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Biosensor for detection of dissolved chromium in potable water: A review

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ABSTRACT

The unprecedented deterioration rate of the environmental quality due to rapid urbanization and industrialization causes a severe global health concern to both ecosystem and humanity. Heavy metals are ubiquitous in nature and being used extensively in industrial processes, the exposure to excessive levels could alter the biochemical cycles of living systems. Hence the environmental monitoring through rapid and specific detection of heavy metal contamination in potable water is of paramount importance. Various standard analytical techniques and sensors are used for the detection of heavy metals include spectroscopy and chromatographic methods along with electrochemical, optical waveguide and polymer based sensors. However, the mentioned techniques lack the point of care application as it demands huge capital cost as well as the attention of expert personnel for sample preparation and operation. Recent advancements in the synergetic interaction among biotechnology and microelectronics have advocated the biosensor technology for a wide array of applications due to its characteristic features of sensitivity and selectivity. This review paper has outlined the overview of chromium toxicity, conventional analytical techniques along with a particular emphasis on electrochemical based biosensors for chromium detection in potable water. This article emphasized porous silicon as a host material for enzyme immobilization and elaborated the working principle, mechanism, kinetics of an enzyme-based biosensor for chromium detection. The significant characteristics such as pore size, thickness, and porosity make the porous silicon suitable for enzyme entrapment. Further, several schemes on porous silicon-based immobilized enzyme biosensors for the detection of chromium in potable water are proposed.

1. Occurrence, refining and Industrial use of chromium

Chromium (Cr), derived from the Greek word *Chroma* meaning color (Liddell et al., 1940), is the first element of atomic number 24 placed in group VI. Chromium on its various oxidation states such as Cr(0), Cr(II), Cr(III), Cr(IV), Cr(V), and Cr(VI) are useful for the manufacturing of a vast and varied group of chemicals (Richard and Bourg, 1991; Mertz, 1992; Papp and Lipin, 2001). Chromium is one of the most available elements on the earth and ranked 22 based on the availability (Barnhart, 1997). The occurrence of chromium deposits in earth crust depends on the geochemistry of the region. Chromite (iron chromium oxide) and magnesiochromite are two essential native minerals of the chromium and obtained by either open cast or underground mining methods (Greenwood and Earnshaw, 2012). The annual world chromite producing trend has shown in Fig. 1 (USGS, 2016).

South Africa contributes for almost half of the global chromite production, and India covers 14% of total output in the world (USGS, 2016). Sukinda mines valley of Odisha alone produces 98% of the total chromium in India (Fig. 2), and the Valley is recognized as the fourth most polluted place in the world by Black Smith Institute Report (2007) (USGS, 2016; Alok and Shikha, 2011). Two-step roasting and leaching process separate iron from the chromium and the process is as follows in Table 1:

Table 2 shows the percentage wise industrial application of chromium (Dhal et al., 2013). In the steel industry, chromium is used to manufacture stainless steel or to give a polished finish to steel (Report on Carcinogens, 2016). Other applications of chromium include metal finishing or electroplating with chromium, alloying, leather tanning and finishing, textile dyes, and mordants (Avudainayagam et al., 2003). Chromium compounds are used as industrial catalysts magnetic tape, colored glass, paints, and pigments as oxidizing agents, catalysis, ceramic coatings, safety matches, glues, and adhesives, enchant for plastics, wood preservatives, and inhibition of water corrosion (Mohan and Karthikeyan, 1997; Mohan and Pittman, 2006). Due to its widespread industrial applications of chromium, large quantum is being discharged in the form of Cr(VI), chromic acid and other oxidizing products cause numerous problems in

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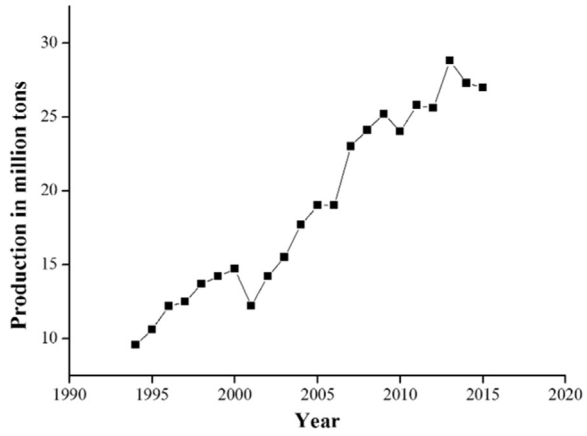

Fig. 1. Global trend of chromite production in million tons (USGS, 2016).

all media such as soil, air, and water (James and Stephen, 2005).

1.1. Effect of chromium on the ecosystem

Chromium pollution occurs in the environment and is found in two major stable oxidation states: Cr(III) and Cr(VI) (Richard and Bourg, 1991; Mertz, 1992). Chromium toxicity is dependent on metal speciation which is determinant for its uptake, translocation, and accumulation (Peralta-Videa et al., 2009). Hexavalent chromium or chromate occurs rarely, and its compounds are oxidizing agents. Cr(VI) is a highly water-soluble and exist as hexavalent chromate ions or chromate (CrO_4^{-2}) oxyanion. CrO_4^{2-} is considered as toxic and excessive presence of chromate ions in the body affect the health of living organisms in various ways. Cr(VI) is 1000 times more toxic at a lower concentration than Cr(III) because of its higher solubility and bioavailability (Zayed and Terry, 2003). Most of the mammals can bear with almost a 100 times more Chromium in the Cr(III) state without any toxic effects, but an excess of Cr(III) may cause an adverse effect (Forstner and Wittmann, 2012).

1.1.1. The biological role of chromium in the human body

Cr(VI) with the principal characteristics of low density, highly toxic and non-biodegradable nature, enters into the alimentary track of living organism through either breathing in dust, fumes or mist or skin contact with solutions or solids or by swallowing it and creates greater health risks. Cr(VI) causes health issues such as irritation in upper respiratory tract, inflammation in the nose, injury in the nasal septum, cancer in respiratory track. Cr(VI) also causes skin problems (allergic skin reactions, skin burn), gastrointestinal problems (chronic ulceration, dermatitis, gastrointestinal ulcer), weaken the immune system, kidney, and liver damage may lead to other carcinogenic effects (Sharma et al., 2012). Table 3 reveals the health hazards caused by Cr(VI) along with the mode of intake and route of exposure. Around 16

Table 1
Two-step roasting and leaching process for refining chromium from its ore.

Step-I Roasting	The iron in the chromite ore forms a stable product ferric oxide (Fe_2O_3) while aerial oxidation of chromite in molten alkali gives sodium chromate (Na_2CrO_4).

$$4\ FeCr_2O_4 + 8\ Na_2CO_3 + 7O_2 \rightarrow 8\ Na_2CrO_4 + 2\ Fe_2O_3 + 8CO_3 \quad (1)$$

Step-II Leaching	Molten sodium chromate reacts with sulfuric acid and water is precipitated reducing sodium chromate to Cr(III) oxide using carbon. Cr(III) oxide is further reduced either by aluminum (known as an alumina-thermic process) or silicon.

$$2\ Na_2CrO_4 + H_2SO_4 \rightarrow Na_2Cr_2O_7 + Na_2SO_4 + H_2O \quad (2)$$

$$Cr_2O_3 + 2Al \rightarrow 2Cr + Al_2O_3 \quad (3)$$

$$2Cr_2O_3 + 3\ Si \rightarrow 4\ Cr + 3SiO \quad (4)$$

Table 2
Use of chromium in various industries (Dhal et al., 2013).

Industry	Application
Metallurgical Industry (90%)	Ferrous alloys (cast iron, steel) Non-ferrous alloys (Al, Cu, Ni)
Refractory and Foundry (5%)	Cement Kiln, Fiberglass Furnace, Glass Tank regenerator, Mag-Chrome refractories
Chemicals (5%)	Chrome plating, Corrosion control, Metal Finishing, Tanning

Table 3
Health hazards caused by Cr(VI) and route of exposure.

Route of exposure	Mode of Intake	Health hazards
Air	Breathing	Respiratory tract cancer, lung cancer, tuberculosis, nasal irritation, nasal ulcer, cough and cold
Water	Drinking and eating	Alimentary tract cancer, stomach cancer, bronchospasm, pneumonia, diarrhoea
Dermal	Skin penetration	Dermatitis, irritation, skin lesions

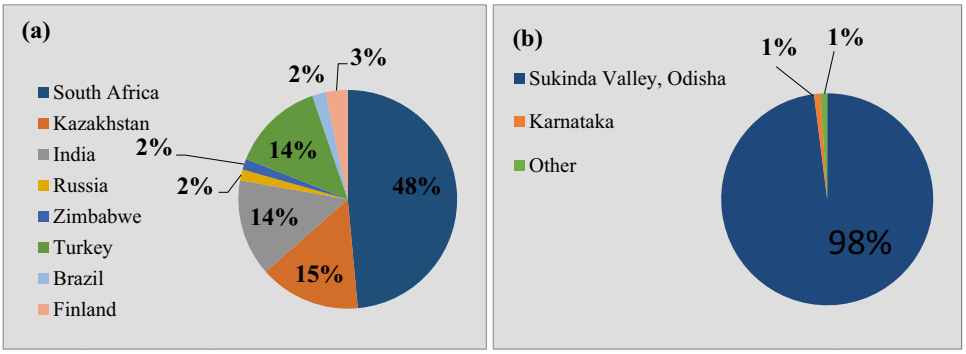

Fig. 2. (a) Country wise chromium production (%) in the year 2014-15 (USGS, 2016), (b) Chromium production (%) in India in the year 2010-11 (Alok and Shikha, 2011).

million people are at risk globally due to contamination caused by chromium and also estimated the number of individuals affected by related diseases is around 3 million DALYs[1] (Fuller, 2015).

The requirement of Cr(III) for adult human being is around 50–200 mg/day and acts as a cofactor for the action of insulin to regulate the sugar levels (Fendorf et al., 2000). Toxicity of Cr(III) is considered relatively low because of its inability to cross cell membranes by the formation of octahedral coordination compounds. However, when in excess, Cr(III) reacts with cellular macromolecules such as deoxyribonucleic acid (DNA) and intracellular reducers, e.g., growth stimulating hormone or cysteine causing genomic instability and cell mutation.

The redox transformation of Cr(III) to Cr(VI) or vice versa (Fig. 3) only take place in the presence of another redox couple, such as H_2O/O_2, Mn(II)/Mn(IV). Due to the low kinetic energy potential of Cr(III), it acts as a weak oxidizer which in turn reacts to form complexes slowly and also the stomach's acidity is sufficient enough to keep it in this state (Valko et al., 2005).

Reactive oxygen species (ROS) produced during reduction of Cr(VI) are responsible for different toxic effects causing health hazards (Smith, 2008). Unlike Cr(III), Cr(VI) is a strong fast reacting oxidizer, which is highly subjected to form complex chromium compounds and also can readily cross cell membranes through the sulfate anion transport system (Fig. 4).

2. Detection methods for chromium in water

The unprecedented release of chromium containing effluents in the environment causes a non-negligible threat to the ecological system. Excessive use of chromium in the industries produces Cr(VI), and natural or induced degradation leads to the increased level of Cr(III). The detection of the two chromium variants in soil and water are essential to implement preventive measures to avoid the ill-effects of Cr(VI) and Cr(III). Access to safe drinking water is a key developmental issue at both global and regional levels. The nature and forms of drinking water standards given chromium concentration vary among countries and regions. World Health Organisation (WHO) recommended a maximum acceptable concentration of Cr(VI) as 0.05 mg/L in drinking water based on health concerns (WHO, 2003).

