

## **Mini Recap on electricigens Biosensor and their applicability:**

**From Rishika Khanagwal, Prof. Shamama Ahmed, Prof Dr Mohd. Muazzam**

### **ABSTRACT:**

Microbial fuel cell (MFC) is a favourable platform for continuous energy generation and monitoring wastewater treatment due to the electrochemically active microbes backing. Their considerable usage is in removing pollutants or toxic elements as a water softener in biosensors and formation of a few compounds. Despite various drawbacks predominantly including insufficient power supply, frequent noticeable growth in microbiological, chemical, and electrochemical viewpoints enables envisioning microbial fuel cell (MFC) biosensors as standard future analytical technology. This review summarizes the basic concepts, general understanding of the important associated terms with examples. The in situ online monitoring approach draws a special attention to the applicability part that majorly entails quantitative analysis of BOD and toxicants, along with additional benefits like monitoring specific microbial activity, MIC sensing, powering other sensors externally, antibiotic detection etc. Insight to modern strategies including miniaturization along with modified versions for better sensing is also provided. This paper briefs the microbial fuel cell (MFC)s as biosensors including the challenges and its future perspective.

**Keywords: Microbial fuel cells, Exoelectrogens, Biosensors, Microbial fuel biosensors, environmental monitoring, Scaled down approach etc.**

### **INTRODUCTION:**

With per day increase in the environment depleting activities globally follows various risks denying sustainable and healthy planet to live in. Thus, exploring in how to extract maximum benefits from resources that are ecofriendly and are renewable in nature should be the current topic of discussion. Microbial fuel cell-based biosensor is a promising technology where biofilm grown at the electrode is its functional basis. Such cells widely broaden the sensing applicability in biotechnology, later discussed in this mini review. This paper provides a quick recap regarding microbial fuel cell (MFC) biosensors and relevant terms connected with it.

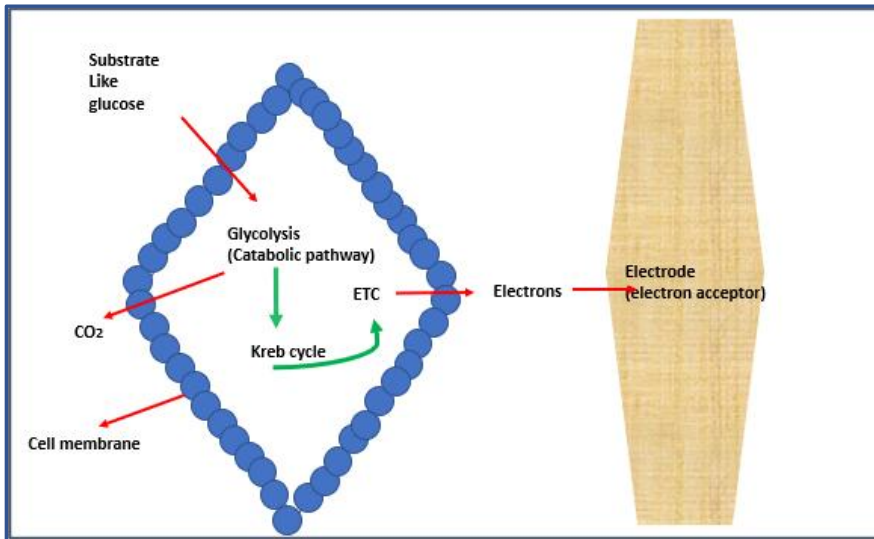
#### **1. Exoelectrogens**

They are the captain of the so-called ship microbial fuel cells.(1). The prominent characteristic of Electricigens is it oxidizes organic, certain inorganic matter and shifts the flow of electrons outside the bacterial cells (hence termed Exo) to the electron acceptor for power production. These microbes can be easily isolated from anaerobic sludge and wastewater treatment plants, anaerobic sediment, primary and municipal effluent, or even farm soil.

