Biosensors and its applications in Water Quality Monitoring

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Abstract

Even if terrestrial life may be carbon-based; but most of its mass is filled with water. Clean water is indispensable to all aspects in maintaining life. Mainly due to human activity, pressure on the water resources of our planet has increased significantly, that lead a demanding action in water management and treatment. Water quality sensors are desired in order to measure the problem and validate the success of corrective actions. This paper highlights recent development of biosensor engaged in controlling water quality parameters. To do this first the basic concept of biosensor, their type and application is discussed. Then biosensors advancement for parameters BOD, E. coli, heavy metal, toxic materials, and EDC are reviewed.

Keyword: Biosensor, BOD*, E. coli, EDC*

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1. INTRODUCTION

Massive urbanization, industrialization, reliance on irrigated agriculture, and the increase living standards all together changed the environment significantly, resulting to all sorts of pollution. For the past several decades, the quality of surface waters has been changing as the result of the mentioned anthropogenic activities, which has led to a significant increase in hazardous substance concentration. Ongoing depositions of pollutant affect the biodiversity and functioning of aqueous ecosystems. Appropriate steps should be applied to minimize this negative phenomenon, including water quality monitoring.

Sensors can detect the properties and events occurring around and convert the sensed information into signals. Different electrochemical sensors for the detection of pollutants in water have been well established, which can be categorized as (i) potentiometric sensors, (ii) amperometric sensors, (iii) voltammetric sensors, and (iv) conduct metric sensors.

Nowadays, sensors have great significance in many industries, providing quantitative information for counting, sorting, reading, and robotic guidance. For environmental monitoring, stricter regulations and higher standards have resulted in growing demand for sensors that can detect pollutants quickly and sensitively. Due to the feasibility of portability, of working on-site and determining biological effects, biosensors have been emerging as appropriate analytical tools for environmental monitoring

Biosensors can be classified according to their transduction principle such as optical (including optical fibre and surface plasmon resonance biosensors), electrochemical (including amperometric, and impedance biosensors), and piezoelectric (including quartz crystal microbalance biosensors) or based on their recognition element as immunosensors, aptasensors, genosensors, and enzymatic biosensors, when antibodies, aptamers, nucleic acids, and enzymes are, respectively, used. In environmental monitoring, the majority of biosensors are identified as immunosensors and enzymatic biosensors, but recently the development of aptasensors has been increased, due to the advantageous characteristics of aptamers such as easiness to modify, thermal stability, in vitro synthesis, and possibility to design their structure, to distinguish targets with different functional groups, and to hybridize.

2. Material and methods

The term ‘‘biosensors’’ was searched in Science direct, and Google, and the articles published on the water quality and environmental monitoring are received priority to be reviewed.

3. Basic concepts of Biosensors

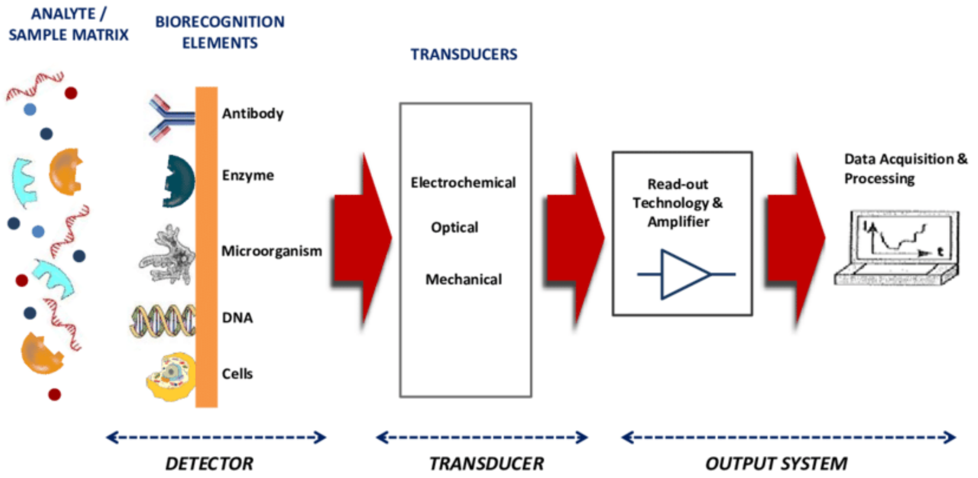
In fields of industry, medicine and pharmaceutical, agriculture, environment monitoring and biotechnology research, routine analyses using physical instruments are conducted for assessment and controlling the levels of given analytes. The traditional physical methods for this routine analysis do not involve the use of any living organism or molecule of biological origin. However, in the near past; for the same purpose, biological molecule or living cells have been used to develop sensitive devices that are labeled as “Biosensors”. A biosensor measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction (Joseph wang 2011; Bhalla, Jolly, et al. 2016). The biosensor can be defined as a device that utilizes biological molecules or living organisms such as antibodies and enzymes to identify chemicals; means; it converts a biological response into an electrical signal. According to Collings & Caruso (1997) “*it is an analytical device, which exploits a biological detection or recognition system for a target molecule or macromolecule, in conjunction with a physicochemical transducer, which converts the biological recognition event into a useable output signal”* (Peter Kruse 2018; S. M. ZakirHossain et al., 2019).

The biosensor was first made known in the 1960s and patent the application of enzyme originated bio-electrodes and their bio-catalytic action (Zoski, C.G., 2002). Afterward; a biosensor based model involving an oxygen electrode for the detection of electrochemical such as hydrogen peroxide or oxygen for the bio analytical application is presented in 1962. It is used to measure the dissolved oxygen in blood; this is famously known as Clark electrode. Later, the 'glucose oxidizes' enzyme in a gel was coated and immobilized on the oxygen electrode to measure blood sugar. Then the use of biosensors has stretched in the frontier of interdisciplinary research, i.e., analytical chemistry, bioelectronics, combines biology, and physics (Gonzalez-Rodriguez J, Raveendran M, 2015). After this point; a number of biosensors are being approved, which includes DNA biosensors, immune-sensors, enzyme-based, tissue-based, piezoelectric, and thermal biosensors (Mehrotra P, 2016).