Among heavy metals, detection of chromium using biosensor attracted much less attention compared to other heavy metals, e.g., Arsenic, Cadmium, Iron, lead. Two possible reasons for little research interest can be (Samborskaa, 2004): 1. Chromium is considered as a "local source" contaminant, thus not constituting a widespread environmental problem 2. The naturally occurring form of chromium is the Cr III considered essentially immobile in the environment whereas Cr VI is highly mobile and is considered acutely toxic. The methods available for the detection of chromium in potable water is categorized into the following sections: (1) Standard or Conventional method (2) Experimental methodology / Lab based methods (3) Sensors and (4) Biosensors (Fig. 5). To date, spectroscopic techniques are broadly used, and experimental / lab based methods are in a nascent stage of very limited use. However, biosensor-based detection methods are emerging due to its selectivity, specificity, and simplicity.

2.1. Standard/conventional method for detection of chromium

The globally acknowledged standard methods for chromium detection are spectroscopic techniques using diphenyl carbazide method, atomic absorption spectroscopy, and ion chromatography (BIS, 2003a), Indian Standard Methods of sampling and test (Physical and Chemical) for water and waste water Part 52 Chromium: IS 3025 (Part 52):2003, 2003).

[1] Disability-adjusted life year, measure of overall disease burden, expressed as the number of years lost due to ill-health, disability of early death.

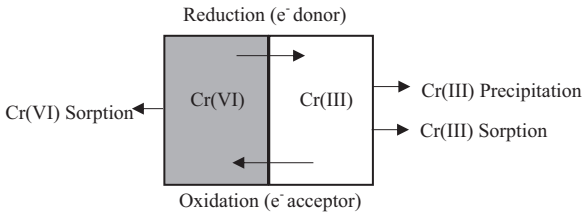

Fig. 3. Conversion of Cr(VI) to Cr(III) by reduction-oxidation.

2.1.1. Diphenyl carbazide colorimetric method

Diphenyl carbazide (DPC), with Cr(VI) in acid solution, produces a red-violet colored solution, based colorimetric method is a standard procedure for the detection of Cr(VI) only. For calibration standard, the photometric analysis of colored reaction product gives the qualitative measurement and quantitation of Cr(VI) with its concentration by linear regression of either peak area or peak height. The generated unknown solution obeys Beer-Lambert law providing spectroscopic measurements at 540 nm. The applicable range of chromium for DPC method is 30–20000 µg/L (BIS, 2003b), Methods of Sampling and Test (Physical and Chemical) for waste and waste water, 2003).

$$\text{Chromium} \xrightarrow[\text{with } K_2MnO_4]{\text{Oxidation}} Cr^{6+} \xrightarrow{\text{Diphenyl carbazide}} \textit{Red-coloured unknown solution}$$

$$\rightarrow \textit{Spectrophotometric detection}$$

A qualitative study shows reaction between chromium in its different oxidation states and pure DPC, diphenyl carbazone (DPCO), and diphenyl carbadiazone. A magenta colored complex from the reaction of Cr(III)/Cr(II) with DPCO and Cr(VI) with DPC (Willem et al., 1977). The unique properties of DPC were utilized to fabricate a carbon paste electrode to improve the analytical determination of Cr(VI) and Cr(III) by using voltammetric techniques (Paniagua et al., 1995). The application of DPC technique limited the testing of drinking water that requires high detection sensitivity (< 1 µg/L) and short testing time. However, the limitation of sensitive nature of DPC method is up to 20 µg/L (Marczenko, 1976).

2.1.2. Atomic absorption spectroscopy

Atomic absorption spectroscopy (AAS) is a quantitative spectro-analytical procedure for the determination of chemical elements using the absorption of optical radiation by free atoms in the gaseous state (Welz and Sperling, 2008). AAS analyses the concentration of the specific metal constituent in a sample. The rapid and sensitive methods are useful for species-selective determination of Cr(III) and Cr(VI) in water (Michel et al., 1992). Feldman and Purdy evaluated the variables such as solution matrix, flame composition, and extraction procedures for chromium analysis with a detection limit of 0.006 mg/L (Fredric and William, 1965). A graphite furnace AAS were demonstrated for chromium determination in food samples against aqueous standards by quantifying the peak area in low ionic strength water (Miller-lhli, 1996). Based on co-precipitation with Cerium VI hydroxide, the total chromium with a detection limit of 0.18 µg/L was achieved by quantifying the absorption signal (Umit et al., 2008). Sperling et al. (1992) revealed the speciation of Cr(III) and Cr(VI) in water using flow injection on-line preconcentration coupled with electrothermal AAS (ET-AAS) using sodium diethyldithiocarbamate as the complexing agent. A recent study combined the ET-AAS with electro membrane extraction for the speciation, preconcentration, and quantification of Cr(VI) and Cr(III) for achieving higher detection limit. The analysis was performed by selective complexation of Cr(VI) with 1,5-diphenylcarbazide (DPC) as a complexing agent, and the detection limit was 0.02 ng/L (Tahmasebi and Davarani, 2016).

2.1.3. Ion chromatography

Ion chromatography (IC) is a process to separate ions or polar

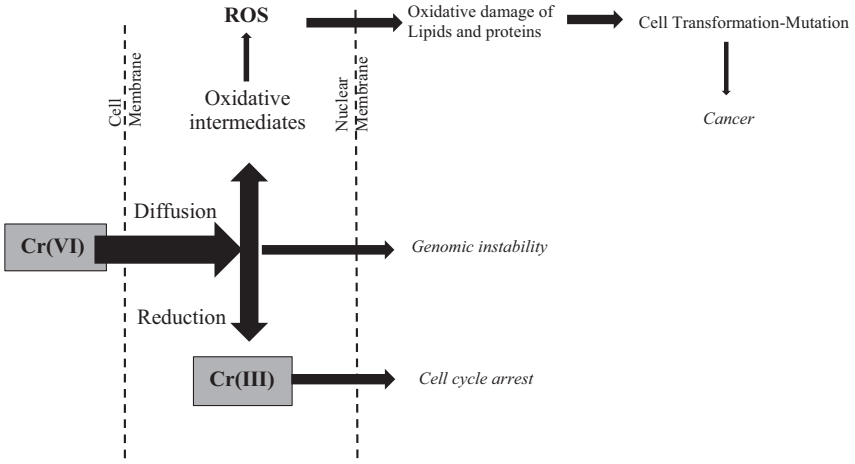

Fig. 4. Biological role of chromium causing cancer.

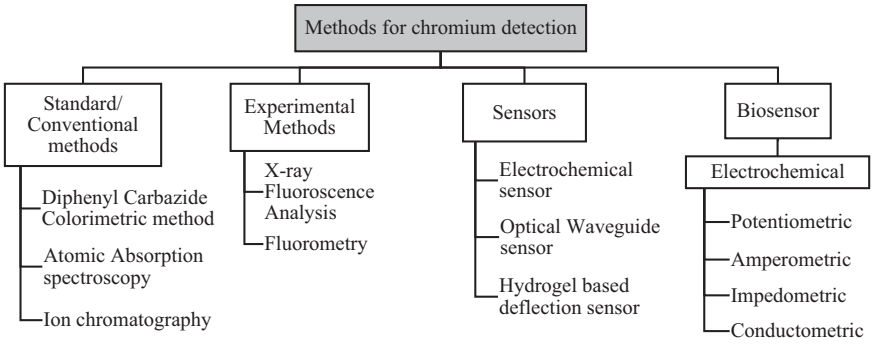

Fig. 5. Classification of methods for chromium detection.

molecules based on their affinity to the ion exchanger. The water soluble charged molecules bind to oppositely charged moieties by forming covalent bonds to the insoluble stationary phase (Luqman and Inamuddin, 2012). IC is useful for the determination of Cr(VI), and possible interferences include iron, copper, nickel, vanadium. Ten μM of any of these interference causes an absorbance of equivalent to approximately 0.02 μM Cr(VI) due to the formation of colored complexes. However, interference due to reducing agents, such as Fe, Fe^{2+} could be minimized by alkaline extraction. A study shows the determination of Cr(VI), and Cr(III) by IC using Pyridine-2,6-dicarboxylic acid (PDCA) based eluent system (Thunyarat et al., 2012). Jin et al. (2016) demonstrated the use of low-pressure ion chromatography with inductively coupled plasma-mass spectrometry (LPIC-ICP-MS) for the detection of dissolved Cr(III) and Cr(VI) in water with the detection limit of 3.5 and 9.8 ng/L. The quaternary ammonium groups present in low-pressure anion exchange column separate the Cr(III) from Cr(VI) (Jin et al., 2016).

2.2. Experimental or lab based non-standard methodology for chromium detection

The standard or conventional approaches are the right choices of a method for the qualitative and quantitative determination of chromium. However, the discussed methods are time-consuming, labor-intensive, and requires a special setup for every analysis. Non-standard methods are a collection of experiments which uses manipulation and controlled testing to understand or to determine the unknown factor. The experimental method for chromium detection includes fluorescence analysis and fluorimetry.

X-ray fluorescence spectrometry is a non-destructive elemental analysis technique where the working principle depends on the emission of characteristic secondary X-ray from the material when excited by an external energy source having energy greater than the ionization potential (Van et al., 2001; Beckhoff et al., 2007). The X-ray fluorescence analysis is useful for chromium detection because of non-destructive screening, fast and accurate results, ease of operation and portability. However, the method could not differentiate between Cr(VI), Cr(III), and Cr(0). Currently, there is a huge demand and necessity for chemical sensors (discussed in Section 2.3) that could distinguish Cr species in the field of environmental and biochemical application, as most of the standard methods determine the total Cr concentration rather than its oxidation state. Fluorimetry is another useful technique for the detection of heavy metals based on the phenomenon of fluorescence. A study shows detection method of chromium using glutathione-capped Cadmium telluride quantum dots as fluorescent probes (Zhang et al., 2009). The selectivity of quantum dots for the heavy metal ions depends on the size of the quantum dots.

Conventional and non-conventional lab-based techniques including simple spectroscopic analysis using DPC method, AAS, ICP-MS are practically used and commercially available due to the beneficial properties of reliable and accurate analytical data with sensitivity and selectivity. However, these techniques have several disadvantages such as necessitate of sophisticated high-end instruments, skilled operators, non-functional outside the analytical laboratory, dedicated sample collection, and other associated pre-treatment for routine analysis. At this juncture, sensors are an attractive and promising choice to conventional methods due to simple, cost-effective, portable, real-time as well as online monitoring capability, relatively less detection time after few complex instrumentation strategies.

2.3. Detection of chromium in water using sensors

The non-standard techniques (as discussed in Section 2.2) employed for the detection of chromium have limitations regarding the

cost in comparison with routine analysis, tedious sample preparation and time-consuming (Zamani et al., 2009). The experimental/non-standard lab-based methods are useful techniques for the determination of metal ions, but the methods are applicable only for elemental analysis. Speciation of different oxidation states is also a challenge for lab-based methods. To overcome the issues mentioned above, various chemical sensors are used for the detection and speciation of chromium dissolved in water. Additionally, chemical sensors have numerous benefits such as simple instrumentation, faster response, ease of sample preparation, cost effective along with wide dynamic range, excellent selectivity, and non-destructive analysis (Gupta et al., 2006).