##### **1.1. Metabolism of electricity generating microbes.**

Generally, a bacterium has its own choice for oxidizing a sort of substrate. In addition to this, exoelectrogens choose specific type of pathway, genes, or proteins responsible in degradation of the substrate. Therefore, a suitable selection of electron loving bacteria along with its preferred substrate is immensely important for the required output in an

Microbial Fuel Cell (MFC). Evidently when anaerobic-aerobic sludge inoculum with glucose was added as substrate to Microbial Fuel Cell (MFC), within three months the electricity production increased 7 times. (2).



**Figure 1: This is a general Electron Transport Chain pathway of how exoelectrogens regulate catabolism and cellular respiration.**

Substrate level phosphorylation and oxidative phosphorylation are the ways with which a microbe generally earns ATP for gaining energy. In former case ADP either combines with phosphorylated intermediate or inorganic phosphate to yield energy. While latter involves ETC dependent ATP synthesis where oxidation occurs [both inorganic ions (in chemo-lithotrophs) and organic compounds (in organo-heterotrophs)] of lesser redox potential associated with reduction of electron accepting electrode of higher (more positive) redox potential. However, in phototrophs light is the driving factor for ETC dependent formation of ATP. Microbial colonization (mostly at anode) is the chief attribute of electricigens. The electrode acts as the electron acceptor when bacteria oxidize organic matter in anodic chamber. The electron acceptor can be molecular oxygen, or dissolvable compounds like (or iron/ manganese oxides) depending on aerobic and anaerobic breathing of cell resp. (3).

## 1.2. Electron transfer trackways to anode

There are three ways of electron transfer to the electrodes i) Direct contact with electrodes. On the outer surface present redox-active proteins like cytochromes in cell membrane regulates this short range transfer. II) Through

soluble electron shuttles secreted by microbes. iii) Long range transfer- for single layer biofilm it is easy to interact with electrode surface but in multilayer biofilm most cells are not in close proximity to electrode surface and that is why they choose long range electron transfer mechanism where transfer of electrons occur with conductive pili (dense network) called nanowires. A tabular description for this topic is as follows:

Exoelectrogens	Pathway name	Pathway carriers	Ref.
<b>G.sulfurreducens</b>	Short range	..C-cyts present electrons to different electron acceptors. ..OmcZ-regulates(homogeneous) direct electrontransfer. It is an Electrochemical gate that lies betweenelectrode adhered microbial cells and electrodesurface. ..OmcF helps in transcription of genes involvedin electricity production	(4),(5), (6),(7)
<b>T.potens</b>	Short range	MHC's(c-cyts) are involved in electron transportation across gram positive cell envelop.	(8)
<b>Pseudomonas aeruginosa</b>	Electron shuttle	Pyocyanine and phenzamine-1-carboxamide-self-secreted electron shuttles	(9)
<b>Shewanella.sp</b>	Electron shuttle	Produce flavin and riboflavin	(10)
<b>Klebsiella pneumoniae</b>	Electron shuttle	2,6-di-tert-butyl-p-benzoquinon	(11)
<b>G. metallireducens</b>	Long range	The fliC (Flagellin protein) and type 4 Structural protein pili (pilA) deletion resulted in declineof electron transfer toelectrode.	(12)
<b>S. oneidensis</b>	Long range	Electrically conductive- MR-1nanowires	(13)

**Table 1: List of few Exoelectrogens mostly studied and their observed preferred pathway**

### **DIET –**

As the name suggests it is the direct inter species electron transfer method for microbial fuel cell (MFC) containing co-cultures. Example: Conductive nanowires by P.thermopropionicum for efficient energy distribution and electron transfer setup bonds with M.thermautotrophicus. DIET also is seen with G.metallireducens and Methanosaeta harundinacea in anaerobic digesters (14).

## 2. Microbial Fuel cells:

Electricity generation by microbes is way older than one can imagine, still it took past few years to bring its applicability as novelty in laboratory (15). MICROBIAL FUEL CELL (MFC) employs chemical reactions in a setup like any other commercial battery that involves electrodes and electrolysis which can be visualized in Fig 2.