Often; biosensor comprises of three elements; a bio-recognition (detector), bio-transducer, and an output system, which include a processor, signal amplifier, and display (NurAzuraMohd Said, et al., 2014; Mehrotra P, 2016). Bio-recognition also named as a bio-receptor, which exploits bimolecular as receptors to interact with a specific analyte. High selectivity of the analyte with bio-receptor is necessary. For more see table below

Table 1: Components of biosensor

Figure: 1 Schematic representation of a biosensor (NurAzuraMohd Said, et al., 2014).



Element	Description
Sensor	A sensitive biological element (biological material). Example: glucose is an 'analyte' in a biosensor designed to detect glucose NurAzuraMohd Said, et al., 2014; Malhotra et al., 2017; Bhalla et al., 2016; Rajpoot K ,2017))
Bio-receptor	A molecule that specifically recognizes the analyte is known as a bio-receptor. Enzymes, cells, deoxyribonucleic acid (DNA) and antibodies are some examples of bio-receptors. The step of signal generation in the form of light, heat, pH, charge or mass change, etc. during interaction of the bio-receptor with the analyte is termed bio-recognition (NurAzuraMohd Said, et al., 2014; Malhotra et al., 2017; Bhalla et al., 2016; Rajpoot K ,2017).

Transducer	It is the detector element that transforms the signal resulting from the interaction of the analyte with the biological responsible for the display of the results in a user-friendly way. The function of traducer In a biosensor is to transform the bio-recognition event into a measurable signal. This process of energy conversion is known as signalization (NurAzuraMohd Said, et al., 2014; Malhotra et al., 2017; Bhalla et al., 2016; Rajpoot K ,2017). Example: works in a physicochemical way; optical, piezoelectric, electrochemical, etc.
Electronics	This is the part of a biosensor that processes the transducer signal and prepares it for display (NurAzuraMohd Said, et al., 2014; Malhotra et al., 2017; Bhalla et al., 2016; Rajpoot K ,2017)
Display	The display comprises of signal conditioning circuit, processor and a display unit. This part usually contains a combination of hardware and software that generates output of the biosensor in a user-friendly manner. The output signal on the display can be numeric, graphic, tabular or an image, depending on the requirements of the end user (NurAzuraMohd Said, et al., 2014; Malhotra et al., 2017; Bhalla et al., 2016; Rajpoot K ,2017).

Based on the nature of bio-recognition element, the biosensor detector can be differentiated into different categories namely catalytic, affinity and hybrid receptors (DNA). A range of physical and chemical transducers has been used to monitor these biological recognition events. It can be broadly classified into electrochemical, optical and mechanical devices (Fig. and table 1).

Bio-catalytic devices integrate enzymes, whole cells or tissue slices that identify the target analyte and generate electro-active species. Affinity sensors rely on a selective binding interaction between the analyte and a biological component such as an antibody-antigen, nucleic acid, or a receptor (Ronkainen et al., 2015; Rajpoot K, 2017). The DNA biosensors is devised on the property that single-strand nucleic acid molecule is able to recognize and bind to its complementary strand in a sample. The interaction is due to the formation of stable hydrogen bonds between the two nucleic acid strands (Wang J.1998, Kavita V. 2017). Seeing the biosensor progress over the last fifty years, the biosensors have been classified into "first-generation", "second-generation" and "third generation" biosensors (NurAzuraMohd Said, et al. 2014). First-generation biosensors - the electrons are transferred to molecular oxygen and the resulting decrease in the oxygen concentration and/or the produced hydrogen peroxide is measured. Second-generation biosensors **-** use artificial, partially toxic mediators or nanomaterial to transport the electrons to the electrode. The redox mediator should provide reversible electrochemistry, specificity for the selected electron transfer pathway, be stable in both oxidized and reduced forms; and produce no side reactions. Third generation biosensors - the electrons are transferred directly from the enzyme to the electrode without any intermediate stages or use of nanoparticles.

Comparing with physical instruments, the biosensors have been considered to be superior and more sensitive: the reasons might be

1. In the biosensor; the presences of intimate contact between the immobilized biological materials a suitable transducer, so that these speeds up the conversion of the biochemical signal into an electrical signal.
2. The immobilization of bimolecular allows reuses of these molecules and helps to simplify the entire apparatus.

3. The biological sensing is very sensitive and placed only in some areas, thus facilitating the analysis of the substance in small quantities.
4. Biosensors can be developed according to specific requirements and can be highly specific or broad spectrum.

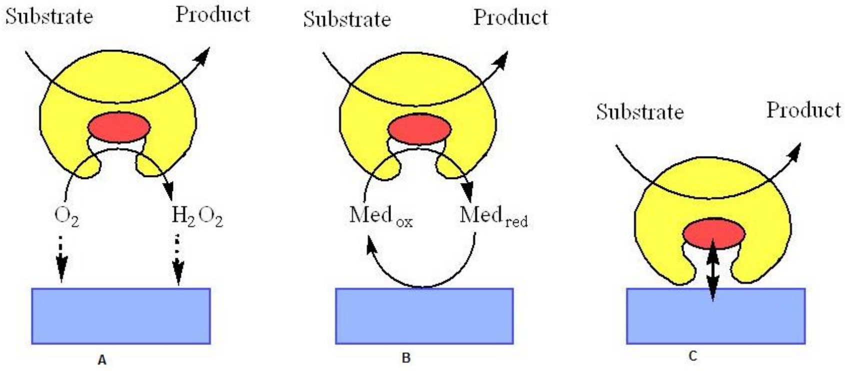


Figure: 2 Electron transfer mechanism A-first-generation, B-second-generation and C-third generation biosensors.

