# CHAPTER II
# THEORETICAL BACKGROUND AND LITERATURE REVIEW

## 2.1 Biosensor

Biosensor-related research has experienced explosive growth over the last two decades. A biosensor is generally defined as an analytical device which converts a biological response into a quantifiable and processable signal (Lowe *et al.*, 1987). Biological and biochemical processes have a very important role on medicine, biology and biotechnology. However, it is very difficult to convert directly biological data to electrical signal, the biosensors can convert these signals and the biosensors over this difficulty. The first biosensor was described in 1962 by Clark and Lyons who immobilized glucose oxidase (GOD) on an amperometric oxygen electrode surface semipermeable dialysis membrane in order to quantify glucose concentration in a sample directly. They described how to make electrochemical sensors (pH, polarographic, potentiometric or conductometric) more intelligent" by adding "enzyme transducers as membrane enclosed sandwiches (Sassolas *et al.*, 2011). According to a recently proposed IUPAC definition, "A biosensor is a self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is in direct spatial contact with a transducer element. A biosensor should be clearly distinguished from a bioanalytical system, which requires additional processing steps, such as reagent addition. Furthermore, a biosensor should be distinguished from a bioprobe which is either disposable after one measurement, i.e. single use, or unable to continuously monitor the analyte concentration" (Koyun *et al.*, 2012). A biosensor can include, for example, an enzyme, an antibody, or a microorganism that in turn is connected to an electronic element. With this in mind one could define a biosensor as a self-contained integrated device consisting of a biological recognition element (enzyme, antibody, DNA or microorganism) which is interfaced to an analytical device and together

respond in a concentration-dependent manner to a given analyte. Thévenot's definition of a chemical biosensor from 2001 is often quoted in biosensor research: "An electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element.

A biosensor is a device composed of two elements:

1. A bioreceptor that is an immobilized sensitive biological element (e.g. enzyme, DNA probe, antibody) recognizing the analyte (e.g. enzyme substrate, complementary DNA, antigen). Although antibodies and oligonucleotides are widely employed, enzymes are by far the most commonly used biosensing elements in biosensors.

2. A transducer is used to convert biochemical signal resulting from the interaction of the analyte with the bioreceptor into an electronic one. The intensity of generated signal is directly or inversely proportional to the analyte concentration. Electrochemical transducers are often used to develop biosensors. These systems offer some advantages such as low cost, simple design or small dimensions. Biosensors can also be based on gravimetric, calorimetric or optical detection (Sassolas *et al.*, 2011).

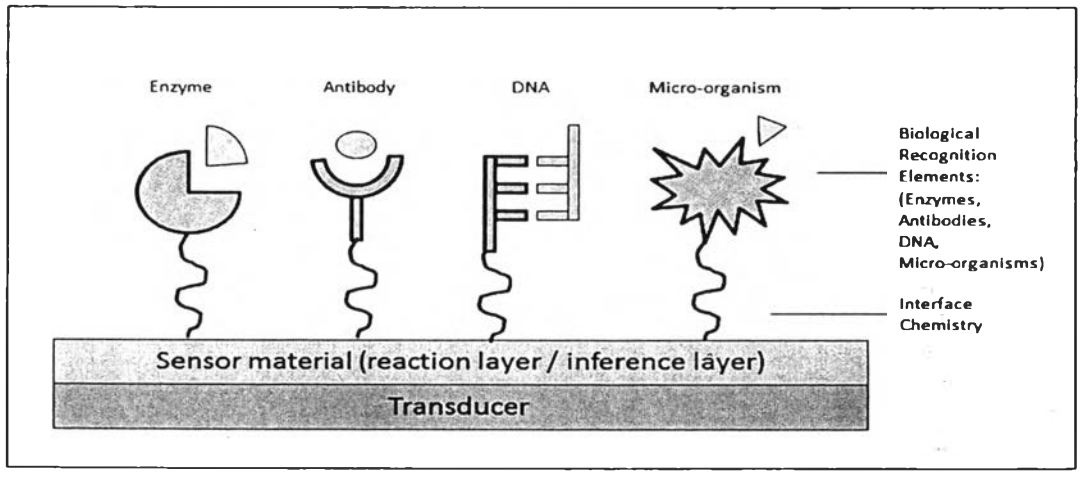


**Figure 2.1** The major elements of a biosensor. (Ahmet *et al.*, 2012)

Biosensors are categorized according to the basic principles of signal transduction and biorecognition elements. According to the transducing elements, biosensors can be classified as electrochemical, optical, piezoelectric, and thermal sensors (Thevenot *et al.*, 1999). Electrochemical biosensors are also classified as potentiometric, amperometric and conductometric sensors.

The application of biosensor areas are clinic, diagnostic, medical applications, process control, bioreactors, quality control, agriculture and veterinary medicine, bacterial and viral diagnostic, drag production, control of industrial waste water, mining, military defense industry, etc. A few advantages of biosensors are listed below:

1. They can measure nonpolar molecules that do not respond to most measurement devices
2. Biosensors are specific due to the immobilized system used in them
3. Rapid and continuous control is possible with biosensors
4. Response time is short (typically less than a minute) and
5. Practical

There are also some disadvantages of biosensors:

1. Heat sterilization is not possible because of denaturaziation of biological material,
2. Stability of biological material (such as enzyme, cell, antibody, tissue, etc.), depends on the natural properties of the molecule that can be denaturalized under environmental conditions (pH, temperature or ions)
3. The cells in the biosensor can become intoxicated by other molecules that are capable of diffusing through the membrane.