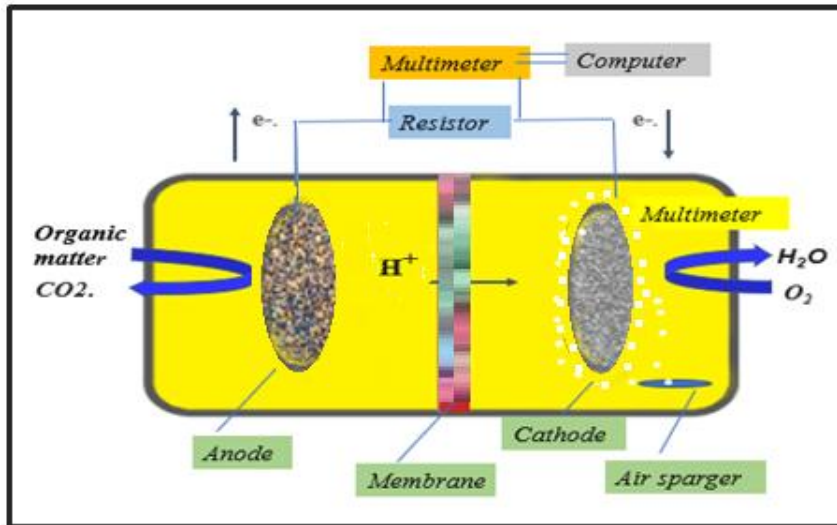
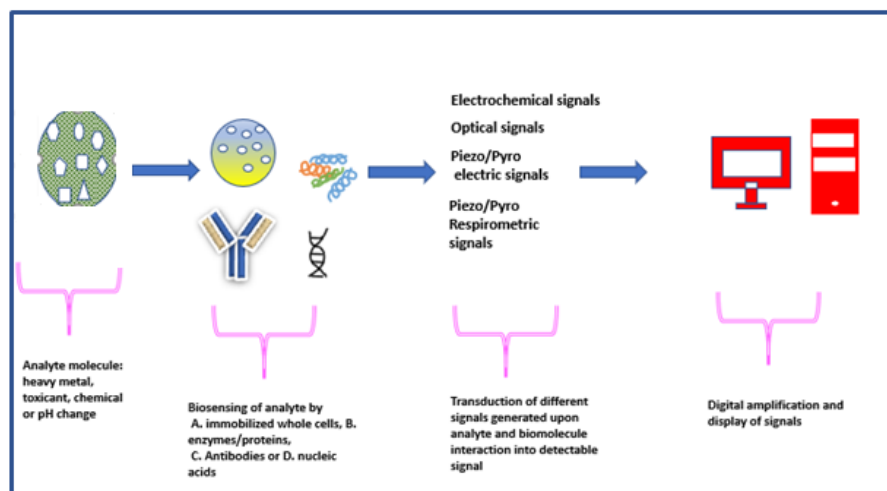


Figure 2: It is a pictorial representation of a Microbial fuel cell activity.

## 3. Introduction to Biosensors:

Leyland C. Clark in 1962 gave birth to the concept of biosensors. Glucose oxidase (Immobilized enzyme) that converted platinum electrode to powerful instrument for glucose detection in diabetic human samples is a classic example build by Clark (16). Biosensors are scientific devices that use bio-sensing components in concurrence with physiochemical transducers for bioanalytic measurements in easy to use formats (17). Drug discovery, food safety, environment monitoring, defence are major areas where its applications exist.

**3.1 Fundamentals of Biosensors:** Biosensors predominantly comprise three constituents- biorecognition element that detects the analyte based on which it generates signal. Second part is the transducer that is present in any biosensing gadget and third is a detector that catches and amplifies the signals before presenting on electronic screens (Fig 3 represents the process of biosensors). Bio element can be of any organic body that senses an analyte from the medium of interest and could be polysaccharide, microbial nucleic acid, tissue, enzyme or an antibody whereas sensor part entails the signal transduction section and can be of optical, electrochemical or magnetic type etc with components such as viscosity, temperature, mass, electric current, electric potential, electric impedance, EM radiations (18).