5. Application of Biosensor

With the aim of improving life; biosensors found a very wide range of applications. Some of the common fields applying the use of biosensors are food industry to monitor on its quality and safety, to help differentiate between the natural and artificial; in the fermentation industry and in the scarification process to identify precise glucose concentrations; in metabolic engineering to enable in vivo monitoring of cellular metabolism. The roles of biosensor in medical science including early stage detection of human interleukin-10 causing heart diseases, rapid detection of human papilloma virus, etc. are important aspects. Fluorescent biosensors play a vital role in drug discovery and in cancer. Biosensor applications are prevalent in the plant biology sector to find out the missing links required in metabolic processes. Other applications are involved in defense, clinical sector, and for marine applications.

Table: 2- show a summary of the application of Biosensor.

Figure: 3- Application of biosensor

Area	Discussion
Food Analysis	Biosensors for the measurement of carbohydrates, alcohols, and acids are commercially available. These instruments are mostly used in quality assurance laboratories or at best, on-line coupled to the processing line through a flow injection analysis system. Their implementation in-line is limited by the need of sterility, frequent calibration, analyte dilution, etc. Potential applications of enzyme based biosensors to food quality control include measurement of amino acids, amines, amides, heterocyclic compounds, carbohydrates, carboxylic acids, gases, cofactors, inorganic ions, alcohols, and phenols. Biosensors can be used in industries such as wine beer, yogurt, and soft drinks producers. Immunosensors have important potential in ensuring food safety by detecting pathogenic organisms in fresh meat, poultry, or fish (Malhotra et al., 2017).
Medical diagnosis	One of the main and the most important applications of biosensors is the detection of biomolecules that are either indicators of a disease or targets of a drug (medical diagnostics). For example, electrochemical bio sensing techniques can be used as clinical tools to detect protein cancer biomarkers. The electrochemical variety is used now in clinical biochemistry laboratories for measuring glucose and lactic acid. One of the key features of this is the ability for direct measurement on undiluted blood samples. Consumer self-testing, especially self-monitoring of blood components is another important area of clinical medicine and healthcare to be impacted by commercial biosensors. Nowadays reusable sensors also permit calibration and quality control unlike the present disposable sticks where only one measurement can be carried out. Such testing will improve the efficiency of patient care, replacing the often slow

	and labour intensive present tests. It will bring clinical medicine closer to bedside, facilitating rapid clinical decision-making (Malhotra et al., 2017).
Agricultural Industry	Enzyme biosensors based on the inhibition of cholinesterases have been used to detect traces of organophosphates and carbamates from pesticides. Selective and sensitive microbial sensors for measurement of ammonia and methane have been studied. However, the only commercially available biosensors for wastewater quality control are biological oxygen demand (BOD) analyzers based on micro-organisms like the bacteria Rhodococcuserythropolis immobilized in collagen or polyacrylamide (Malhotra et al., 2017).
industrial application	Along with conventional industrial fermentation producing materials, many new products are being produced by large-scale bacterial and eukaryotes cell culture. The monitoring of these delicate and expensive processes is essential for minimizing the costs of production; specific biosensors can be designed to measure the generation of a fermentation product (Malhotra et al., 2017).
Environmental field monitoring	Environmental water monitoring is an area in which whole cell biosensors may have substantial advantages for combating the increasing number of pollutants finding their way into the groundwater systems and hence into drinking water. Important targets for pollution biosensors now include anionic pollutants such as nitrates and phosphates. The area of biosensor development is of great importance to military and defense applications such as detection of chemical and biological species used in weapons (Malhotra et al., 2017).

6. Biosensors for water quality monitoring

Scientific literature is abounded with different biosensor technologies for offline or on line evaluation of biological and/or ecological quality elements or for the monitoring of chemical contaminants (inorganic and organic). Even though contaminants are generally classified based on their chemical structure, they can also be classified based on their nature of action, such as endocrine disruption, carcinogenicity cytotoxicity, genotoxicity or mutagenicity.

6.1 BOD

The Biochemical oxygen demand (*BOD*) is the amount of dissolved oxygen consumed by aerobic biological microorganisms to digest/assimilate organic material present in given water sample, at given temperature, usually 20ºC, over a specific time period. An Important part of standard water quality analysis is identifying the biological oxygen demand (BOD). In the standard method of BOD determination; it is required to incubate an oxygen-saturated sample, after activated sludge, that is mixture of various microorganisms, is introduced, for 5, 7, 10 or 20 days (BOD_5, BOD_7, BOD_{10} or BOD_{20}, respectively) at 20°C (Standard Methods, 1992), and like other chemical analysis BOD test also need well-furnished laboratories, a trained technician to obtain reproducible results, that make the monitoring difficult. Thus, the method is not suitable for process control in wastewater treatment (because it takes 5 days to complete) and the real-time monitoring of water environments, such as rivers, streams, ponds, and ground water.

The biosensors for measuring biochemical oxygen demand (BOD) first developed in the early stages of the emerging technology. In 1962, Clark and Lions developed an amperometric sensor for the measurement of dissolved oxygen. “The algal oxygen sensor” A cathode made of a thin gold grid and a silver / silver – chloride anode in a KCI electrolyte are biased with a 0.7V voltage. When an

Oxygen molecule migrates through the PTFE membrane, the following reaction takes place. Since the electron current is proportional to oxygen. The algal is deposited on the porous membrane tightly fitted PTFE membrane algal illumination is provided through a light emitting diode from inside Clark cell (Karube et al., 1997b; Hikuma et al., 1979, Jean Marie ORy.1996). And these systems continue to be actively developed at present (Rodriguez-Mozaz et al., 2006) See figure 4 for algal oxygen sensor.

$$\text{Cathode:}\quad O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$$

$$\text{Anode:}\quad 4Cl^- + 4Ag \rightarrow 4AgCl + 4e^-$$

Based on this sensor, fast measurement of BOD_5 using biosensors relied on the determination of the bacterial respiration rate near a transducer (Rodriguez-Mozaz et al., 2006). An optical fiber biosensor based on yeast has been developed for monitoring low BOD values in river waters in 15 min (Dhewa, 2015; Rodriguez-Mozaz et al., 2006). Pasternak and his coauthors have reported a self-powered, autonomous BOD biosensor for online water quality measurement based on signal frequency (Pasternak, et al., 2017). Contamination of water with urine can be detected using this sensor. As electro active microorganisms are used to produce this biosensor, it is self-powered and can operate autonomously for five months. Figure -.5Illustrate Schematic illustration of the biosensor and the principle of operation. Recently, Di Nardo et al. (2018) have developed an integrated smart meter with sensors connected to a cloud computing system for smart management of a water network, allowing the efficient online monitoring of water quality.