2.3.1. Electrochemical sensor

The basic principle of an electrochemical sensor relies on the electrocatalytic reaction between electrodes with analyte present in the test sample. Electrochemically cleaned gold electrode (immobilized with methylene blue) is useful for the detection of chromium. Methylene blue (MB) is then reduced at the electrode to form leucomethylene blue (LMB). The electrochemically generated LMB reacts with Cr(VI) to form Cr(III) and reduces to methylene blue as shown in Eqs. 5 and 6. (Korshoj et al., 2015; Kamburova, 1993; Lee and Choi, 2005).

$$MB + 2H^+ + 2e^- \rightarrow LMB \quad (5)$$

$$3LMB + 2Cr(VI) \rightarrow 3MB + 2Cr(III) \quad (6)$$

The use of surface immobilized MB enables the sensor to attain excellent specificity, selectivity, and reusability without any bio-recognition element (Wu and Lai, 2014; Yu and Lai, 2013). The sensitivity and specificity of the MB-based electrochemical sensor are suitable for Cr(VI) without the interferences of Cr(III) (Korshoj et al., 2015).

A recent study by Salimi et al. (2015) shows a novel electrochemical sensor for detection of Cr(III) using nanocomposite containing chitosan and multi-walled carbon nanotube (MWCNTs) as a platform for immobilization of electrodeposited nanostructured manganese oxide (MnOx). MnOx electrodeposited onto chitosan/MWCNTs modified glassy carbon electrode using a combination of constant potential step (0.6 V) and cyclic voltammetry (0.3–0.6 V) techniques. The modified electrode showed excellent electrocatalytic activity towards oxidation of Cr(III) at natural pH solutions. Detection limit, sensitivity and linear concentration range of the sensor were 0.3 μM, 18.7 nA/μM and 3–200 μM, respectively (Salimi et al., 2015).

2.3.2. Optical waveguide sensor

An optical waveguide is a physical structure, includes an optical fiber or rectangular waveguides, guides electromagnetic waves in the optical spectrum (Fluitman and Popma, 1986). Intrinsic distributed sensors are used for monitoring a single measurand at a large number of points or continuously over the path of the fiber. The fiber acts as a conduit to transmit light from the sensing region and sensing take place outside the fiber. The physical property of the fiber undergoes changes with external sensors.

Raquel et al. (2007) show selective and sensitive detection of Cr(VI) using optical membrane sensor (Raquel et al., 2007). The response of the optical film for different anions (usually present in natural waters at high concentrations, e.g., Cl^-, SO_4^{2-} and NO_3^-) gives the selectivity of the optode membrane towards Cr(VI). The proposed sensor shows an optical response in the range of 1.1×10-5 – 1.0×10–3 M. Yang et al. (2013) uses a hollow-core metal-cladding optical waveguide sensor for detection of Cr(VI) (Yang et al., 2013). A change in extinction coefficients in the waveguiding layer due to the reaction of chromate ions with diphenyl carbazide leads to a significant shift in light intensity in the reflection spectrum. This method proposes a more sensitive detection procedure than surface plasmon field-enhanced resonance light scattering method, fluorimetry spectroscopy or flame absorption spectrometry. Hydrogel coated Fiber Bragg grating sensor incorporated with tetraalkylammonium salts for chemo-mechanical-optical sensing approach which is sensitive to Cr(VI) ions. The mechanical strain experienced by various concentrations of Cr(VI) shifts the 'Fiber Bragg grating peak.' The sensor can estimate Cr(VI) of 10 μg/L and the resolution of the sensor system is found to be 0.83 μg/L (Kishore et al., 2017).

2.3.3. Polymer-based electrochemical sensor

Susan and Aziz (2013) reported a screen-printed carbon electrode (SPCE) modified with quercetin (QH2) to detect Cr(VI) in the water. Quercetin forms complex with Cr(VI) due to the presence of carbonyl and hydroxyl groups in its structure (as shown in Eq. (7)), which could not produce a readily electroactive complex with the hydrated Cr(III) (Susan and Aziz, 2013).

$$[QH_2]_{surf} + Cr(VI)_{sol} + Cl^- \rightarrow [Q - Cr(III) - Cl]_{surf} + 2H^+ \quad (7)$$

SPCEs are useful for the detection of pollutants because of its advantages including reproducibility, reliability, and low cost. A selective SPCE was modified by multi-walled carbon nanotube (MWCNT), and the measurement was done based on the electro-reduction of Q-Cr(III)/Q-Cr(II) (Bobrowski and Zar, 2000). Eq. (8) shows the reduction reaction of Q-Cr(III) producing Q-Cr(II).

$$[Q - Cr(III) - Cl]_{surf} + e^- \rightarrow [Q - Cr(II) - Cl] \quad (8)$$

The MWCNTs were used to modify the surface of bare SPCE and acts as a promoter to enhance the electrochemical reaction after functionalized with –COOH groups (Bobrowski and Zar, 2000). The interaction of Cr(VI) with quercetin provides the pre-concentration of Cr(VI) on the QH_2/MWCNT-SPCE surface.

2.3.4. Hydrogel-based deflection sensor

A cantilever is a rigid structure with one end fixed to the firm support, and another end free (Thundat et al., 1997). Hydrogel-based microcantilever uses the principle of structural change to measure the quantity under measurement (Yifei et al., 2003). The deflection of microcantilever (with concentration of CrO_4^{2-}) is linear over the concentration of 10^{-11} M. The microcantilever undergoes bending in presence of CrO_4^{2-}due to the shrinkage of hydrogels, resulted by molecular adsorption or binding-induced change in surface tension (as shown in Fig. 6). The chemical reactions involved in the process are shown in Eqs. (9) and (10).

$$HCrO_4^- \rightarrow CrO_4^{2-} + H^+ \quad (9)$$

$$2CrO_4^- \leftrightarrow Cr_2O_7^{2-} + H_2O \quad (10)$$

Yifei et al. (2003) used a commercially available V-shaped silicon microcantilever coated with a layer of copolymerized gel ATAC [(3-acrylamidopropyl) trimethylammonium chloride] for the detection of Cr in water. The deflection measurements detect the bending of the cantilever by observing the position of the laser beam reflected from cantilever onto a four-quadrant photodiode (Yifei et al., 2003).

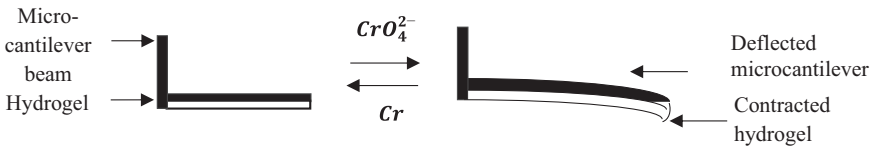

Fig. 6. Hydrogel based microcantilever sensor.

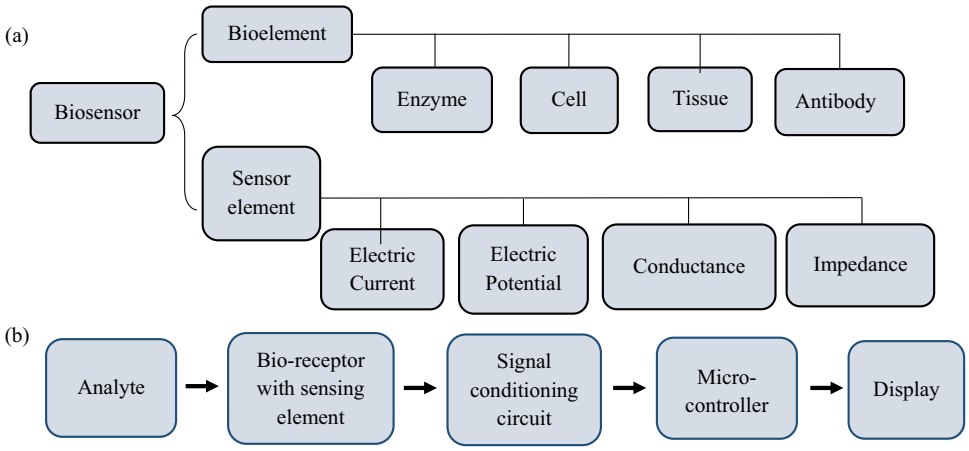

Fig. 7. (a) Classification of biosensor based on bioelement used and the property sensed (b) Working principle of biosensor.

Although sensors are developed given minimizing the cost factor and extend its applications to real-time situations, the net quantum of heavy metal is not always directly linked to its toxicity. The ionic form of the metal, their physiological state, and bioavailability dictates its toxicity demand the need for determining the speciation of metal in its real-time environmental conditions. Hence, attempts are made to develop a biosensor to increase the selectivity, sensitivity, and specificity by having a biological recognition element such as enzymes, microbes or other in the conventional sensor to achieve a rapid, on-site measurement due to the biological role associated with the target analyte. Indeed, the biosensors could offer similar sensitivity and accuracy as the conventional sensors but with much higher detection limit.

2.4. Electrochemical biosensor for chromium detection

The biosensor is an analytical device that uses biomolecular interactions on its surface for the detection of any biochemical change and rejects unintended nonspecific signal (Mohanty and Kougianos, 2006). The biosensor (Fig. 7(a)) is a combination of two parts- (i) bioelement and (ii) sensor element. The bioelement may be an enzyme, living cells, tissue or antibody, and the sensor element may be electric potential, electric current, electric conductance or impedance. The bioelement is responsible for the detection of a specific analyte, and the sensor element executes the transduction of biochemical change into another form of the signal (Mohanty and Kougianos, 2006). The sensing element is coupled with the bioreceptor to transform the generated signal for the interaction between analyte and bioelements (Fig. 7(b)) (Borgmann et al., 2011). A signal conditioning circuit is added to enhance the transducer output and then displayed using any microcontroller. There are four different categories of biosensor based on the type of biological signaling mechanism and their mechanism of transduction.

Electrochemical biosensors are well-commercialized biosensors (Mohanty and Kougianos, 2006), working based on the principle of a chemical reaction between target analyte with the immobilized biomolecule that affects the electrical properties of the solution (Borgmann et al., 2011). The biochemical signals are used to generate a current/voltage signal resulting in a quantitative measure of the analyte present in the sample. There are four types of transduction possible under electrochemical categories: potentiometric, amperometric, conductometric and impedimetric. The major advantages of the electrochemical biosensors are compatibility with modern macro-fabrication technologies, minimal power requirements, economic costs, and independence of sample turbidity and color (Mohanty and Kougianos, 2006).

2.4.1. Potentiometric biosensor

Potentiometric biosensors use the gas sensing electrode, ion selective electrode, and ion selective field effect transistors to transmit the biological reaction into an electrical signal (Kost et al., 1995). The potentiometric biosensors, in its simplest term, consist of immobilized enzymes. The accumulation of ions at the ion selective membrane interface results in the form of the output signal. The current flowing through the electrodes is nearly zero, and then the potential difference is measured between a working electrode and a second reference electrode. There is a direct logarithmic relationship between the charge generated at the electrode and the activity of ions of interest. Nernst equation (refer Eq. (11)) shows the relation between the potential and the concentration of a particular analyte in the solution as follows:

$$E_{cell} = E^{0}_{cell} - \frac{RT}{nF}\ln Q \tag{11}$$

Here, E_{cell} represents the observed cell potential at zero current. E^{0}_{cell} is a constant potential contribution to the cell, R is the universal gas constant and T is the absolute temperature in kelvin, n represents the number of charge transferred during electrode reaction. F is Faraday constant and Q is the ratio of concentration of anode to the ion concentration at the cathode. The analyte is not being consumed during the electrochemical process and the type of measurement is non-destructive.