### 2.1.1 Electrochemical Biosensors

Bioelectroanalysis with electrochemical biosensors is a new area in rapid development within electroanalysis. In biosensor development studies, suitable bioreceptor molecule, suitable immobilization method and transducer should be selected firstly bioelectroanalytical sensors permit the analysis of species with great specificity, very rapid, sensitive, highly selective and cheap cost in principle. They can be used in clinical analysis, in on-line control processes for industry or environment, or even in vivo studies. The difference between biosensor and physical

or chemical sensors is that its recognition element is biological. The investigated bioelectrochemical reaction would generate a measurable current (amperometric detection), a measurable potential or charge accumulation (potentiometric detection) or measurable conductivity change of a medium (conductometric detection) between electrodes. When the current is measured at a constant potential this is referred to as amperometry. If an electrical current is measured while a controlled variation of the potential is being applied, this is named as voltammetry. Firstly, Potentiometric, amperometric and conductometric measurement techniques form the kinds of electrochemical biosensors. Potentiometric sensors have an organic membrane or surface that is sensitive to an analyte. The reaction between them generates a potential (emf) proportional to the logarithm of the electrochemically active material concentration. This potential is compared with the reference electrode potential. Enzyme immobilized electrodes reacts with substrate and products are detected by electrodes. In addition, Amperometric sensors measure the current change resulted by chemical reaction of electroactive materials while a constant potential is being applied. The change of the current is related to the concentration of the species in solution. Generally biological compounds (glucose, urea, cholesterol, etc.) are not electroactive, so the combination of reactions to produce an electroactive element is needed. This electroactive element leads a change of current intensity. This change is proportional to the concentration of analyte. Finally, Conductometric biosensors can measure the change of the electrical conductivity of cell solution. Most reactions involve a change in the composition of solution. Thus conductometric biosensors can detect any reactive change occurring in a solution. Electrochemical biosensors have advantages that they can sense materials without damaging the system. The use of biosensors for industrial and environmental analysis is very important. The control of food manufacturing processes, evaluation of food quality, control of fermentation processes and monitoring of organic pollutants are some of the applications of biosensors. The present popularity of analytical biosensors is due to their specific detection, simple use and low cost. For example an electrochemical biosensor can be used to detect Salmonella and E. coli O157:H7 in less than 90 minute. Electrochemical biosensor studies are performed with electrochemical cells.

*2.1.1.1 Electrochemical Cells*

An electrochemical cell is used in electrochemical sensor studies. The electrodes themselves play an important role in the performance of electrochemical biosensors. The electrode material, its surface modification or its dimensions affects the detection ability of the electrochemical biosensor. There are three kinds of electrodes in the electrochemical cell:

- Working electrode
- Reference electrode
- Auxilary (counter) electrode

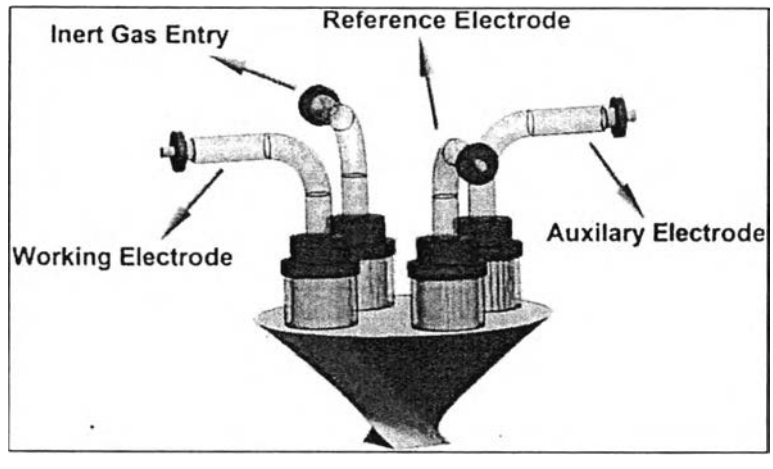


**Figure 2.2** Electrochemical cell. (Ahmet *et al.*, 2012)

*2.1.1.1.1 Reference Electrode*

The other electrodes in the cell are referred to this electrode, the hydrogen electrode, the calomel electrode, glass electrodes. Reference electrode is a kind of standard hydrogen electrode. Hydrogen is potentially explosive and is not very suitable using an electrode with hydrogen gas for routine measurements. So there are two common use and commercially available reference electrode types:

- Ag/AgCl Electrode: There is a Ag wire that coated with AgCl and dipped into NaCl solution.

$$AgCl + e^- \rightarrow Ag + Cl^- \qquad (E^0 = +0.22V)$$

- Saturated-Calomel Electrode: Calomel is the other name of mercurous chloride ($Hg_2Cl_2$)

$$Hg_2Cl_2 + 2e^- \rightarrow 2Hg + 2Cl_2 \qquad (E^0=+0.24V)$$

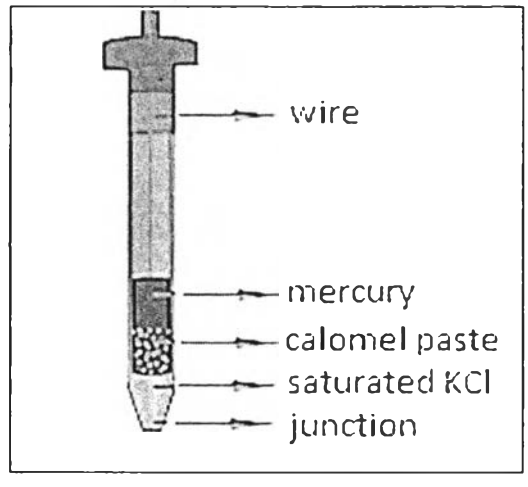


**Figure 2.3** Reference (calomel) electrode. (Ahmet *et al.*, 2012)

Calomel electrode is consist of mercury, paste (mixture of mercury(I) chloride powder and potassium chloride) and saturated potassium chloride solution.