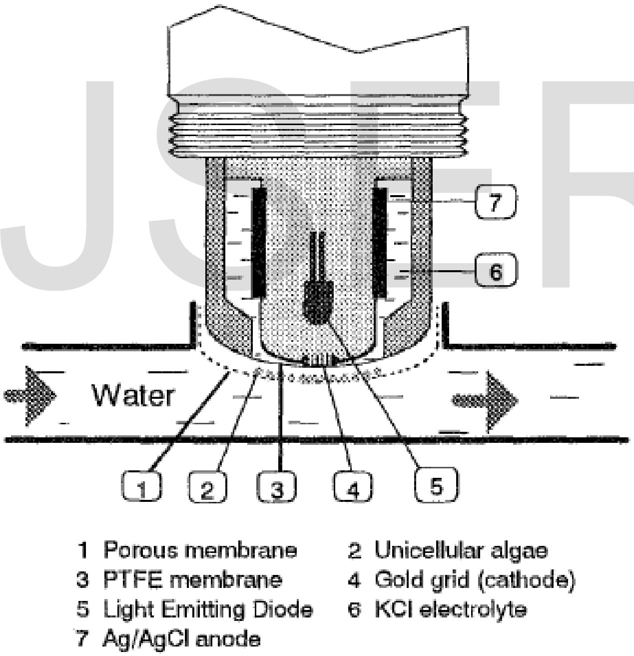


Figure: 4- The algal oxygen sensor -Modified Clark electrode (Jean Marie O.1996).

A microbial fuel cell (MFC) uses bacteria to produce electrical energy (Yang Cui et al. 2019). The bacteria oxidize a carbon source and donate their electrons to the first electrode, the anode. This causes a current to pass through an external circuit, thereby causing a reduction reaction at the second electrode, the cathode. A chemical signal the oxidation of a chemical substrate is thus translated directly into an electrical signal by billions of bacteria. The electrical signal produced depends on several environmental factors including pH, substrate availability and the concentration toxins (Modin, O. &Wilen, B.-M., 2012, Yang Cui et al. 2019). As shown in figure 6, the signal produced by the bacteria can therefore be used as a measure of water quality because a change in water quality can be measured by a change in electrical current or potential by passing a sample through the anodic compartment of the cell, any toxins present will affect the bacteria. This technique could be developed into a continuous, on-line, on-site system for monitoring water quality (Takahiro Yamashita, et al 2016, Dengbin Yu, Lu Bai, JunfengZhai, et al., 2017, Yang Cui et al. 2019). Bacteria gain energy from the release of electrons to the anode. When the anode potential in the MFC-based biosensor changes, the bacteria that grow on the anode experience a new energy level Most MFC-based biosensors, which are used to detect either biological oxygen demand (BOD) or toxicity, use an external resistor in the external electrical circuit (Modin, O. &Wilen, B.-M. 2012, Yang Cui et al. 2019).

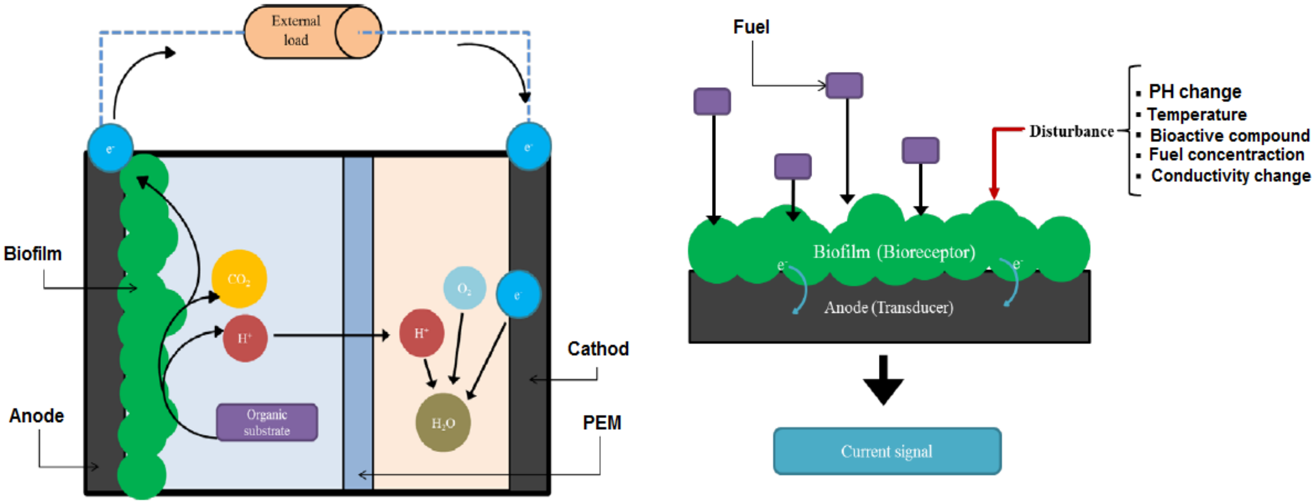


Figure: 5 .A operating principles of a two-chamber microbial fuel cell (not to scale). The electro-active bio-film at the anode breaks down an organic substrate to produce electrons, protons and CO_2. The electrons pass through an external load is reduced at the cathode. **B**. Basic principle of an MFC as a biosensor (Pasternak, Greenman, & Ieropoulos, 2017).