To investigate the presence of chromium, many chemical and biological materials are used to modify the working electrodes of electrochemical biosensors. Microbial fuel cell (MFC)[2] is one of the bioelectrochemical compounds of interest. MFCs have four unique features as they do not require an external enzymes/pure microorganism to be loaded, the prompt response of anaerobic electrogenic bacteria and rapid signal change, no requirement of the external power source as the MFCs generate electricity from wastewater, and the electrogenic bacteria are expected to have different types of responses to various shocks. Bingchuan et al. (2014) explored novel self-sustained biosensor using a cube microbial fuel cell (CMFC) for real-time chromium toxicity monitoring of wastewater (Bingchuan et al., 2014). The study uses a single chamber CMFC of a 4-layer polytetrafluoroethylene (PTFE) treated carbon cloth facing air and platinum-coated side facing water in the CMFC. The Platinum works as the cathodic catalyst for oxygen reduction reaction, and the anode and cathode are connected using a copper wire with an external resistance. The CMFC effectively gave distinct sharp voltage drop within 30 min for 8 mg/L concentration of Cr(VI). The CMFC biosensor was able to

[2] Bio-electrochemical device that harnesses the power of respiring microbes to convert organic substances directly into electrical energy. Sensors are developed by compacting two filter membranes.

distinguish the shock of toxins from non-toxins based on a change in voltage signal, which is predominantly due to the activity of the electrogenic bacteria on the anode surface. The suggested sensor recovered its sensing function after 65 h (recovery time) of start-up (anodic bacteria acclimation) time with feeding waste water. Zhiheng et al. (2015) have proposed another study, to minimize the recovery time using membrane-based MFC with carbon ink coated two filter membranes for the detection of chromium. The electrode offered high sensitivity, high stability, high microporosity, the hydrophilicity of membranes and simple, compact configuration at micron range (Zhiheng et al., 2015). The high microporosity and hydrophilicity of membranes suggest the distinct advantages of short acclimation period and hydrophilic microporous membranes give a simplified operation avoiding long time incubation for the MFC sensors.

Chung et al. (2016) used two chamber MFCs instead of one chamber MFC. However, the detection is restricted to acidic water of pH 1–2 with the concentration ranges from 0.1 to 15 mg/L. The suggested method was applicable in the co-presence of Fe(II) up to Cr(VI)/Fe(II) molar ratio 1:15. The generated voltage was used to specify the change in Cr(VI) concentration of the cathode under changing conditions of ionic strength, pH, co-existing Fe(II) concentration, and organic matter concentration at pH 1 and 2, but not at upper pH conditions. The voltage varies with a stepwise upsurge in the Cr(VI) concentration at different pH conditions and the rising voltage generation with increased Cr(VI) levels is followed by the Nernst equation. An increase in Cr(VI) concentration leads to a higher cathode potential, and hence, higher voltage and power generation in an MFC (Chung et al., 2016). To overcome the limitation of pH range, Wang et al. (2016) proposes a method using MFC-based biosensor inoculated with a facultatively anaerobic, exoelectrogenic, and Cr(VI)-reducing *Ochrobactrum anthropi* YC152 giving a high adaptability to temperature, pH, salinity, and water quality under anaerobic conditions for pH ranges 5–8 with detection time of 45 min. The MFC voltage decreases as the Cr(VI) concentration in the MFC increased, and for various Cr(VI) concentration, the voltage output ranges 0.0125–0.3 mg/L and 0.3–5 mg/L (Wang et al., 2016).

MFCs could be useful shock sensors for wastewater qualities as the voltage/power output of MFCs are directly dependent on the metabolic activities of the anaerobic electrogenic bacteria. The single-chamber batch-mode CMFC was used for real-time monitoring the toxicity shocks but the recovery time was too long (Bingchuan et al., 2014). Later two-chamber MFCs have utilized for the Cr(VI) detection, but the usage was limited only to the acidic medium of pH 1–2 (Chung et al., 2016).

2.4.2. Amperometric biosensor

The amperometric biosensor is a device in which current change determines the analyte concentration on the application of a constant potential between a working electrode and a reference electrode (Borgmann et al., 2011). The oxidation or reduction of an electroactive material at the electrode surface results in current flow between the electrodes. The magnitude of the current signal depends on the mass transfer of electroactive species to the electrode surface.

Electrochemical biosensors with interfaces such as microorganisms, microspheres, nanomaterials, and enzymes were reported to detect the dissolved chromium in potable water. Among these, enzymes are considered to be most promising due to fast response, high stability, and biocompatibility. Caroline et al. (2003) developed an amperometric biosensor for chromium detection by using the chromate reductase activity of cytochrome c_3 isolated from a sulfate-reducing bacterium (Caroline et al., 2003). There are four methods for the immobilization of Cyt c_3, such as entrapment with a dialysis membrane, adsorption, entrapment with cellulose nitrate and entrapment in poly 3,4-ethylenedioxythiophene films by electropolymerization. This biosensor uses the principle of metal reduction by oxidation of the electrochemically regenerated immobilized cytochrome, and the signal comes from the cytochrome regeneration current at an applied reduction potential. The advantages of Cyt c_3 immobilized biosensor are rapid quantification, sensitivity ranges 35 nA/mg (1.82 nA/μM) and detection ranges between 0.20–6.84 mg/L. However, interference with other metals or metalloids are not known, and enzyme activity reduces at high Cr(VI) concentrations. Aiken et al. (2003) demonstrated the use of nitrate reductase for the detection of heavy metal using enzyme inhibition assays. The activity of nitrate reductase as a function of metal concentration, such as Cd(II), Cu(II), Ni(II), Pb(II), Zn(II), Cr(III), and Cr(VI) was assayed, and the approach is also useful for individually quantifying Cr(III) and Cr(VI) (Aiken et al., 2003). Dominguez et al. (2004) immobilized the tyrosinase on polypyrrole film based biosensor for selective amperometric determination of Cr(III) through enzyme inhibition kinetics (Domınguez et al., 2004).

A bacterial sensor was developed based on the oxidizing ability of acidophilic bacterial strain *Acidithiobacillus ferooxidans* (*A. ferrooxidans*)[3] of ferrous and thiosulphate ions with oxygen probe as a transducer (Roumen et al., 2006). However, the biosensor response is influenced by the nature of biomass support, O_2 diffusion coefficient, a technique for biomass immobilization and utilization of the mediator. Using a cellulose filter with bacterial biomass fixed by filtration gives high sensitivity (816 μA L/mol) and good response time (51 s for 2.0×10−5 mol/L Cr_2O_7). Caroline et al. (2006) demonstrated chromium quantification in groundwater with the enzyme-based amperometric biosensor where cytochrome c_3 (*M*r 13,000) from *D. norvegicum* (DSM 1741) were evaluated for the influences of pH, temperature, and ionic strength. The working glassy carbon electrode (GCE) holds the cytochrome c_3 through entrapment with a dialysis membrane (Caroline et al., 2006). The pH of groundwater usually ranges from 6.8 to 8.5. The temperature increases with a sensitivity of the biosensor (Bianco et al., 1986). Ionic strength alters the sensitivity of biosensor due to the electrostatic interaction between Cr(VI) and cytochrome c_3 (Assfalg et al., 2002). Also, sensitivity decreases with increasing ionic strength in the 0–0.1 M range. However, sensitivity was stable for values higher than 0.1 M (Lojou et al., 1998). The lowest detection limit of Cr(VI) with Cyt c_3-based biosensor was 0.2 mg/L, which is greater than the regulatory standard (0.05 mg/L) of Cr(VI) in drinking water.

Nepomuscene et al. (2007) proposed a sol-gel immobilization sensor to detect chromium in water based on the enzyme inhibition activity and evaluated the effect of free and immobilized enzyme on biosensor activity (Fig. 8). The biorecognition element, crude urease (50 μl of activity of 32.4 units per mg) obtained from *Dolichos uniforms* were immobilized on non-woven cellulose using a swab. The maximum velocity (V_m) decreased from 10.62 mM/min for free urease to 3.28×10−3 mM/min for immobilized urease, whereas the change in saturation constant (K_m) was from 20.44 mM to 22.049 mM.

The stability of the enzyme in an immobilized state is determined by measuring the activity of the same biosensor up to several times at different test conditions. For chromium concentration of up to 50 mg/L, a change in production current observed was around 200 μA. Restoration of immobilized urease activity after inhibition by chromium ions was done by soaking the sensor chip in a buffer solution containing ethylene diamine tetraacetic acid (EDTA). The kinetic parameters were also estimated, and the activity along with the stability of the biosensor remained same upon using the strip for six times and four days. The modified sol-gel immobilized biosensor is a reliable means of chromium ions determination in liquid samples, especially in laboratories (Nepomuscene et al., 2007). Maria et al. (2008) developed glucose oxidase (GOx) based amperometric biosensor for the detection of chromium by immobilizing GOx in electro synthesized poly-o-

[3] Gram negative rod shaped chemolithoautotrophic bacterium which can use many different electron donors to support growth. In aerobic conditions, electron donors may include ferrous ions or sulfur compounds which are oxidized into ferric iron and sulfuric acid, respectively, yielding high energy.

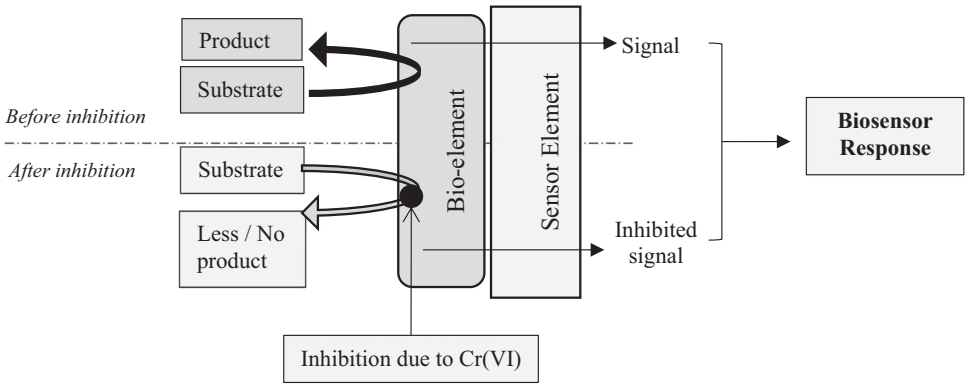

Fig. 8. Working principle of enzyme inhibition-based biosensor.

phenylenediamine. The proposed biosensor also able to distinguish two different oxidation states of chromium by rejecting Cr(III) and detecting toxic Cr(VI) (Maria et al., 2008). The study represents the first example of the decomposition of hydrogen peroxide by heavy metal ions to estimate the interferences on an enzymatic inhibition biosensor. The performance of GOx immobilized biosensor about sensitivity, detection limit, linear range and standard deviation makes it suitable for quantitative detection of chromium.