**Figure 3: Stepwise pictorial description of working of a biosensor**

Difference in a biosensor is mainly due to difference in its transducer and therefore the classification majorly falls into electrochemical, optical and other transducers (baroxymeter and infrared analyzer based sensors for pressure change and CO<sub>2</sub> detection resp.). Electrochemical sensors on analyte interaction with bioelement utilize the fluctuations in the form of voltage, current, capacitance and conductance that eventually differentiates the types from amperometric, conductimetric to impedimetric sensors. Changes in the optical properties attained if due to the interaction between biocatalyst and analyte then they are referred as bioluminescence sensors, fluorescence and even as colorimetric sensors. Other type is piezoelectric or gravimetric biosensor that detects the resonating frequency change of piezoelectric material based on surface molecules adsorption or desorption (19). Table 1 illustrates few examples of biosensors.

Biosensors	Features with Examples	Ref.
<b>Amperometric</b>	..Holds great potential to be used in food industry ..Monitors Ethanol, glucose and lactate in wine.	20
<b>Potentiometric</b>	..Ion selective electrodes for electrical output. ..Initially used ammonia for urea detection. ..Also determines carbon, sugars, and pesticides.	21
<b>Conductimetric</b>	..Thin film electrodes as cost effective approach towards miniaturization. ..No reference electrodes and light sensitive transducers needed. ..Detects protein markers and heavy metals	22
<b>Microbial fuel cell</b>	Self-powered portable devices capable for good online monitoring of environment (BOD, chemical pollutants) ex-Cr <sup>+6</sup> was sensed in 1-8mg/L range within 74 mins.	23.

<b>Bioluminescence</b>	..Uses Recombinant bioluminescent cell. ..Cells responds to genotoxic agents by transferring bioluminescence signals from analyte through optical fibers. ..Modified E.coli produced luminescent signals in genotoxic agents presence.	24
<b>Immunosensors</b>	..Immunochemical reactions assisted with transducers. ..Antibodies bind to their specific antigens and interact with pathogens toxins. Ex-PfHRP2 biomarker for malarial detection	25
<b>Fluorescence</b>	..Molecular fluorescence is very beneficial as it can detect a single molecule also, no damage to host system, both fluorescence intensity and decay can help in measurements. Ex. Concanavalin A, ..Glucose oxidase and dehydrogenase are few fluorescence based glucose sensors.	26
<b>Baroxymeter</b>	..They are microbial pressure sensors. ..Pressure drop in close vessel due to microbial intake of oxygen is termed as Bacterial Respirometry which is the basis for toxicity assessment in wastewater.	27
<b>Biodefense</b>	..Biological attack, in detecting biowarfare agents by recognizing chemical markers. Ex- HPV can be detected by nucleic acid biosensors	28
<b>Impedimetric biosensors</b>	..Can be enzyme, Nucleic acid, Immuno and microbe based sensors. ..Can monitor catalyzed reactions of enzyme along with characterizing biorecognition events of specific lectins, proteins, receptors, antibodies etc.	29

**Table 2** :Different types of biosensors with their important examples.

#### **4. MICROBIAL FUEL CELL (MFC) based biosensors:**

The exoelectrogen's property of converting the substrate into generating electric current empowers an microbial fuel cell (MFC) to be a microbial transducer that replaces the need for an external transducer. A Dual chambered microbial fuel cell (MFC) is compartmentalized into two sections named anode and cathode separated by proton exchange membrane. Microbes are allowed to digest the fuel provided and colonize at anode electrode for generating electrons and protons. Electrons are transferred through external circuit and proton through the semi permeable membrane in cathodic chamber where in contact with oxygen these ions ultimately produce current which can be used for different purposes. With this working mechanism the current generation or depletion in a microbial fuel cell based on analyte addition also allows an Microbial Fuel Cell (MFC) exhibit biosensing potential. Easy portability, environmental sensing at remote areas, self- powerhouse and long term operation are