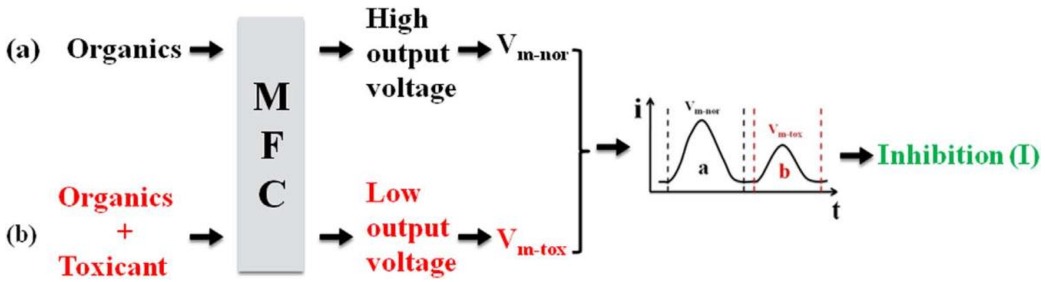


Figure: 6- Schematic illustration of toxicity detection in water based on self-powered MFC (Dengbin Yu, Lu Bai, JunfengZhai, et al., 2017).

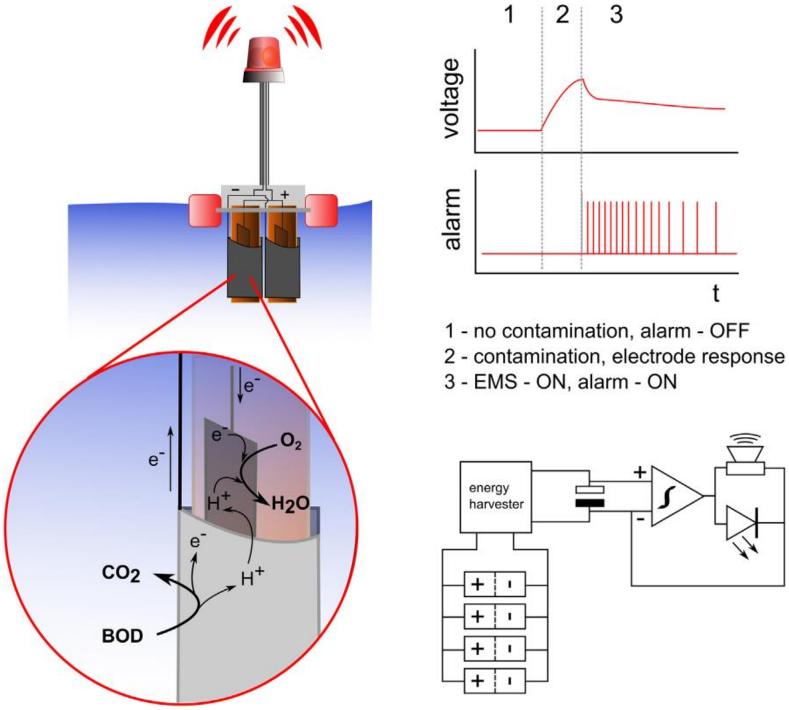


Figure: 7- Schematic illustration of the biosensor and the principle of operation. 1–biosensor operates in uncontaminated freshwater under open circuit conditions, 2–in the presence of urine, the sensor open circuit voltage increases, 3–the energy management system (EMS) switches ON, resulting in charging the capacitor. The system is able to repeatedly charge/discharge the capacitor (Grzegorz Pasternak, et al., 2017).

BOD MFC based Biosensors were performing *for ex situ monitoring* under anaerobic conditions is repeatedly reported. One was by Kim, B. H., Chang, I. et al. (2003), this biosensor was a two-chambered mediator-less, and showed a strong linear correlation of charge with BOD_5 concentrations up to 206 mg/L (Chang, I. S. et al., 2004). A biosensor based on an air-cathode, single-chambered MFC displayed a linear correlation with BOD_5 concentrations up to 350 mg/L is also reported by Chang, I. S. et al. (2004); additionally, a BES-based biosensor with voltage input was demonstrated to have a wide detection range from 32 to 1,280 mg/L with a linear correlation of charge with BOD_5 (Modin, O. & Wilen, B.-M. 2012).

Takahiro Yamashita, et al (2016) published a novel open-type biosensor for the *in-situ* monitoring of biochemical oxygen demand in an aerobic environment, in this study, a bio electrochemical open-type biosensor was designed for *in-situ* monitoring of BOD during intermittent aeration. The open-type anode, without any protection against exposure to oxygen, was directly inserted into an intermittently aerated tank filled with livestock wastewater. Anodic potential was controlled using a potentiostat. Interestingly, this biosensor generated similar levels of current under both aerating and non-aerating conditions, and showed a logarithmic correlation of current with BOD concentrations up to 250 mg/L (Takahiro Yamashita, et al., 2016). This study suggested that, as a result of a thick biofilm on the anode, the application scope of BES-based bio devices is extended from anaerobic to aerobic environments**.**

Nisshin Electric Co. Ltd in 1983 was the first to put commercial model biosensor for BOD analysis. Later, a number of commercial BOD biosensor analyzers were manufactured by other Japanese (Central Kagaku Corp.) and European (Dr. Lange GmbH, Aucoteam GmbH, PrufgeratewerkMedingen GmbH) companies (). The working principles for these sensors were Clark's oxygen electrode-based biosensors, but made use of activated sludge as receptor element substrate (Liu &Mattiasson, 2002).