*2.1.1.1.2 Auxilary (Counter) Electrode*

In a two electrode system, when a known current or potential is applied between the working and auxiliary electrodes, the other variables may be measured. The auxiliary electrode functions as a cathode whenever the working electrode is operating as an anode and vice versa. The auxiliary electrode often has a surface area much larger than that of the working electrode. The half-reaction occurring at the auxiliary electrode should occur fast enough not to limit the process at the working electrode. The potential of the auxiliary electrode is not measured against the reference electrode but adjusted to balance the reaction occurring at the working electrode. This configuration allows the potential of the working electrode to be measured against a known reference electrode. Auxiliary electrode is often fabricated from electrochemically inert materials such as gold, platinum or carbon.

*2.1.1.1.3 Working Electrode*

It is the electrode on which the reaction occurs in an electrochemical system. In an electrochemical system with three electrodes, the working electrode can be referred as either cathodic or anodic depending on the reaction on the working electrode is a reduction or an oxidation. There are many kind of working electrodes. Glassy carbon electrode, screen printed electrode, Pt electrode, gold electrode, silver electrode, Indium Tin Oxide coated glass electrode, carbon paste electrode, carbon nanotube paste electrode etc. Screen printed electrodes are prepared with depositing inks on the electrode substrate (glass, plastic or ceramic) in the form of thin films. Different inks can be used to get different dimensions and shapes of biosensors.

Screen printed electrochemical cells are widely used for developing amperometric biosensors because these biosensors are cheap and can be produced at large scales. This could be potentially used as disposable sensor that decreases the chances of contamination and prevents loss of sensitivity. Figure 2.4 exhibits an electrochemical biosensor as screen printed electrode. Performance factors of an electrochemical biosensor are: selectivity, response time, sensitivity range, accuracy, recovery time, solution conditions and the life time of the sensor.

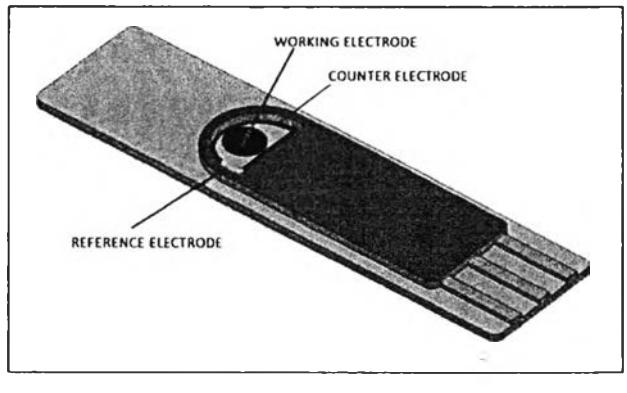


**Figure 2.4** Electrochemical biosensor as screen printed electrode. (Ahmet *et al.*, 2012)

*2.1.1.2 Cyclic Voltammetry (CV)*

Cyclic voltammetry is a type of potentiodynamic electrochemical measurement. In a cyclic voltammetry experiment, the working electrode potential is changed linearly versus time. Cyclic voltammetry experiment ends when it reaches a set potential value. When cyclic voltammetry reaches the set potential, potential ramp of the working electrode is inverted back. This inversion can happen multiple times during a single experiment until a set cycle number is obtained. The plot of the current at the working electrode vs. the applied voltage gives the cyclic voltammogram of the reaction. Cyclic voltammetry is a general way to study the electrochemical properties of an analyte in a solution.

*2.1.1.3 Electrochemical Impedance Spectroscopy (EIS)*

Electrical resistance can be described as the ability of a circuit element to resist the flow of electrical current. This is defined with Ohm's law:

E=IxR for DC conditions

While this is a well-known equation, its use is limited to only the ideal resistor. An ideal resistor follows Ohm's Law at all current and voltage levels and its resistance value is independent of frequency. Impedance is a measure of the ability of a circuit to resist the flow of electrical current like resistance, but electrochemical impedance is usually used by applying an AC potential to an electrochemical cell and then measuring the current through the cell. When we apply a sinusoidal potential, the response to this potential is an AC current signal. This current signal can be considered as a sum of sinusoidal functions (a Fourier series). For AC conditions: E = IxZ, where Z is the impedance of the system. The impedance can be calculated by setting the input potential and measuring the induced current.

Electrochemical impedance spectroscopy (EIS) is a technique well suited for evaluating coating permeability or barrier properties for corrosion control of steel structures based on the electrical resistance of the coating. EIS has been widely used in the lab to determine coating performance and to obtain quantitative kinetic and mechanistic information on coating deterioration (Eggins 2002).

*2.1.1.4 Immobilization Methods*

Electrochemical detection techniques use predominant enzymes, because enzymes have specific binding capabilities and biocatalytic activity. Some of the other biorecognition elements are antibodies, nucleic acids, cells and micro-organisms. Biorecognition elements should be immobilized on the electrode surface. Adsorption, microencapsulation, entrapment, covalent attachment and cross linking methods are the most well-known immobilization methods.

*2.1.1.5 Adsorption Methods*

Physisorption is weaker than chemisorption. Adsorption is the simplest way for immobilization of organic material. However, the bonding is weak and life time of electrode is short. Microencapsulation method is more reliable for adsorption. In this method, an inert membrane traps the biologic material on the working electrode. Most used membranes are cellulose acetate, collagen, gluter aldehyde, chitosan, nafion, polyurethanes, etc.. In entrapment method, generally a solution of polymeric materials is prepared containing biologic material that will be entrapped onto the working electrode. The solution is coated on the electrode with various coating methods. Starch gels, nylon and conductive polymers such as polyaniline or nafion are used for. Covalent attachment immobilization is important particularly for the advantage that the enzyme is not been released from the electrode surface when it is used. However, covalent bonding should not decompose or hide the active site of the enzyme. The functional groups that may take part in this binding are $NH_2$, COOH, OH, $C_6H_4OH$ and SH groups (Eggins 2002). Cross linking is bonding two or more molecules by covalent bonds. In cross-linking method bifunctional agents such as gluteraldehyde are used to bind the biological materials. The disadvantage of this method is high ratio of enzyme activity loss.