Ana et al. (2014) estimated the Cr(III) and Cr(VI) concentration using chronoamperometric assays. The study uses tyrosinase (EC 1.14.18.1, from mushroom, 3130 units per mg) and GOx (EC 1.1.3.4, type X-S: from *Aspergillus niger*, 129.9 units per mg) for identification of Cr(III) and Cr(VI) separately (Ana et al., 2014). Tyrosinase was immobilized on screen-printed carbon electrodes (SPCEs) by cross-linking with electron transfer mediator tetrathiafulvalene (TTF) (Fig. 9(a)). The inhibition of tyrosinase/SPCE response to pyrocatechol is noted for the detection of Cr(III). However, the same process is not being affected by Cr(VI) allowed the monitoring of Cr(III) with a detection capability of 2.0 ± 0.2 mM and a reproducibility factor of 5.5%. Moreover, for Cr(VI) determination, GOx modified SPCEs were fixed using ferricyanide as a redox mediator (Fig. 9(b)). The chronoamperometric response of the biosensor towards glucose decreases in the presence of Cr(VI) with a detection limit of 90.5 ± 7.6 nM and a reproducibility factor of 6.2%. The advanced sensor based on electrochemical techniques incorporates the simplest procedure for speciation of Cr(III) and Cr(VI) along with the utilization of redox mediators to exhibit high-level selectivity towards different metals at relatively low cost.

Horseradish peroxidase (HRP, E.C. 1.11.1.7, 500 units per mg) was used by Aisha et al. (2014) for the chromium detection with hydrogen peroxide in amperometric enzyme inhibition biosensor. HRP was immobilized on the surface of carbon film electrodes (CFE) directly or on the electropolymerized neutral red crosslinking with glutaraldehyde (GA) along with bovine serum albumin (BSA) as shown in Fig. 10. The amount of immobilized enzyme was varied to get the best response towards hydrogen peroxide, and the detection limit ranges 0.4 and 2.5 μM respectively for Cr(III) and Cr(VI). This study first reported the utilization of HRP for Cr(III) and Cr(VI) detection through chromium inhibition effect on the activity of the enzyme. The inhibition constants

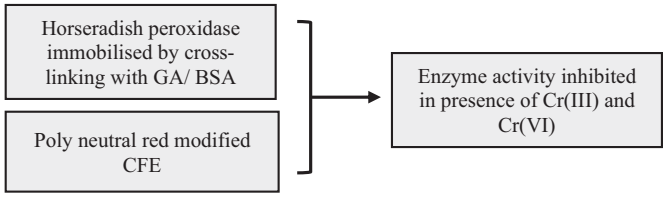

Fig. 10. Enzymatic response of horseradish peroxidase for the detection of Cr(III) and Cr(VI).

were determined from Dixon plots, and Cornish-Bowden plots and the inhibition mechanisms were found to be mixed inhibition for Cr(III) and uncompetitive inhibition for Cr(VI) (Aisha et al., 2014).

The Section 2.4.2 shows different approaches for an amperometric biosensor for the detection of Cr(VI) and speciation of Cr(III) and Cr(VI). The enzyme immobilized amperometric biosensors work on two basic principles, direct measurement method and indirect enzyme inhibition method (as shown in Fig. 11(i)).

The direct method analysis involves enzyme cytochrome c_3 which shows a linear increase of current with chromium concentration (Caroline et al., 2003) whereas the indirect enzyme inhibition method using glucose oxidase shows an exponential decay of current with chromium concentration (Maria et al., 2008). The changes of current with time for different biorecognition elements such as cytochrome c_3, *A. ferroxidans*, glucose oxidase and urease gave detection limit and sensitivity were reported in Table 4. The amperometric biosensor using urease gives the highest detection limit among the compared studies (as the range varies from 10×10^3–50×10^3 μmol/L).

2.4.3. Conductometric biosensor

Compared to potentiometric and amperometric biosensors, the conductometric biosensors are created on the fact that most of the enzymatic reactions include either consumption or production of charged species leading to an alteration in the ionic composition of the tested sample (Jaffrezic-Renault and Dzyadevych, 2008). The conductometric biosensor uses two noble-metal electrodes immersed in the solution, and the conductance is measured. Enzymatic reactions are used to convert neutral substrates into charged products to change the conductance of the medium. Although these transducers are not in

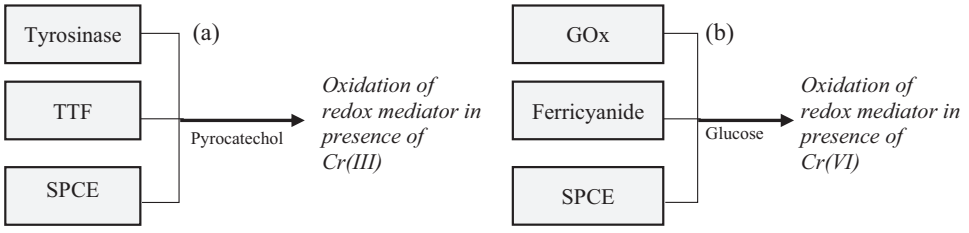

Fig. 9. Enzymatic mechanism of Tyrosinase/ SPCEs and GOX/SPCEs biosensor for detection of (a) Cr(III), (b) Cr (VI).

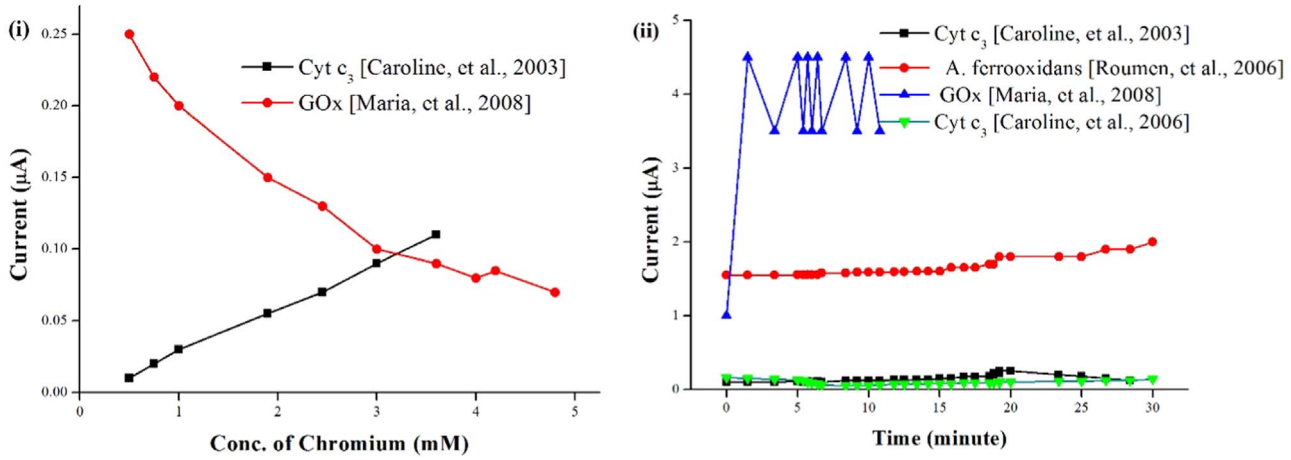

Fig. 11. (i) Current vs. Conc. of Chromium (mM) plot for direct method using cytochrome c_3 (Caroline et al., 2003), and Enzyme inhibited indirect method using glucose oxidase (Maria et al., 2008) (ii) Current vs. Time plot for amperometric enzyme based biosensor using different enzymes at different test conditions using cytochrome c_3 (Cyt c_3), A. ferrooxidans and glucose oxidase (GOx) (Caroline et al., 2003, 2006; Roumen et al., 2006; Maria et al., 2008).

Table 4
Comparative study of amperometric biosensor for Cr(VI) detection.

No.	Sensing element	Detection limit (μmol/L)	Sensitivity limit (μA L/mol)	References
1	Urease	10×10^3–50×10^3	–	(Nepomuscene et al., 2007)
2	*Acidithiobacillus ferrooxidans*	0–400	816	(Roumen et al., 2006)
3	Glucose oxidase	48–4800	–	(Maria et al., 2008)
4	Cytochrome c_3	0–200	Sensitivity inversely varies with ionic strength 0–0.1 M	(Caroline et al., 2003)

widespread use, the technique is routinely used to measure the salinity of marine environments. The conductometric biosensors are chosen over other types of electrochemical biosensors as the thin-film electrodes without the requirement of reference electrode resulted in size miniaturization, large-scale application at cost effective technology. As the transducers are not light sensitive, the low driving voltage can decrease the overall power consumption of the biosensor. However, a broad spectrum of compounds can be determined by different reactions and mechanisms based on the chosen biorecognition element (Jaffrezic-Renault and Dzyadevych, 2008). Enzyme immobilized ensemble gold electrodes conductometric biosensor were proposed for detection of heavy metal ions (Zhylyak et al., 1995). The study showed membrane formation by crosslinking the urease (EC 3.5.1.5, type B, activity 12 units per mg) with BSA in saturated glutaraldehyde vapor on the transducer surface. Positive result observed for the metal ions including Hg(II), Cu(II), Cd(II), Co(II), Pb(II), and Sr(II) respectively as per their toxicity towards urease, although no significant results were found for Cr(VI). The calibration curves for the analysis of heavy metal concentration were obtained for both free and immobilized urease. The benefits for immobilized enzyme over free enzyme such as the minimal requirement of the enzyme ($< 10^3$ times), minimizing the possible interferences in the non-specific output signal, easier reactivation of enzyme, rapid analytical response and low acclimation time.

Apart from other conventional chemical analysis, such as atomic absorption spectroscopy or, chromatography, bioassays[4] are one of the most suitable technology for the detection of toxicity monitoring (Oh et al., 2011). Bioassays are helpful in monitoring the physiological response of living organisms such as fish, daphnia, algae, plant tissue, animal cells, and several bacterial species (Palma et al., 2009). Microbial bioassays are preferred over other methods due to ease in detection, reproducibility, and cost-effectiveness. Microbial bioassays based on aerobic bacteria, bioluminescence bacteria, nitrifying bacteria, iron oxidizing bacteria, sulfur oxidizing bacteria, and algae were reported. Each of them utilizes some specific inherent property of the microorganisms such as growth rate, biochemical properties, respiration, amperometry, bioluminescence, and conductivity. A biosensor established on the metabolic properties of sulfur-oxidizing bacteria (SOB) to oxidize elemental sulfur to sulfuric acid under aerobic conditions resulting in an increase in electrical conductivity (EC) and decrease in pH (Gurung et al., 2012). The chemoautotrophic bacteria use reduced sulfur compounds as an energy source to produce sulfuric acid. Under aerobic condition, these SOBs use aerobic elemental sulfur (S^0) particles and O_2 as an electron donor and an electron acceptor, respectively, as shown in Eq. (13).

$$S_0 + H_2O + 1.5O_2 \rightarrow SO_4^{-2} + 2H + , \Delta G0' = -587kJ/reaction \quad (13)$$

SOB oxidize S^0 to sulfate (SO_4^{2-}) and protons (H^+) causing a decrease in pH and increase in EC. The presence of toxic chemicals in dissolved water inhibits the activity of SOB to stop the oxidation of sulfur to sulfate. As a result, pH changes from acidic to neutral led to monitoring the toxicity in semi-continuous mode and detection time is on the order of minutes to hours. Similarly, Hassan et al. (2012) used the SOB inhibition property to evaluate the effect of different physical factors such as hydraulic retention time, the particle size of S^0, and the temperature on biosensor activity. The biosensor sensitivity was improved by decreasing the hydraulic retention time from 30 to 10 min and increasing S^0 particle size from 1 to 4.75 mm. The SOB sensor was active over a broad range of temperature and for higher temperature, the detection time was shorter providing maximum growth at temperature 45°C for a detection limit of 50 μg/L (Hassan et al., 2012).