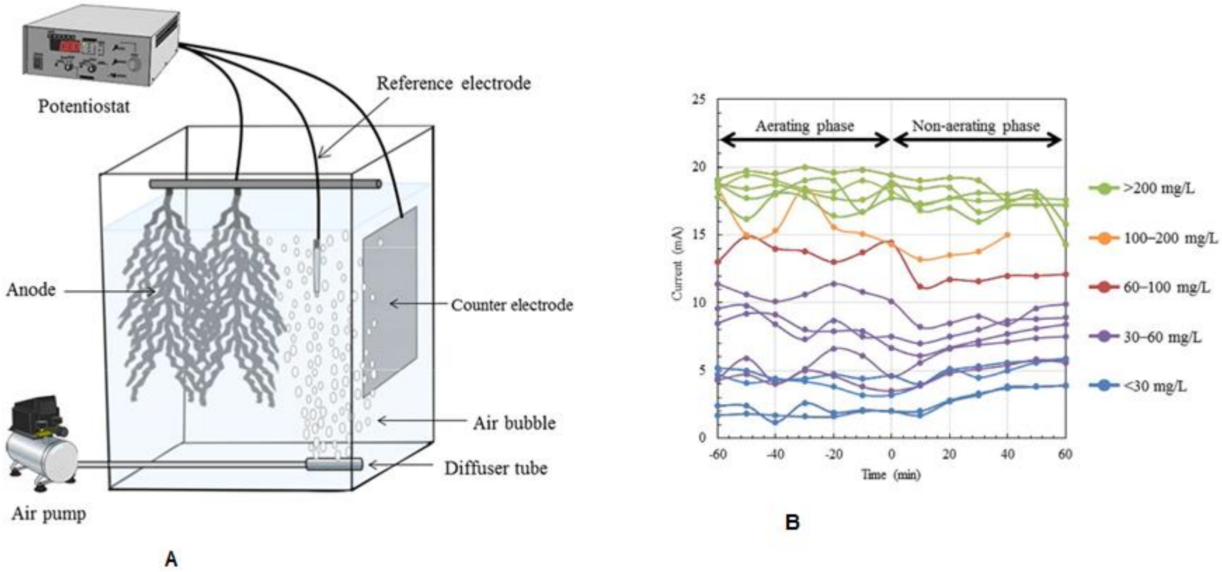


Figure: 8- A) Schematic representation of the iBOB biosensor inserted into an intermittently aerating tank. B) Trend of current generation by the iBOB biosensor at different BOD_5 concentrations in the intermittently aerated tank. The zero value on the horizontal axis shows the time point of switching from the aerating to the non-aerating phase. BOD_5 concentrations are indicated by color (Takahiro Yamashita, et al., 2016).

The method of BOD analysis using biosensors was included into the Japanese Industrial Standard in 1990 (JIS K3602). Several models of BOD biosensors are sold on the market at present: QuickBOD α1000, BOD-3300, HABS-2000 (https://aqua-ckc.jp/company/english.html).

Even if lots of researches are done, still the BOD-biosensor systems have a number of drawbacks that hinder their use. These include inadequacies of legislation in most countries, limitations the standardization procedure, complicated service requirements and defiance in stability of used microbial cultures with respect to heavy metals and various toxic substances present in the water (Rodriguez-Mozaz et al., 2006).

Some reviews done on microbial sensors (D'Souza, 2001; Liu &Mattiasson, 2002; Sara Rodriguez-Mozaz . et al., 2006; Lei et al., 2006; Xu& Ying, 2011; Ponomareva et al., 2011), and the use of biosensors for environment and for ecological monitoring (Rodriguez-Mozaz et al., 2006; Baeumner, 2003) provide examples of BOD sensors advancements . In the reviews, it is noted that, an important role of bio-recognition elements based on eukaryotic microorganisms in biosensors for solving environmental problems is mentioned, including for determining the BOD of water bodies (Walmsley& Keenan, 2000).

Yang Cui et al. 2019 noted, more investigations on the stability of MFC-based biosensors are urgent, which are often unnoticed. MFC use bacteria as the catalysts, which are self-renewable. But during long term operation, bacteria can evolve rapidly in response to environmental changes. As a result, the sensitivity, selectivity, and reproducibility of the biosensors will be adversely affected, screening of bacteria having high extracellular electron transfer rate might be a good strategy to improve the MFC-based biosensors.

6.2 Heavy Metals and Toxic

Relatively the half life time of heavy metals is long, tens to hundreds of years, means it is barely reduced by microorganism. Despite some of the metals are essential for human health. The harmful once could cause health problems, if it is collected in human body along with the food chain. Progress has been made in the development of biosensors depending on intact bacterial cells to analysis toxic metals. Heavy metals are well known to inhibit the activity of enzymes, and application of this phenomenon for the determination of these hazardous toxic elements offers several advantages, such as simplicity and sensitivity the other advantage of the whole-cell sensors is their ability to react only with the available fraction of metal ions whereas the analytical methods are not able to differentiate between fractions of metals that are available and non-available to biological systems (Rasmussen LD, Sorensen SJ, et al, 2000). Durrieu C, Tran-Minh C (2002) described a biosensor for the determination of heavy metals based on inhibition of the alkaline phosphatase (AP) present on the external membrane of Chlorella vulgaris microalgae .Bullough et al. (2013) have developed different methods of measuring arsenic using genetically modified whole-cell biosensors.

Figure: 9 WaterPOINT 870

An amperometric sensor that integrates bacterial cells, E. coli is used for the determination of toxicity and genotoxicity. As reported by different authors, cell sense is used for rapid toxicity measurement []. A similar configuration was used by Kvatinsky, Leibowitch, et al., 2008. Enzyme-based sensors have also been developed for measuring toxicity (Saleem, 2013; Hesari, et al., 2016) investigated E. coli enzyme can hydrolyzed 4-methylumbelliferyl glucuronide (MUG) to form a fluorogenic output. The fluorogenic intensity is detected, and was directly related to the number of E. coli bacteria existed in water (Hesari et al., 2016)

MFC)-based biosensor, that is illustrated in figure is recently developed sensor for the detection of toxic components in water, it is the microbial fuel cell for onsite monitoring of toxicants in water (Jon Chouler et al., 2019). The MFCs are simple to operate and use microalgae to form a sensitive, portable and cost-effective bio-electrochemical sensor. This photo MFC has been reported to be more efficient than other MFCs for onsite detection of formaldehyde and other contaminants, such as herbicides and pharmaceuticals, which pose a global environmental concern.

