only at a specific time for measuring a process effluent discharge, which may contain the metals. As the biosensor could be used only at the time of necessity and at different places, it is necessary to store the biosensor and use it at a later stage. Hence, the shelf life of a biosensor with monitorable activity was also carried out as a long-range methodology for sensing.

4.6.2.1 Study methodology

Sensing of each metal was carried out with specific biosensor over a period of 5 weeks, with the sensing operation being done once every week. The different concentrations of toxins sensed were 1µg/ml, 5µg/ml and 10µg/ml in aqueous medium (volume 50ml). The methodologies of sensing were the same as that for the short range sensing.

4.6.2.2 Maintenance of stability

The activity of long range sensing biosensor was maintained over time by storing it in a Tris buffer (pH 7.4) as mentioned earlier, at 4°C. The response was maintained for nearly 4 to 5 days (based on one day sensing per week).

4.6.2.3 Data comparison - probe biometry with instrumental method

The metal concentration biosensed, with standard graph was compared with instrumental analysis for analytical significance of the results.