some important traits that favor microbial fuel cell (MFC)s as biosensors. They are preferably involved in online monitoring for biotoxicity and biological oxygen demand measurements. The linearity observed in current production and BOD concentrations is the reason why we have Microbial Fuel Cell (MFC) BOD biosensors. Let us look at a few examples for microbial fuel cell (MFC) biosensors. Glucose single chamber MICROBIAL FUEL CELL (MFC) biosensor showed linearity up to 25g/L of glucose with detection limit of 0.025g/L (30). Wall jet microbial fuel cell (MFC) was used as a sensor in order to detect volatile fatty acid and gas contents (31). First BOD detected in microbial fuel cell (MFC) was till 100mg/ml limit; later a packed bed microbial fuel cell (MFC) with carbon cloth anode detected enhanced BOD range up to 350mg/ml (32). Components of microbial fuel cell (MFC) biosensors can also be seen in figure 4.

#### **4.1 Parameters that influence a MICROBIAL FUEL CELL (MFC) sensor:**

The rate of electron extracellular transfer (EET) is of paramount importance for it regulates the operation and determines the efficiency of microbial fuel cell (MFC) sensors. A healthy biofilm at anode was observed to efficiently enhance the EET even when mediators were not present. Although adding riboflavin to individual *shewanella* can increase voltage by minimizing internal resistance (33), it is also believed that mediators added externally have a tendency to create toxicological issues and that's why this external addition might not be appropriate in the actual application of microbial fuel cell.

*Shewanella* produces riboflavin for its EET but this component can be adversely affected by change in electrolyte's pH resulting in voltage variation due to internal resistance created in the system (34)

Anode type is also a relevant factor. The fact that surface modification of anode can result in improving efficiency of the system by providing high surface area for biofilm growth is supported by Kong et al who used a novel niobium doped electrode (lanthanum calcium ferrite perovskite) that showed convincing results (35).

Even though metals are better conductors, still they are ruled out for their corrosive nature but apt surface modifications like mixing manganese sulfate with graphite powder for replacing simple graphite brush anode increased the output 1000 folds (36). Factors like conductivity, chemical balance, biocompatibility makes an anode influence the system's operational efficiency.

Another study also revealed that sensitivity of microbial fuel cell (MFC) sensors showed no significant impact by ion exchange membrane types on it; including cation, monovalent cation, bipolar and anion membranes. High sensitivity characteristic is depicted by increased overpotentials and therefore at elevated current density (37). Performance of PNP biosensors was influenced by other parameters like temp, pH, and PNP concentration. (38) Different catalysts used for cathode working also have an influence as expensive nanoparticles like FePO<sub>4</sub> in place of carbon or platinum have shown promising results (39). Electrolyte pH,

system sensitivity, anode and cathode type along with saturated organic matter amount are some basic parameters that must be controlled in order to have their positive influence over the microbial fuel cell sensors.