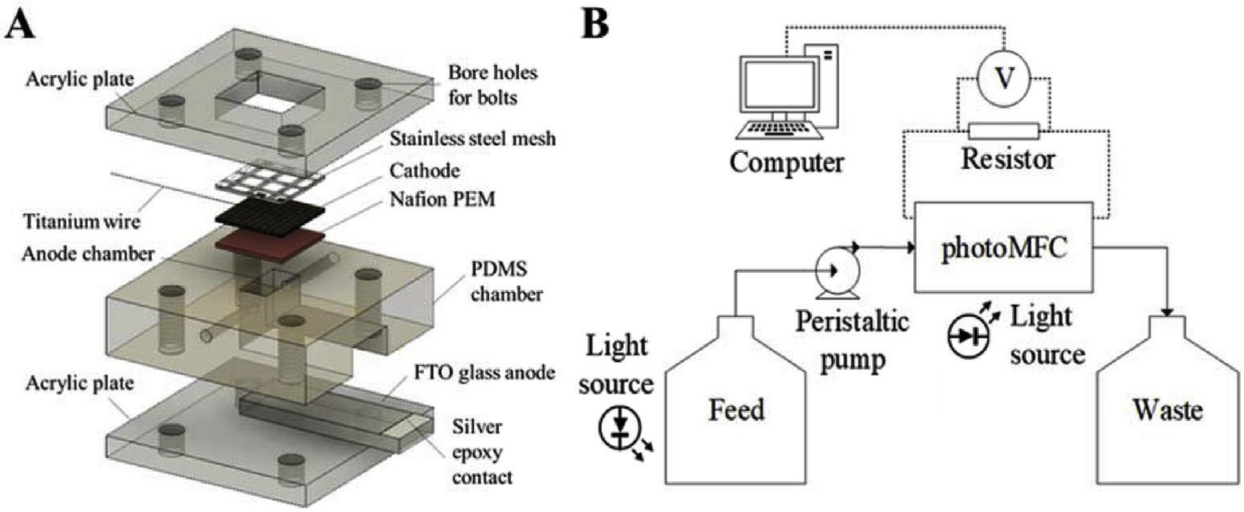


Figure:10- A Schematic of the photo-MFC construction (to scale); B- schematic of the experimental set up for operation of the photo-MFC, 'feed' may constitute of algae þ AW, AWor AW þ formaldehyde depending on the mode of operation (Jon Chouler et al. 2019).

Hassan et al. (2019) have published the on-line detection of heavy metals and other toxic chemicals in water using a sulfur oxidizing bacteria (SOB)-based biosensor Generally, in nontoxic water, SOB oxidizes elemental sulfur (S_{-}) to sulfuric acid (H_2SO_4) resulting in a decrease of the water pH, while $SO4_{-}^{2}$ increases the electrical conductivity (EC) of water. However, in the presence of contaminants, activity of SOB is inhibited, which results in an increase of the pH and a decrease of EC. Therefore, changes in EC can be used as an indication of toxicity. WaterPOINT 870, which now offers 35 different physical-chemical parameters tests in a single handheld device, one of the most versatile devices available. Using Water POINT it is possible to test for manganese, sulfide, zinc, molybdate, bromine, silica, and chloride.

6.3 E. coli

Pathogens are infectious agents that cause disease. Pathogens contain microorganisms, such as fungi, protozoan, and bacteria, and molecular-scale infectious agents, together with viruses and priors. Food borne, waterborne, and airborne pathogens enter the body through various modes of infection and are accountable for over 15 million deaths per annum worldwide (Dye 2014). Some of the most common pathogens include viruses, such as nor virus and influenza virus, and bacteria, such as *E. coli* and *S. aureus*. Pathogens vary in many regards, such as virulence, contagiousness, mode of transmission, and infectious dose. For example, the world is currently dealing a global pandemic related with the COVID-19 virus, for which virulence and infectious dose data are still emerging. Techniques for sensitive and rapid detection of pathogens in complex matrices, such as body fluids and aerosols, and on surfaces are critical to the treatment of infectious diseases and the spread of infection.

Surface water acts as main vector for the transmission of pathogens. Bacteria, viruses, and other microorganisms are widely found in polluted, untreated and treated waters and causing worldwide public health problem. The proper monitoring of the water supply for the presence of pathogens can help in controlling diseases from these sources, and thus, new technologies like biosensors have been developed to deliver fast identification of contamination by microorganisms at source and in real time, whereas in case of conventional analytical methods, days or weeks are required to get a result (Nasser AM, Oman SD 1999).

The conventional method for quantitative microbiological analysis is counting visible microbial colonies grown on semi-solid agar-based growth media, for a broad spectrum of prokaryotic and eukaryotic microbes. The advantages of colony-counting assays are the simple protocols and the high sensitivity, a single cultural cell in a sample can develop into a visible colony. However, since the development of colony-counting assays long ago, the technique has improved little. Microbial colonies are still grown in conventional Petri dishes or multi-well plates. Visual plate counting is commonly applied using aliquots of liquid cultures and plating out of serial dilutions onto culture plates. Following incubation under conditions appropriate for the microorganism of choice, the colonies are counted to determine the number of colony forming units. This is done manually by counting colonies on plates illuminated using transmitted light, which is a time-consuming process that is vulnerable to human error.

In near recent, 2019, Peixoto, Machado et al., worked on bioactive paper sensors for on-line monitoring of water quality. In this work it is reported a method for selective and ultra-sensitive multiplexed detection of *Escherichia coli* (non-pathogenic or pathogenic) using a lab-on-paper test strip (bioactive paper) based on intracellular enzyme (β-galactosidase (B-GAL) or β-glucuronidase (GUS)) activity. The sensor is recognized by its' desirable future, rapid, sensitive, on-line monitoring of toxins, heavy metals in addition to bacteria and no need of advanced instrumentation and/or trained personnel (Patrícia S. Peixoto, Ana Machado, et al. 2019).This biosensor works as standalone equipment with online data transfer for detecting and controlling the water quality as well as enhancing water treatment procedures. The instrument is capable of detecting at least two colony forming units (CFU) of E. coli in 8 h.

Jung and Lee (2016) developed an optical biosensor for automated, real-time monitoring of microbial colony formation and growth in water. The system can dynamically detect individual micro-colonies using sub-pixel sweeping microscopy with a high-resolution. Real-time bacterial micro-colony-counting system implemented on a wide field-of-view (FOV), on-chip microscopy platform, termed ePetri.