The effect of Cr(VI) contamination was studied using the same inhibition principle for thiosulfate-oxidizing bacteria (TOB) by determining EC, pH and sulfate production based on thiosulfate oxidation (Qambrani et al., 2014). TOB utilize thiosulfate as an electron donor producing sulfate by decreasing pH along with an increase in EC could detect less than 100 μg/L of Cr(VI) concentration in dissolved water.

[4] Biological assay or assessment is a type of scientific experiment (both *in vivo, in vitro)* to determine the biological activity of a substance.

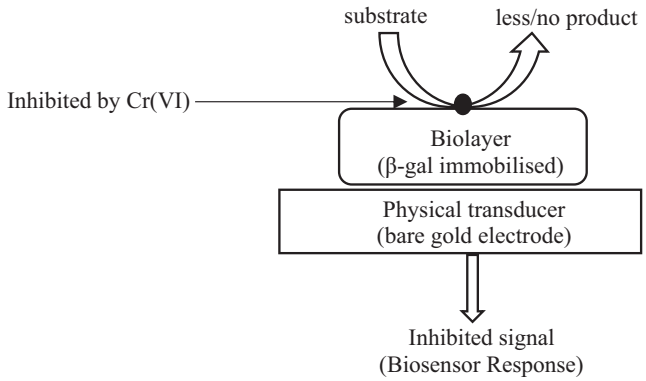

Fig. 12. Inhibition of immobilized β-gal in presence of Cr(VI).

Qambrani et al. (2016) studied a comparison of Cr(III) and Cr(VI) toxicities of water using SOB bioassays in both batch and fed-batch conditions. Cr(III) was found to be nontoxic for concentration up to 100 mg/L in both conditions where Cr(VI) at very low concentrations, 0.1–2 mg/L. The SOB activity was inhibited by Cr(VI) concentration of 0.5, 1.0 and 2.0 mg/L by 30, 60% and 98% respectively. At the lowest concentration level of 0.1 mg/L, Cr(VI) did not have any effect on the SOB activity (Qambrani et al., 2016). Fourou et al. (2016) developed a simple, sensitive, selective biosensor for the detection of heavy metal including Cr(VI) through inhibition of β-galactosidase (β-gal) from *Aspergillus oryzoe* after its immobilization on an electrochemical transducer by cross-linking with glutaraldehyde (as shown in Fig. 12). The inhibition of β-gal by Cr(VI) was noted by the decrease in β-gal activity monitored by the conductometric signal, and the detection limit ranges 3.12×10^{-10} M (91.7 ng/L) in the presence of ferri-ferrocyanide redox probe (Fourou et al., 2016).

The enzyme immobilized gold electrode ensemble conductometric biosensor proposed by Zhylyak et al. (1995) uses urease as bioelement. The biosensor can be utilized for overall analysis of water but lacks the speciation of heavy-metal ions. Bioassay based detection technique has been used further by utilizing different microbes such as SOB and TOB. For instance, SOB based biosensor detects chromium in the water on semi-continuous mode and the detection time is of the order of minutes to hours (Gurung et al., 2012). Similarly, SOB were used in another study at the working temperature of 450 C with the detection limit of 50 μg/L (Hassan et al., 2012). Qambrani et al. (2014) uses TOB for detection of Cr(VI) where detection limit is 100 μg/L and SOB for speciation of Cr(III) and Cr(VI) (Qambrani et al., 2016). The Section 2.4.3 shows the response of different conductometric biosensors for the detection of Cr(VI) using various biorecognition elements such as TOB, SOB and urease enzyme (Qambrani et al., 2014; Gurung et al., 2012; Zhylyak et al., 1995). Fig. 13 shows the EC and pH change with time for using different bacteria. Table 5 gives the comparative study of various parameters such as EC, pH, and inhibition of the different enzymes on conductometric biosensor for Cr detection.

2.4.4. Impedimetric biosensor

Electrical impedance (*Z*), the quotient of the voltage-time function *V(t)* and the resulting current-time function *I(t)*, is defined as the ratio of incremental change in voltage (*V(t)*) to the resulting current change (*I(t)*) (Wang et al., (2012) New trends in impedimetric biosensors for the detection of foodborne pathogenic bacteria, 2012; Guan et al., 2004). Impedimetric techniques have been performed to characterize the fabrication of the biosensors as well as to monitor the catalyzed reactions of enzymes. Electrochemical impedance spectroscopy method used for observing dynamics of biomolecular interaction describing the response of amplitude of sinusoidal voltage signal of the electrochemical cell as a function of frequency. A cell-based complementary metal-oxide-semiconductor chip uses impedance of microelectrodes for the detection of toxicity in wastewater, and measured impedance could encode in the form of the frequency of the digital output signal (Mucha et al., 2011). A study shows detection of chromium toxicity using mammalian cell cultures on impedance sensors as well as physiological *in vitro* systems. The sensor is proved to be a useful tool to detect Cr(VI) within several hours and can distinguish between toxic Cr(VI) and non-toxic Cr(III) (Bohrn et al., 2013). Table 6 shows comparative of the different sensing element with the detection limit where catecholics ranges 0–20 μmol/L (Ensafi et al., 2014).

3. Enzyme immobilized Porous silicon-based biosensor for chromium detection

The Porous Silicon (PS) is a spongy material, usually formed by the electrochemical anodization of Si substrate with pores running perpendicular to the substrate (Unagami, 1980). PS is an anisotropic nanocrystalline material with a high surface area, controllable pore size, tunable band gap (Bisi et al., 2000; Canham (1990), Silicon quantum wire array fabrication by electrochemical and chemical dissolution of wafers, 1990), and compatibility with microfabrication based processing (Gupta et al., 2013). Due to the quantum confinement[5] at the nanoscale the material properties such as optical, electrical also enhanced further by making it porous nanostructured material.

3.1. PS formation methods

There are three techniques for the formation of PS: electrochemical anodization, strain etching, and plasma dry etching. In electrochemical anodization, under constant direct current, silicon reacts with an electrolyte solution of hydrofluoric acid (HF), ethanol and/or acetic acid and PS formed by the anodization process (as expressed in Eq. (14)) inside the PS generator (Kale et al., 2012). The parameters responsible for the formation of PS are current density, anodization time, the resistivity of the substrate, type and orientation of the wafer, and electrolyte composition (Canham (1997) Properties of Porous Silicon, 1997). SEM images of PS films produced by electrochemical anodisation are shown in Fig. 14 exhibits porosity of about 30% with pore sizes ranging in between 20–30 nm.

$$Si+6HF + h^{+} \rightarrow SiF_6^{2-} + 4H^{+} + e^{-} \quad (14)$$

Lift-off technique separates the fragile and solid PS film from the substrate. There are three methods to lift-off the film from the Si wafer: one-step, two-step, and multi-step process (Campbell et al., 2008). In strain etching process, the silicon wafer is etched in the presence of the solution of HF, HNO_3, and H_2O (Dimova et al., 1997). The reaction of acid solution with the silicon during the incubation time produces pores (Steckl et al., 1993).

In plasma dry process the masked pattern is removed from the semiconductor material by the bombardment of ions, which consists of three major steps (Han et al., 2001) including the deposition of the polycrystalline silicon film on the silicon wafer followed by thermal oxidation of the silicon film in the next step. Since the oxidation rate of polysilicon grain and grain boundary are different, the oxide at grain boundaries is thicker than the grain surface. The reactive ion etching is further performed for the formation of PS. Plasma dry etching produces a high-quality PS with natural and stable microporous surface.

3.2. Porous silicon properties

PS is a porous material with nanocrystalline pores and large surface area (Canham (1990), Silicon quantum wire array fabrication by

[5] Phenomenon results from electrons and holes being squeezed into a dimension that approaches a critical quantum measurement

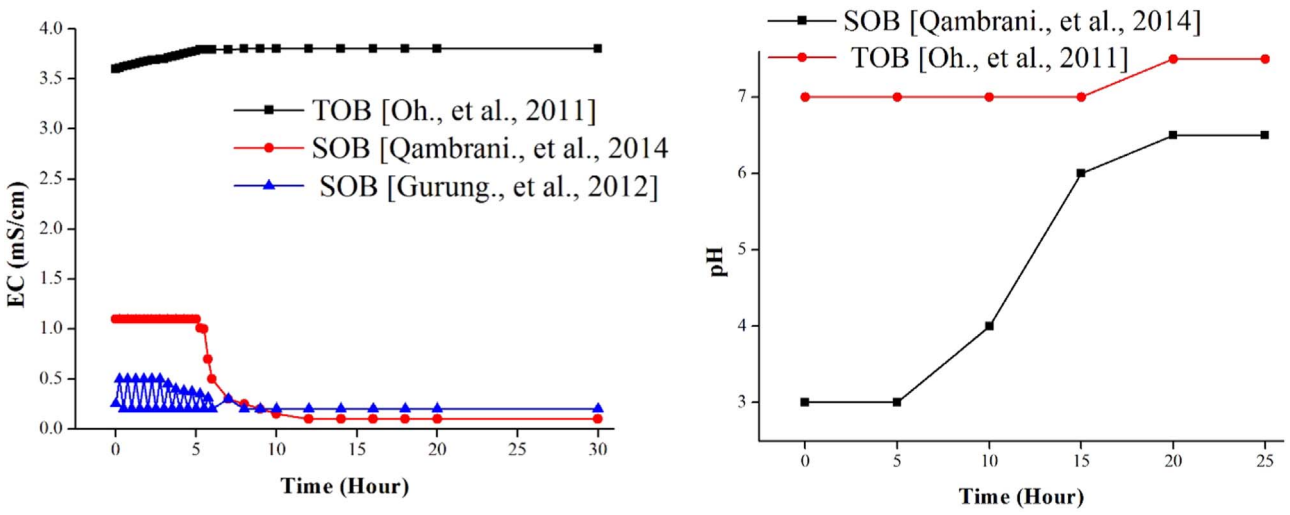

Fig. 13. (i) EC and (ii) pH response with respect to time for conductometric biosensor (Oh et al., 2011; Qambrani et al., 2014; Gurung et al., 2012).

electrochemical and chemical dissolution of wafers, 1990). The controllable pore size, porosity, high surface reactivity, thickness and luminescent properties of PS are critical parameters for the development of PS based biosensor. The following section includes the modification of PS properties on controlling the formation parameters for biosensing applications.

3.2.1. Physical properties of porous silicon

Three most important morphological characteristics of PS are pore size, thickness, and porosity which need to optimize for the efficient entrapment of the enzyme (Chaudhari et al., 2005). The pore size of PS depends on the current density and electrolytic concentration. Porosity is the fraction of the void within the PS layer (Dhanekar and Jain, 2013). Table 7 shows the effect of the PS layer on the anodization parameter on porosity, etch rate, thickness and electropolishing threshold. Fig. 15 illustrates the effect of current density, electrolyte concentration, and the resistivity of the wafer on the porosity of PS film measured by gravimetric analysis.