*2.1.1.6 Detection of Analyte*

Detection principle of analytes changes according to transducer type of the biosensor. Electrochemical biosensors use electrical signals as output data. Thus detection of an analyte is related with the changes of electrical signals. For example; the intensity of the current, potential energy and electrical conductivity of the electrode change.

2.1.2 Optical Biosensor

Optical biosensors are a powerful detection and analysis tool that has vast applications in biomedical research, healthcare, pharmaceuticals, environmental monitoring, homeland security, and the battlefield (Narayanaswamy *et al.*, 2004). Moreover, optical biosensors, as a powerful alternative to conventional analytical techniques, enable the highly sensitive, real-time, and high-frequency monitoring of pollutants without extensive sample preparation (Long *et al.*, 2013). Optical biosensors that exploit light absorption, fluorescence, luminescence, reflectance, Raman scattering and refractive index are powerful alternatives to conventional analytical techniques (Figure 2.5). These biosensors provide rapid, highly sensitive, real-time, and high-frequency monitoring without any time-consuming sample concentration and/or prior sample pre-treatment steps. Although optical biosensors have great potential applications in the areas of environmental monitoring, food safety, drug development, biomedical research, and diagnosis, their use in fields of environmental pollution control and early warning is still in the early stages.

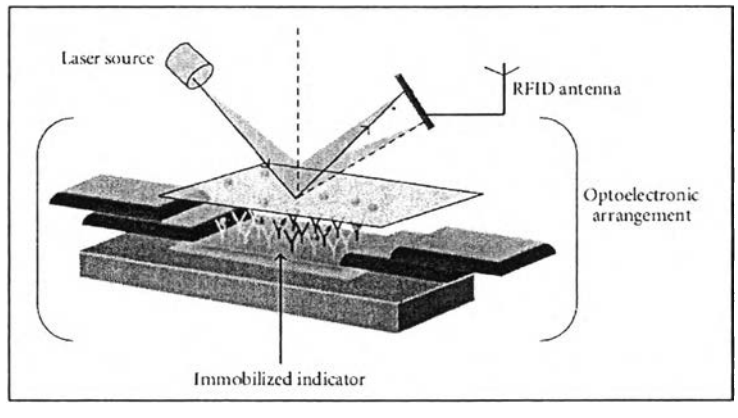


**Figure 2.5** Architecture of an optical biosensor. (Dey *et al.*, 2011)

Generally, there are two detection protocols that can be implemented in optical biosensing: fluorescence-based detection and label-free detection. In fluorescence-based detection, either target molecules or biorecognition molecules are labeled with fluorescent tags, such as dyes; the intensity of the fluorescence indicates the presence of the target molecules and the interaction strength between target and biorecognition

molecules. While fluorescence-based detection is extremely sensitive, with the detection limit down to a single molecule, it suffers from laborious labeling processes that may also interfere with the function of a biomolecule. Quantitative analysis is challenging due to the fluorescence signal bias, as the number of fluorophores on each molecule cannot be precisely controlled. In contrast, in label-free detection, target molecules are not labeled or altered, and are detected in their natural forms. This type of detection is relatively easy and cheap to perform, and allows for quantitative and kinetic measurement of molecular interaction. Additionally, some label-free detection mechanisms measure refractive index (RI) change induced by molecular interactions, which is related to the sample concentration or surface density, instead of total sample mass (Fan *et al.*, 2008).

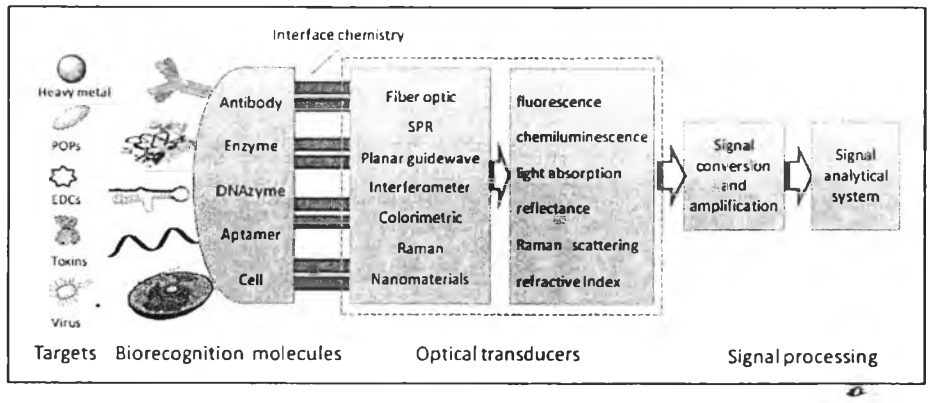


**Figure 2.6** Schematic of an optical biosensor. (Long F., *et al.*, 2013)

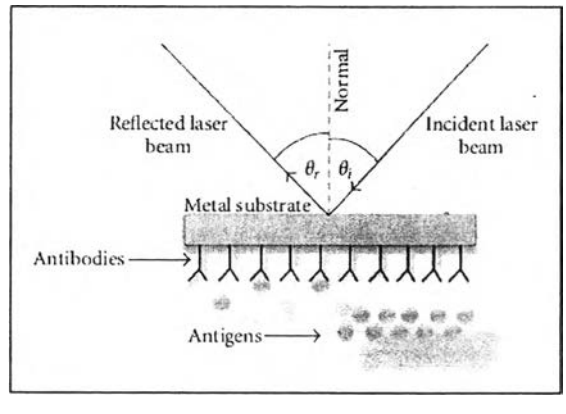


**Figure 2.7** Interaction of biomolecules with light. (Long F., *et al.*, 2013)

There are three types of optical biosensor: Evanescent Wave Fiber Optic Biosensors, SPR Biosensors and Nano-Structured Optical Biosensors.