By using sub-pixel sweeping microscopy (SPSM) with a resolution algorithm; the system exhibited the capability of dynamically track individual bacterial micro colonies over a wide FOV of 5.7 mm × 4.3 mm without requiring a moving stage

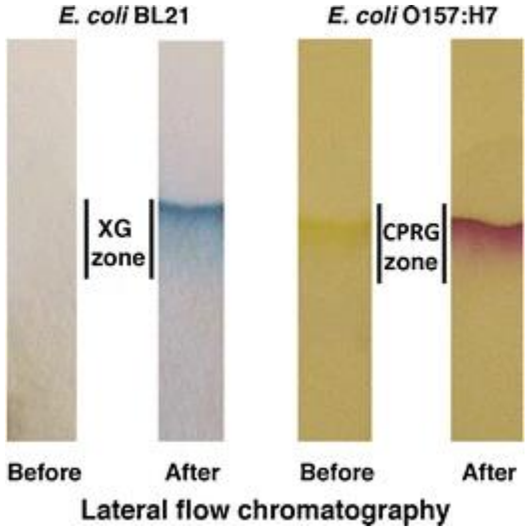


Figure: 11- E. coli -sensor developed using the intracellular enzyme, b- galactosidase (B-GAL) and CPRG or 5-bromo-4-chloro-3-indolyl-b-Dglucuronide sodium salt (XG) substrate (Hossain et al., 2012; S. M. ZakirHossain&Noureddine Mansour, 2019).

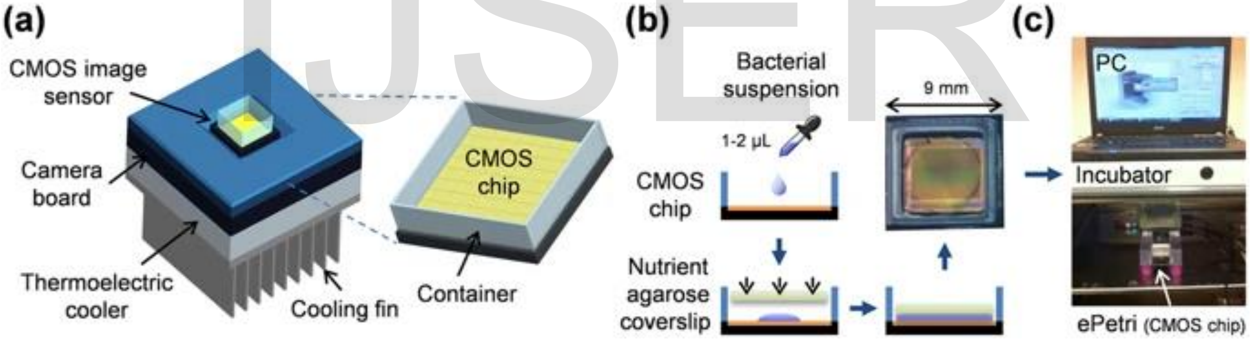


Figure: 12- Schematic diagram showing the real-time bacterial micro colony counter system using on-chip microscopy (**a**) the ePetri platform consisted of image sensor chip, a camera board, and a thermoelectric cooler and cooling fin. (**b**) The preparation procedure of the real-time measurement of bacterial micro colonies consisted of only two steps: 1. loading the bacterial suspension onto the CMOS chip, and covering the CMOS chip with the nutrient agars sheet. (**c**) Then, the e-Petri platform was located inside an incubator to begin the generation of images of the bacterial micro colonies **(**JaeHee Jung & Jung Eun Lee, 2016**)**

6.4 Endocrine Disrupting Compounds (EDC)

Increasing attention has been focused on endocrine disrupting compounds (EDCs) as pollutants in municipal wastewater. Recent studies have shown that these compounds can have a negative impact on the environment, and that in many cases they are not efficiently removed in wastewater treatment plants (WWTPs). Studies have also revealed that their destruction and transport out into the environment depend on the design and operational characteristics of these treatment systems and on the properties of the chemicals themselves.

Biosensors have been developed to determine EDCs by measuring estrogen receptors (ERs). The binding capability of chemicals towards ERs is become possible to be determined using biosensors. An example is the SPR biosensor, which can be used for the measurement of oestrogens and xenoestrogens (Saleem, 2013). The estrogenic activity in water can be determined using optical biosensors, which are produced by recombinant cells to coexpress human ERs (Rodriguez-Mozaz et al., 2006). Aptamer-based electrochemical biosensors have also been reported for monitoring water quality (Rapini&Marrazza, 2017).Also, a multi-channel two-stage mini-bioreactor based on genetically modified bioluminescent bacteria is used to detect the toxicity of some EDCs (Long, Zhu, & Shi et al., 2013; Rodriguez-Mozaz et al., 2006). Other bio-sensing mechanisms are available for the determination of EDCs including cell proliferation, luciferase induction, ligand binding, antigen–antibody interactions or vitellogenin induction (G. Pranjali et al., 2012).

7. Conclusion

In this document, the basic concepts of biosensors, their type and applications are discussed. And after; the recent advancement of the technologies with respect to monitoring the water quality (identification of water pollutant) is also tried to be reviewed.

Depending on the types of biological elements and transducers employed a wide variety of biosensors are available. Biosensors can be used for the rapid monitoring of water quality with a high specificity to a wide range of analytes, both as online and offline systems. Online systems are preferable for obtaining continuous real-time data. They can also provide early warnings of the spread of diseases by monitoring toxins or bacteria in the samples. Commercial-scale application of biosensors is, however, still limited due to various factors such as the size, cost, and detection range, leakage of biological elements, selectivity and online deployment. In order to bring about a novel analytical device revolution, the scientists must consider these factors when designing biosensors. Advances in nanotechnology, biotechnology, microelectronics, microfluidics, telecommunication and other fields are promising and can be used to improve biosensor technologies. Successful integration of nanomaterials with biomolecules on the surface of electrodes or nanofilms may lead to the evolution of a novel generation of biosensors. Such devices can play a crucial role in clinical diagnosis, process control, food analysis and environmental measurement in the coming years. Even though, there are substantial advances in this field, a lot more remains to be accomplished, with a long way to go and explore

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