Electron microscopy is used to determine the pore size and surface morphology (Janshoff et al., 1998). The pore nucleation and propagation in anodized silicon through gravimetric method was reported (Brumhead et al., 1993). Scanning electron microscopy (SEM) is useful for the determination of the thickness of PS film (Janshoff et al., 1998). A non-destructive optical technique named ellipsometry is also used to figure out pore size, thickness, and porosity (Baklanov et al., 2000). Adsorption analysis at cryogenic temperatures yields corresponding information on pore size, as well as surface area and a general idea of the shape of the pores (Janshoff et al., 1998). Photoluminescence spectroscopy (both steady-state and time-resolved) are key tools to characterize the quantum-confined forms of the material. The pore size of PS needs to change depending upon the type of biosensing application. The micropore (< 2 nm) is advantageous for the conductivity based sensing application, but for biosensing application mesopore (2–50 nm) is preferred (Chan et al., 2000). Macropore (> 50 nm) is usually avoided for such application as the pore size becomes large enough to hold the enzyme, and enhancing the chances of leaching (Chaudhari et al., 2005).

Table 6
Comparative study of impedimetric biosensor for Cr detection in water.

Sl. No.	Biosensing element	Detection limit (μmol/L)	Ref.
1	Mammalian cell	0.5–25	(Bohrn et al., 2013)
2	DNA	0–20	(Ensafi et al., 2014)

The freshly prepared PS surface is Si_ySiH_x (where x+ y=4) terminated by hydrogen passivation and reacts slowly with ambient air, affecting both the structural as well as optoelectronic properties (Jarvis et al., 2012). Surface curvature and diffusion within nano-sized spaces influence the surface chemistry of the PS during the biorecognition. The exposure area offers more space for enzyme immobilization, and hence increasing the binding affinity (Mora et al., 2013) and efficiency of the sensor (Chaudhari et al., 2005). Under the exposure of PS for a long time in the open environment, the parameters, humidity, temperature and gasses degrade the surface of the PS material (Mora et al., 2013). While increasing the exposure area to improve the binding capacity of a biomolecule, the chances of the surface to corrode increases and hence, false positive signals may be produced. Therefore, the surface chemistry of PS should design in such a way that provide a maximum desired effect. The response of water is found to be extremely slow as the hydrophobic nature of PS repels the water molecules, and water molecule cannot penetrate easily into the pores (Harraz et al., 2015). The oxidation of PS surface changes the nature of the surface from hydrophobic to hydrophilic. The pore diameters shrink due to the oxidation of the PS surface which is helpful for the trapping of nanoparticles (Granitzer and Rumpf, 2010).

3.2.2. Optical properties

The optical properties of PS are a reflection, refraction, transmission, absorption, scattering, and photoluminescence which are dependent on the pore size, porosity, thickness, and degree of oxidation (Chambon et al., 2005; Kordas et al., 2004; Granitzer and Rumpf,

Table 5
Comparative study of conductometric biosensor for Cr detection.

Sl. No.	Method	EC (mS/cm)	pH	Inhibition (%)	Ref.
1	Assessment of chromium contaminated ground water using thiosulfate-oxidizing bacterial biosensor	5.59 ± 0.03–3.63 ± 0.03	2.02 ± 0.09–7.76 ± 0.07	16.7–100	(Qambrani et al., 2014)
2	Semi-continuous detection of hexavalent chromium using sulfur-oxidizing bacteria	0.65 to 0.412	2–3 to 6	–	(Gurung et al., 2012)

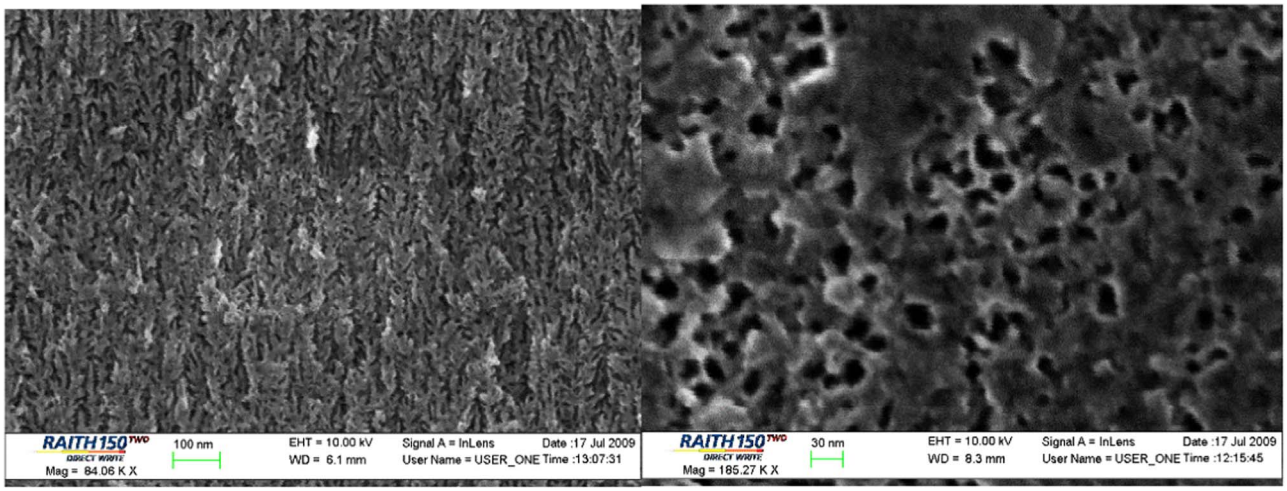

Fig. 14. SEM images of a freestanding PS films fabricated using chemical anodisation of Si wafer – cross-sectional and top view respectively.

Table 7
Effect of anodization parameter on PS formation (Herino et al., 1987; Hussein et al., 1995).

Anodization parameter		Porosity	Etch Rate	Thickness	Electropolishing threshold
Current density	↑	↑	↑	↑	–
Anodization Time	↑	↑	–	↑	–
HF Concentration	↑	↓	↓	↑	↑
Wafer Doping (*p*-type)	↑	↓	↑	↑	↑
Wafer Doping (*n*-type)	↑	↑	↑	X	–
Temperature	↑	*	*	*	↑

↑, ↓, -, x, *, indicates increase, decrease, almost constant, no effect, not known respectively

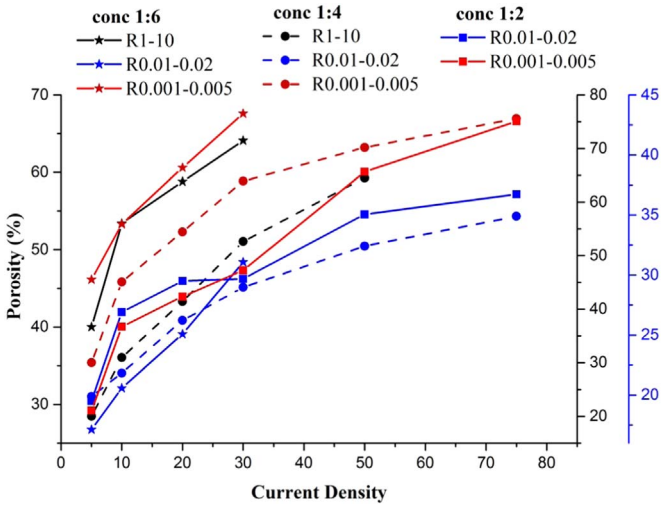

Fig. 15. Effect of current density, HF concentration, and resistivity of wafer on porosity.

2010). When an external analyte is attached to the PS surface, the refractive index (RI) changes accordingly causing a change in interference pattern during transmission of light through the surface (Janshoff et al., 1998; Mora et al., 2013). The external analyte attached to the PS surface for biosensing also alters the effective optical thickness. The reflection of bright white light on the air-PS layer and PS-bulk silicon interface results as a Fabry-Perot pattern. The total amount of analytes attached to the sensing film changes the photoluminescence. A semi-empirical approach shows RIs are measured using spectroscopic ellipsometry (Jellison and Modine, 1994). The transfer matrix method is used to find the best fit model for different values of optical parameters for fabrication of PS Fabry-Perot interferometer (Arnau et al., 1997; He and Cada, 1992).

PL of PS films depends on the production method and the surface chemistry. Molecules adsorption on the PS surface results in quenching of the PL signal, which may or may not reversible depending on the type of adsorbed molecules (Jeyakumar et al., 2015). Variation in pH of the medium change in PL of PS and the phenomenon may be used as a basis for the biosensor system development. Olga et.al. showed PL intensity of PS increased by 1.7 times when increasing glucose concentration in the GOD-containing reaction medium from 0 to 3.0 mM, and decreased by 1.45 times at the same increase in the urea concentration in the urease-containing reaction medium. However in the presence of heavy metal ions (Cu^{2+}, Pb^{2+}, and Cd^{2+}) in the tested solution causes an inhibition of the enzymatic reactions catalyzed by glucose oxidase and urease, which results in a restoration of the photoluminescence quantum yield of PS. The study proves the changes in PL signal on the interaction of enzyme and heavy metal with PS film can be used for detection of heavy metal ions (Syshchyk et al., 2015).

3.3. Porous silicon as a host material for enzyme immobilization

PS is an efficient entrapping tool to immobilize different enzymes where the size of the PS pores plays a vital role in entrapment of the enzyme. The enzyme loading capacity of the material is also critical from the sensing point of view. Therefore, the porosity and the amount of loading on the enzyme for the given pore size and thickness of the PS film of the material needs to be optimized. DeLouise et. al. demonstrates the possibility to quantitatively and predictably immobilize a known amount of glutathione-Stransferase enzyme in a porous silicon matrix. The author provides a simple geometric model to estimate the porous silicon surface area as a function of pore diameter, interpore spacing, and depth (DeLouise and Miller, 2004).

Dilute solutions of metal hydroxides such as KOH or NaOH systematically enhance the pore diameter of the PS films. Sufficiently large pore diameter accommodate the facile diffusion and binding of biomolecular reagents through the PS matrix (DeLouise, 2004). Mesoporous silicon is fabricated from p^+ and n^+-type silicon substrate and mesopores are suitable for the bio-macromolecules. The PS made from p- type silicon is suitable for few nanometer molecules while macro PS from n- type substrate accommodate 100 nm to few micrometer size molecules (Salis et al., 2011). The entrapment of enzyme on the PS surface is dependent on the enzyme concentration and pore size of PS which is explained in Fig. 16.