*2.1.2.1 Evanescent Wave Fiber Optic Biosensors*

When light propagates through a fiber optic on the basis of total internal reflection (TIR), a thin electromagnetic field (the evanescent wave) generated decays exponentially with the distance from the interface with a typical penetration depth of up to several hundred nanometers (Andrade *et al.*, 1985).

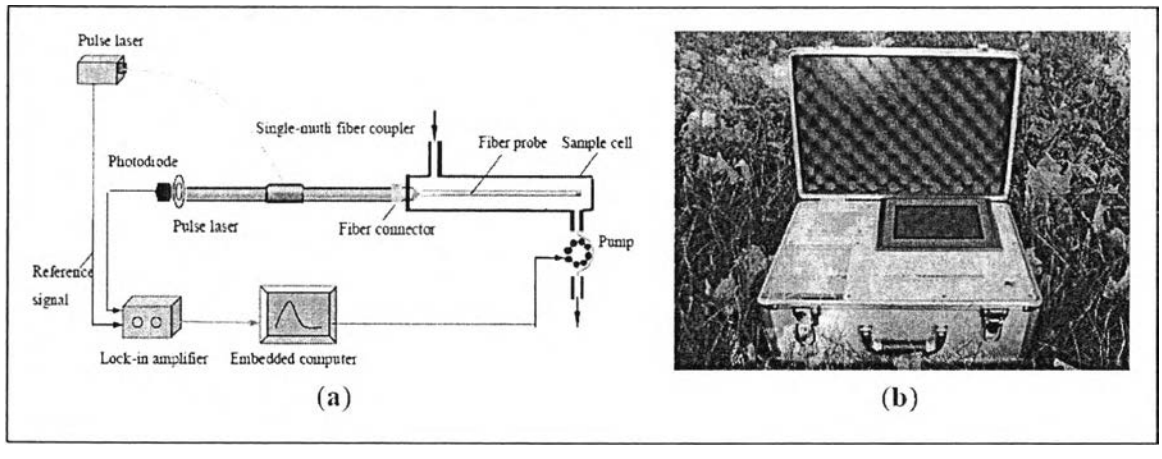


**Figure 2.8** Schematic set-up of the portable evanescent wave optical fiber biosensor (EWAB): (a) principle scheme of the portable optical fiber biosensor and (b) the portable platform. (Long *et al.*, 2009)

This evanescent wave can excite fluorescence in the proximity of the sensing surface, e.g., in fluorescently labeled biomolecules bound to the optical sensor surface through affinity recognition interactions. The short range of the evanescent wave enables it to discriminate between unbound and bound fluorescent complexes, hence eliminating the normally required washing procedures. Moreover, evanescent field-based waveguides are well suited for study and detection of biomolecular interaction. Evanescent wave fiber-optic immunosensors (EWFI) are rapid, specific, sensitive, cost effective and suitable for real-time on-site detection and have been applied to detect a wide variety of pollutants, such as TNT, 2,4-D, atrazine, E. coli O157:H7, and Staphylococcal enterotoxin B. Conventional EWFI have the large size with

numerous optic components (e.g., chopper, off-axis parabolic reflector, and biconvex silica lens) which makes it costly and requires crucial optical alignment restricting its use as portable device.

*2.1.2.2 SPR Biosensors*

Surface Plasmon Resonance (SPR) is a surface-sensitive optical technique that is associated with the evanescent electromagnetic field generated on the surface of a thin metal film when excited by an incident light under total internal reflection conditions (Cooper 2002). Due to the fact the evanscent field diminishes exponentially with increasing distance of penetration from the interface, SPR promotes monitoring of only surface-confined molecular interactions occurring on the transducer surface. Most of the SPR instruments use a Kretchmann configuration working at attenuated total reflectance (ATR) for excitation of surface plasmons, which can detect a small refractive index change at the metal/analyte interface, and the information of the molecular interactions can be obtained by measuring the optical intensity (or phase/polarization) of light reflected from the optical instrument. SPR biosensors allow real-time detection of minute changes in the refractive index when biorecognition molecules (e.g. antibodies) immobilized on a transducer surface bind with their biospecific targets (e.g. analytes) in solution. Since their introduction in the early 1990s, SPR biosensors have seen wide applications including clinical diagnosis, drug discovery, food analysis, environmental monitoring. In general, a SPR biosensor is comprised of several important components: a light source, a detector, a transduction surface (e.g. gold-film), a prism, biorecognition molecule (e.g. antibody/antigen, DNA and aptamer) and a flow system.

*2.1.2.3 Nano-Structured Optical Biosensors*

Progress in nanotechnology, microelectronics and microfluidics could facilitate development of miniaturized, rapid, ultrasensitive and inexpensive nanostructured optical biosensing platforms for rapid toxicity screening and multianalyte testing. These devices are likely to become more compact, robust, smaller and adaptable for in-field and continuous field-based environmental monitoring monitoring. A fiber-optic nanosensor was designed with taper optical fibers, onto which biorecognition molecules (e.g., antibody, peptides, and nucleic

acids) was immobilized. This sensor can probe individual chemical species in a living cell (Vo-Dinh 2013)

### 2.1.3 Piezoelectric Biosensor

The piezoelectric immunosensor is thought to be one of the most sensitive analytical instruments developed to date, being capable of detecting antigens in the picogram range. Moreover, this type of device is believed to have the potential to detect antigens in the gas phase as well as in the liquid phase. Almost all current methods of diagnosing tuberculosis (TB) have drawbacks. They tend to be either nonspecific or too time-consuming. In most cases of pulmonary and extrapulmonary TB, diagnosis depends upon culturing the mycobacterial organism, a process requiring 4-8 weeks (Murray *et al.*, 1990).

The piezoelectric material has ability to produce electricity under pressure (i.e., called direct piezoelectric effect or piezoelectric effect) and vice versa (i.e., called inverse piezoelectric effect). The piezoelectric sensor can be operated in three modes such as transverse, longitudinal, and shear depending on how a piezoelectric material is cut (e.g., A-cut, B-cut, etc.). The most common type unit cell structure of piezoelectric material is perovskite, general formula '$ABX_3$'. Where, the A-site cations are typically larger than the B-site cations and almost similar in size to the X-site anions, i.e., oxygen (O). A general unit cell structure of piezoelectric material is depicted in Figure 2.7. The 'A' and 'B' may be three different combinations of metallic atoms/ions at different lattice position in a unit cell. First, 'A' and 'B' may be both trivalent (e.g., $LaAlO_3$). Second, 'A' may be divalent (Ca, Sr, Ba, Cd, and Pb) and 'B' may be quadrivalent (Ti, Zr, Th, Zr, Hf, Sn, and Ge) (e.g., $CaTiO_3$). Third, 'A' may be univalent and'B' may be pentavalent (e.g., $NaWO_3$). Several natural materials such tendon, bone, dentine enamel, DNA, baleen whale, sugar cane, quartz crystal, topaz, rochelle salt and so on have shown piezoelectric properties. Some of these materials are shown in Figure 2.8. The piezoelectric transduced biosensors have shown several potential advantages in biomedical applications. The piezoelectric crystal (PZC) becomes electrically polarized when it is subjected to a mechanical stress. Conversely, it experiences a strain in response to an applied electric field, proportional to the field strength. An

ideal piezoelectric substrate material for a biosensor has to be nontoxic with outstanding mechanical, thermal, and semiconducting properties (Pramanik *et al.*, 2013).

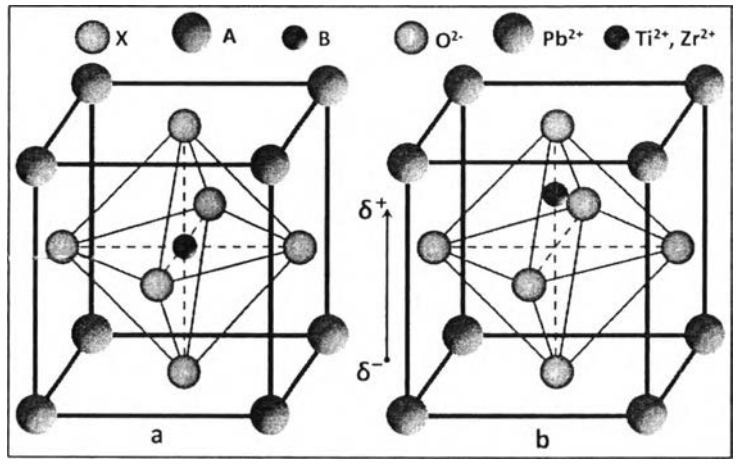


**Figure 2.9** (a) Structure of piezoelectric material (Stolen *et al.*, 2006) and (b) Structure of PZT under an electric field. (Park et al., 1997)

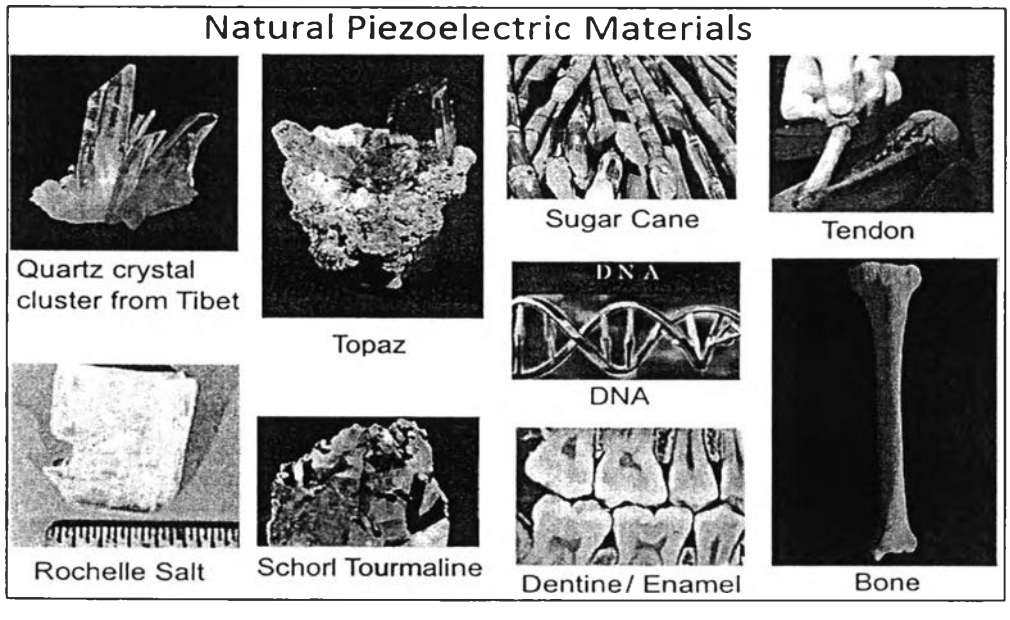


**Figure 2.10.** Natural piezoelectric materials that produce electricity under pressure and vice versa. (Manbachi *et al.*, 2011)