The immobilization of an enzyme inside a porous support such as

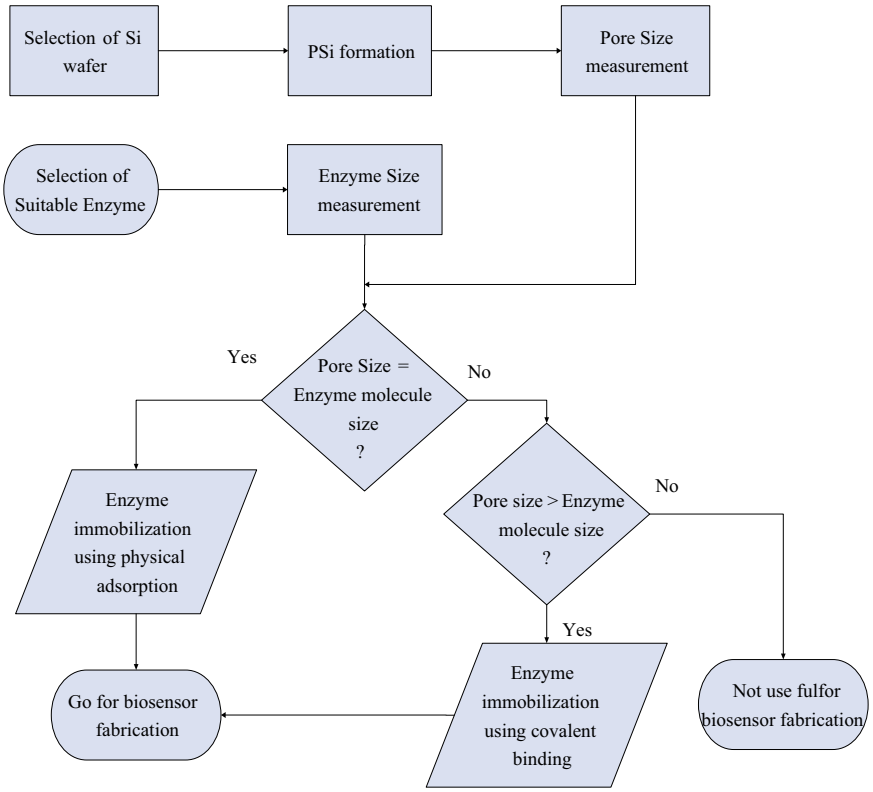

Fig. 16. Flow chart for selection of pore size and porosity for enzyme immobilization.

PS film may have several protective effects on the enzyme structure such as protection against activity loss by enzyme inhibition or by enzyme distortion, complete inactivation of enzymes, and a controlled pH environment (Rodrigues et al., 2013). The mesoporous channels protect the enzyme from leaching and allow the free diffusion of the substrate and product molecule to/ from the catalytic site (Yiu and Wright, 2005). The pore size needs to standardize for the optimal activity of the enzyme. The approaches for immobilizing the enzyme (Brena and Batista-Viera, 2006) inside porous membrane includes physical adsorption[6] via hydrogen bonding, hydrophobic and electrostatic interactions between the enzyme and silica support (Wang and Caruso (2004), Enzyme encapsulation in nanoporous silica spheres, 2004; Wang and Caruso (2005), Mesoporous silica spheres as supports for enzyme immobilization and encapsulation, 2005; ; Brena and Batista-Viera, 2006), enzyme capsulation, membrane confinement or entrapment,[7] covalent binding[8] and copolymerization[9] (Lei et al., 2002; Mohamad et al., 2015).

3.4. Proposed PS-based enzyme immobilized biosensor for Cr(VI) detection

The PS-based biosensor is an analytical device having bioreceptor molecules, used for the detection of an analyte by measuring an electrical or optical signal (Jane et al., 2009). The section includes three proposed models: amperometric, conductometric, and optical biosensor for the detection of Cr in potable water.

The first step for the biosensor fabrication is the development of the sensing layer implemented in all three proposed models. The development of the sensing layer includes four major steps: wafer preparation, electrochemical anodization, oxidization of PS film, and immobilization of enzyme over a porous oxidized film depicting in Table 8. The first step involves the selection of a Si wafer with specific resistivity, and doping level follows the second phase which includes the development of porous layer over the wafer. The water molecules can easily interact with the surface as the porous film is oxidized to change the surface behavior from hydrophobic to hydrophilic (Korotcenkov, 2016). Strong hydrogen-bonding produce a much larger response as it increases the penetration of the solvent into the porous matrix (Liyanage and Blackwood, 2014).

In the fourth and final step, enzymes such as urease (Nepomuscene et al., 2007; Maia et al., 2007; Zhang et al., 2011), cytochrome c_3 (Caroline et al., 2006), glucose oxidase (Maria et al., 2008), and tyrosinase (Domınguez et al., 2004) may be immobilized on the porous layer. The advantages of using urease for Cr detection are The easy availability of C. brasiliensis urease, the easiness of its immobilization on PS layers, and the significantly lower cost of urease obtention and PS fabrication. These advantages provided a basis for biosensor development with cost reduction and improved shelf life (Maia et al., 2007). Samborska et al. showed urease activity decreases with chromium concentration in both forms (Samborskaa, 2004). However, the inhibiting effect of Cr(III) is much stronger than that of Cr(VI). It is further reported that Cr(III) may modify the structure of enzymes through reactions with carbonyl and sulfhydryl groups of enzymes causing modifications of their activities caused by the displacement of magnesium by Cr(III).

After the development of the sensing layer, the next step is the implementation of sensing layer in the proposed biosensors. The PS based electrochemical biosensor uses the sensing surface as the electrode for the transduction of the signal. In the amperometric biosensor as shown in Fig. 17(a) consisting of two electrodes: working electrode and a reference electrode, the interaction of Cr ions (in the water sample) with the enzyme over the working electrode, induced

[6] Enzyme is attached to the outside of an inert material, slowest among all.

[7] Trapping of enzyme in insoluble beads or microspheres and the insoluble substances hinders the arrival of the substrate and the exit of products.

[8] Covalently bound to an insoluble support gives the strongest enzyme/support interaction.

[9] Enzyme molecules are covalently bonded to each other creating a matrix consisting of almost only enzyme. The reaction ensures that the binding site does not cover the enzyme's active site, the activity of the enzyme is only affected by immobility

Table 8
Fabrication process for the sensing layer of PS based Biosensor.

Sl. No	Steps	Schematic diagram of fabrication process
1	Wafer Preparation	Silicon Wafer
2	Electrochemical Anodization	d, w, Pores, PS; d = pore diameter, w = pore depth
3	Oxidation of PS film	Oxygen atoms on pore wall
4	Immobilization of enzyme over oxidized porous film	Enzyme layer

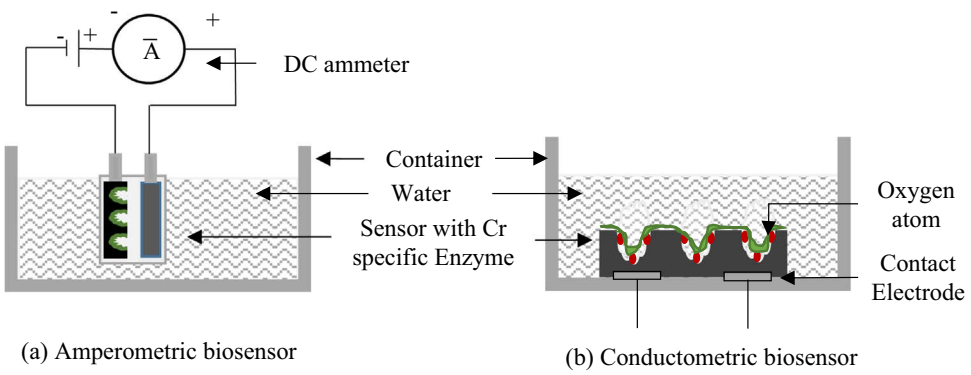

Fig. 17. PS based electrochemical biosensor for Cr detection.

charge (over the PS working electrode) alters the current passing through the circuit. The device can measure the Cr concentration by measuring the change in the current signal which is calibrated regarding the Cr concentration.

The PS based conductometric biosensor as shown in Fig. 17(b) consists of the enzyme immobilized sensing film having the electrical contacts on the non-exposing side. Due to the affinity of Cr towards the enzyme, the Cr molecule present in the water sample binds with the enzymes layer and changes the thickness of PS film which reflects a change in the conductance of the sensor.

The PS based optical biosensor as shown in Fig. 18, consists of three major components; enzyme immobilized PS sensing layer, a coherent light source, and a detector and as the Cr ions come in contact with the PS sensing surface, the RI of the surface changes. The spectral interference pattern of the reactance from the PS film can be detected and analyzed by fast Fourier transform to calculate the effective optical thickness of the PS film. In the presence of analytes, test sample, the effective optical thickness changes. PS-based reflective interferometric Fourier transform spectroscopy is useful for the detection of biological analytes (proteins, DNA, and viruses) (Mirsky et al., 2013; Rossi et al., 2007; Pacholski et al., 2005).

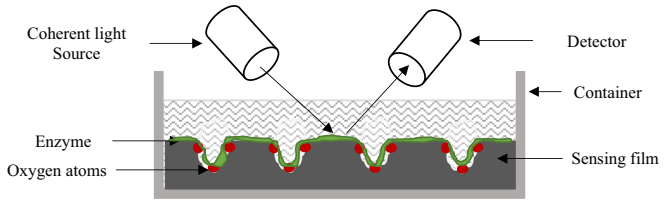

Fig. 18. PS based optical biosensor for Cr detection.

4. Concluding remarks

This paper is an attempt to extensively review the usefulness, environmental issues of chromium and existing detection techniques with particular focus on biosensors for the detection of chromium. Recently, porous silicon (PS) based biosensors have been reported for a variety of applications in the field of monitoring (glucose, urea), detection (pesticides, insecticides, and pathogen) and quantitative measurements. PS is gaining central attention by global researchers as a promising material for sensing application due to its engineered

morphologies of pore size and porosity, surface area enhancement and versatile chemistry. The surface modification of PS array makes it a selective and sensitive substrate for biomolecules (such as enzymes and microbes) to specify the change in one or many properties. Studies with PS as a host/entrapping material to immobilize biomolecules are reviewed. The sensing capabilities of PS based biosensor are evaluated based on the changes in electrical and optical signatures.

The sensitivity of sensor and detection time is the two prime factors that influence the selection of a biosensor for its desired application. Hence, research has been directed on this perspective, and steady improvement was achieved for attaining higher sensitivity with less detection time, which is very crucial for preferring the biosensor over other techniques. Conventionally, cost effective spectroscopic methods and electrochemical techniques are widely practiced for the sensing and detection applications. Subsequently, electrochemical-based biosensors are emerged to detect target molecules due to their selectivity, sensitivity, and stability. Four different categories of electrochemical biosensors for the detection of chromium in water were discussed in detail. Amperometric based transduction mechanisms were preferred over the other techniques due to lesser response time and better sensitivity.

The preparation of PS for biosensing applications are outlined along with the mechanism of surface modification. Through various signal amplification and background noise-reduction techniques, coupled with the improvements in sensitivity promised by miniaturization, enzyme-based detection methods have a high potential for rapid, sensitive analysis of all forms of heavy metals. Surface chemistry in combination with the nanostructures can be fine tuned for optimizing the enzymatic activity. Also, the detection time varies from a few seconds to around 30 min. Improving sensitivity along with minimizing the detection/response time are the biggest hurdles that should overcome to advocate biosensor technology. To recap, state of the art on the sensitivity of PS electrochemical biosensors for different biomolecules should have reached the environmentally significant standard values.

However, these biosensors should be further optimized for sensitivity, specificity, stability, reusability and shelf life before their implementation at field level. The current and future research efforts must be focused on the detection of heavy metals in their original environmental matrix and on the pre-processing steps. It includes miniaturization strategies, materials research, and emphasis on multiplexing so that ideally all relevant heavy metals for a specific scenario can be detected at once. Taken together, this will produce novel methods capable of providing the necessary sensitivity, specificity, and speed to replace the current standards and, hopefully, improve access to safe drinking water and reduce the global health burden of heavy metals.

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