2.1.4 Thermal Biosensor

Thermometric biosensors exploit the fundamental property of biological reactions, i.e. absorption or evolution of heat (Spink *et al.*, 1976). This is reflected as a change in the temperature within the reaction medium. In earlier studies on calorimetry the change in heat was directly monitored to calculate the extent of reaction (for catalysis) or structural dynamics of biomolecules in the dissolved state (Grime 1985). However, its exploitation in biosensors led to the development of thermometric devices (Mosbach *et al.*, 1974). These predominantly measure the changes in temperature of the circulating fluid following the reaction of a suitable substrate with the immobilized enzyme molecules. Thermometry essentially means measurement of temperature. The most basic version of such a device is a thermometer, routinely used for measurement of body or ambient temperature. However, simple mercury based thermometers are limited by their temperature sensitivity in addition to toxicity by metallic mercury. Based on similar principles, in thermometric devices the heat is measured using sensitive thermistors (Danielsson *et al.*, 1981). Such devices are popularly referred to as an enzyme thermistor, ET (Danielsson *et al.*, 1988). Calorimetric devices for routine use were limited by cost of operation and relatively long experimental procedures. However, the invention of the enzyme thermistor based on flow injection analysis in combination with an immobilized biocatalyst and heat-sensing element, circumvented several of these shortcomings (Danielsson *et al.*, 1986). Several instruments were designed in the past two decades and they combined the principles of calorimetry, enzyme catalysis, immobilization on suitable matrices and flow injection analysis.

## 2.2 Self-assembled Monolayers

The word SAM generally denotes a monomolecular thick film of organic compounds on flat (i.e., two-dimensional) metal or semiconductor surfaces. SAM formation provides one of the easiest ways to obtain ordered monolayers through strong chemisorption between the substrate head group of a desired compound and the metal surface leading to the preparation of thermodynamically stable monolayers

as compared to Langmuir–Blodgett and other techniques. Several studies show that long-chain alkane thiols (containing more than six to seven methylene units) form more well-ordered defect-free monolayers than short-chain alkane thiols, disulphides or sulphides. Aromatic (benzene, naphthalene or diphenylene) and hydrogen-bonded molecules with multiple contacts, containing functional groups like thiols, amines, sulphides, selenides etc. provide improved stability (Chaki *et al.*, 2001).

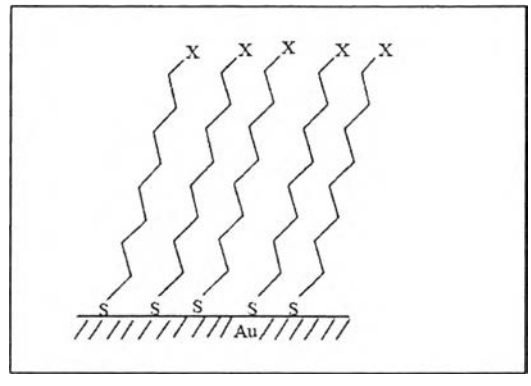


**Figure 2.11** Gold thiolate monolayer. (Margaret 2001)

One of the important advantages of SAM is that they can be prepared in the laboratory by simply dipping the desired substrate in the required millimolar solution for a specified time followed by thorough washing with the same solvent and drying, often using a jet of dry argon. Gas-phase evaporation of the adsorbant can also form good monolayers, although structural control is difficult. Several factors affect the formation and packing density of monolayers, like nature and roughness of substrate, solvent used, nature of the adsorbate, temperature, concentration of adsorbate etc. Cleanliness and crystallinity of the substrate also play a crucial role in determining the compactness, often quantitatively estimated by the pinhole distribution.

## 2.3 Sulfate Reducing Bacteria (SRB)

Sulfate-reducing bacteria (SRB) are bacteria that can obtain energy by oxidizing organic compounds or molecular hydrogen while reducing sulfate to hydrogen sulfide. Most sulfate-reducing bacteria can also reduce other oxidized inorganic sulfur compounds, such as sulfite, thiosulfate/elemental sulfur. SRB is the most important microbe for anaerobic corrosion of buried pipelines. SRB can remove molecular hydrogen from the cathode, leading to cathodic depolarization of the metal surface. Iron sulfide or scale by SRB is accumulated on surfaces of metals, which accelerates the dissolution of the iron.

SRB comprise several groups of bacteria that reduce sulphate to sulphide and produce carbonate which increase the pH. Sulfate-reducing bacteria can create problems when metal structures are exposed to sulfate-containing water by:

- Interacting water and metal creating a layer of molecular hydrogen on the metal surface
- Oxidizing the hydrogen while creating hydrogen sulfide

Sulfate-rich wastewaters are generated by many industrial processes and cause an unbalance in the natural sulfur cycle. Sulfate-reducing bacteria are considered as a possible way to deal with acid mine waters that are produced by other bacteria. SRB are capable of causing severe corrosion of iron material in a water system because they produce enzymes which have the power to accelerate the reduction of sulfate compounds to the corrosive hydrogen sulfide, thus SRB acts as a catalyst in the reduction reaction. SRB occurs naturally in surface waters, including seawater. Bacteria accumulation can lead to pitting of steel, and the buildup of $H_2S$ increases the corrosiveness of the water, thus increasing the possibility of hydrogen blistering or sulfide stress cracking. Some sulfate-reducing bacteria can reduce hydrocarbons such as benzene, toluene, ethylbenzene, and xylene, and have been used to clean up contaminated soils. Their use has also been proposed for other kinds of contaminations. Since it also occurs in sour crude oil, it is the major microbial-induced corrosion causative bacteria in pipeline systems of oil & gas industries. Hydrogen sulfide from sulfate-reducing bacteria also plays a role in the biogenic sulfide corrosion of concrete. SRB can be used to biologically treat sulfate-rich

wastewater. Sulfate-reducing bacteria can cause the external or internal corrosion of water or wastewater pipelines and pipelines for petroleum and natural gas. The formation of galvanic cells by massive growth of sulfate-reducing bacteria under suitable conditions makes the corrosion much worse than just the effect of the hydrogen sulfide on the metal or concrete.

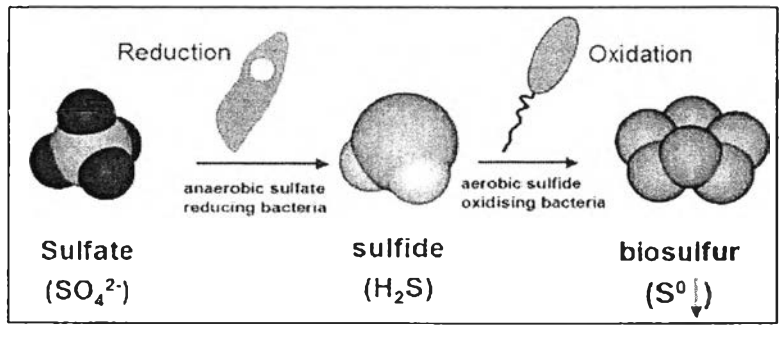


**Figure 2.12** Combining the action of SRBs and sulfide oxidizing microbes. (http://wiki.biomine.skelleftea.se/wiki/index.php/Sulfate_reducing_bacteria#SRB_for_treatment_of_acid_mine_drainage)

Some sulfate-reducing bacteria play a role in the anaerobic oxidation of methane ($CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$). An important fraction of the methane formed by methanogens below the seabed is oxidized by sulfate-reducing bacteria in the transition zone separating the methanogenesis from the sulfate reduction activity in the sediments. This process is also considered a major sink for sulfate in marine sediments. In hydrofracturing fluids used to crack shale formations to recover methane (shale gas), biocide compounds are often added to water to inhibit the microbial activity of sulfate-reducing bacteria in order to avoid anaerobic methane oxidation and to minimize potential production loss efficient monitoring and control strategies.

Most sulfide production result from the sulfate reduction by the sulfate-reducing bacteria (SRB) found in certain reservoirs, well or from inappropriate use of injection water with elevated sulfate concentration and SRB. Until recently, SRB were

considered to comprise two genera, the nonsporulating Desulfovibrio and the spore-forming *Desulfotomaculum*. However, nine additional, new genera of SRB which include *Desulfomonas, Desulfobacter, Desulfobacterium, Desulfobulbus, Desulfococcus, Desulfonema, Desulfosarcina, Desulfomicrobium* and *Thermodesulfobacterium* have been recognized. Also, there are anaerobic extremophilic archaebacteria that reduce molecular sulfur to sulfide. By other side, combustive and oil storage systems, mesophilic species from soil or water such as *Bacillus, Pseudomonas, Proteus, Clostridium, Thermoproteus* and the yeast *Saccharomyces* could be more important than the traditional SRB to produce sulfide gas, in oil and waters in many atmosphere conditions, using different sulfur oxidizing compounds, turning control much more difficult. SRB can thrive under deposits where no oxygen is present even though aerobic conditions exist in the main body of water. Problems with SRB will occur more readily in systems with concurrent fouling problems. Excessive amounts of dirt, algae, oils and other bacteria will provide ideal conditions for growth. Even small amounts of oils and grease will provide nutrients for SRB growth. Stagnant water and low flow conditions will increase the chance of SRB growth. Although in minor quantities, multiple carbon sources commonly found in oil field waters (formate, acetate, propionate, iso- and n-butyrates) with inorganic sulfate as the electron acceptor could serve of substrates for the growth of these bacteria.

From another research, there are more researches about using electrochemical biosensor to detect virus, bacteria, etc.

First In 2008, Ping and his coworker studied an electrochemical impedance immunosensor for the detection of *Escherichia coli.* This sensor was developed by immobilizing anti-*E coli* antibodies on an Au electrode surface. The immobilization of antibodies at the Au electrode was carried out through a stable acyl amino ester intermediate generated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydrosuccinimide (NHS), which could condense antibodies reproducibly and densely on the self-assembled monolayer (SAM). The sensor was directly detected by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) in the presence of $Fe(CN)_6^{3-/}Fe(CN)_6^{4-}$ as a redox probe. They found that a linear

relationship between the electron-transfer resistance and the logarithmic value of E. coli concentration was found in the range of E. coli cells from 3.0×103 to 3.0×107 cfumL$^{-1}$ with the detection limit of 1.0×103 cfumL$^{-1}$. With pre-concentration and pre-enrichment steps, it was possible to detect E. coli concentration as low as 50 cfu/mL in river water samples.

In addition, Mouna *et al.* (2008) described the development of an immunological sensor for rabies viral antigen detection. The biosensor is based on the immobilization of specific anti-rabies polyclonal antibodies onto a functionalized gold microelectrode. The different steps of immunosensor conception were characterized by electrochemical impedance spectroscopy (EIS). They found that the Nyquist diagram for the various steps of the development of the sensor increased from SAM layer, antibody layer, blocking with BSA and injection of the antigen respectively. The increase is due to the change of the electric characteristics of the gold/electrolyte interface. Moreover, the sensitivity of the immunosensor will be improved by using interdigitated gold microelectrodes for industrial development. The Latex beads will be replaced by amines or carboxylic modified particles to created covalent binding and the using of monoclonal antibodies will totally eliminate false positive test.

Finally, Chen *et al.* (2005) reported on the application of Faradaic electrochemical impedance spectroscopy method for the detection of yeast cells immobilized on modified gold-SAM electrodes. An electrochemical impedance biosensor for the rapid detection of Saccharomyces cerevisiae (yeast cells) was developed by immobilizing yeast cells on a gold surface modified with an alkanethiolate SAM. The patterns formed on the gold electrode surface after the assembly of 3-mercaptopropionic acid (MPA) monolayer and the immobilization of yeast cells were clearly observed from atomic force microscopy (AFM) and optical microscope, respectively. The electrochemical impedance spectroscopy (EIS) measurements were based on the charge-transfer kinetics of $[Fe(CN)_6]^{3-/4-}$ redox couple. The SAM assembly and the subsequent immobilization of yeast cells on the gold electrodes greatly increased the electron-transfer resistance (Ret) of the redox couple and decreased the double layer capacitance (Cdl).