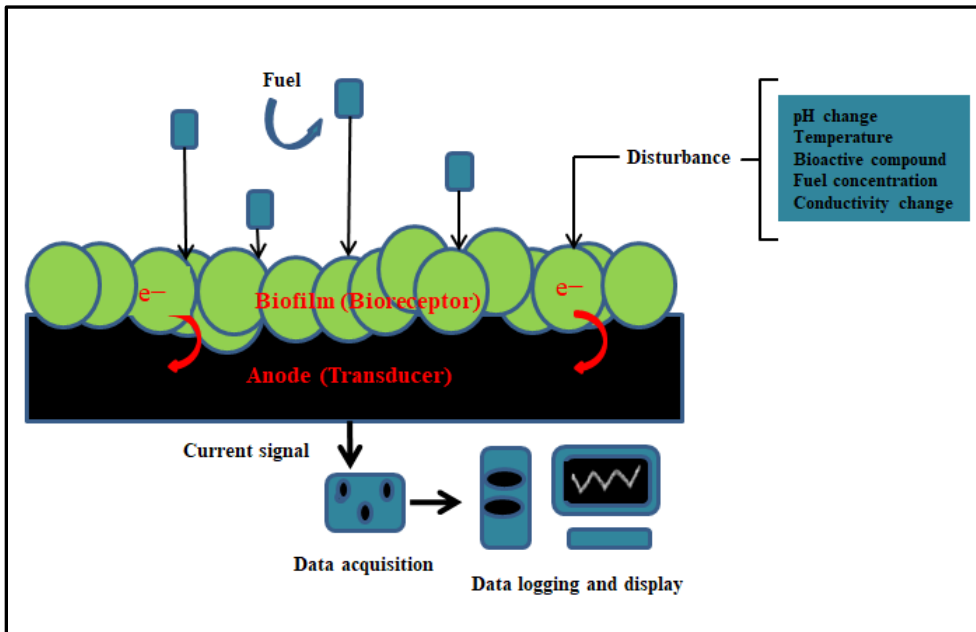


Figure 4 is a pictorial representation of Microbial Fuel Cell (MFC) biosensors.

## 5. An insight to several applications:

### Environmental monitoring:

#### 5.1 Microbial BOD Sensors:

Biological Oxygen Demand is a general variable for assessing sewage water treatment plants and is measured as the biodegradable components quantity present in the water. Expression of contamination with respect to the organic content present in wastewaters is done by analyzing **BOD** and COD levels as a regular schedule. COD relates to chemical oxidation of such organic compounds with added advantage of analysis in lesser time, roughly few hours. Calculation of BOD, however, is based on dissolved oxygen quantity required by aerobic microbes specifically for biological breakdown of organic content and that is why it correlates categorically to biodegradable organic matter present in the sample. According to APHA i.e. American Public Health Association, the sample incubation for 5-7 days with seed microbe grown at roughly 20°C in the dark before DO (dissolved oxygen) measurement is the traditional BOD analysis protocol (40). There is a downside to this conventional analysis along with sizeable time consumption drawback, this offers lesser reproducibility, incomplete accuracy and demands for huge labor input (41-44) that ultimately leads to loss of quick and effective online monitoring of a biosensor. As a result, various biosensors based BOD measurements that encompass speed and accuracy are understood and performed by various researchers.

In BOD Microbial Fuel Cell (MFC)'s current production by the anaerobes upon substrate digestion is exactly implied to sample's BOD load. Initially, fed batch organic load is given to a MICROBIAL FUEL CELL (MFC) for noting highest current generated. A link obtained between generated peak current and the fed organic load allows the evaluation of total charge passed that further contribute in knowing unknown sample's BOD. Hence, Linear Microbial Fuel Cell (MFC) response over BOD range along with quick response and minimum recovery duration are much necessary in such sensors. Conventional ways for BOD calculation involved dissolved oxygen measurements, bacteria's fluorescent and luminescent behavior. Membrane fouling in these sensors and requirement of additional external power were the major drawbacks along with difficult management and unstable time duration. Therefore, Microbial Fuel Cell (MFC)s started gaining attention as an alternative as they offer less analyzing time, broad analysis range, cost effectiveness and real time assessment (45).

Long back microbial fuel cell (MFC) used mediators (like methyl viologen+ or thionine) were replaced with mediator less Microbial Fuel Cell (MFC)s as they were costly, inconsistent, and contributed to toxicity (46). Kim et al. were the first one to report mediator less Microbial Fuel Cell (MFC) (47).

They described mediator less microbial fuel cell (MFC) exhibited a sort of linearity between BOD concentration (up to 206 ppm) and current generation with a stable performance for five years. Their results also revealed that at higher BOD concentration linear behavior was no more observed and in such sensors the reaction time was told to be dependent on BOD concentration as sensors took long to measure the BOD.

Real time monitoring of a sewage water plant through a dual chamber microbial fuel cell (MFC) showed stability in its performance for 60 days with bod range 91-142 ppm (48). In situ monitoring application for microbial fuel cell (MFC) BOD sensors also carry a big architectural drawback i.e. it's closed compartmentalized structure and to overpower this, new design was made that could be completely immersed in wastewater (49) and consequently gave a quick response (30 minute to 10 hour) with linear relation in BOD range 17-78 mg O<sub>2</sub>/L.

There are many disadvantages to Microbial Fuel Cell (MFC) BOD biosensors that include generation of overpotentials and unregulated PH shifts for which external power of 0.1 V-0.8 V reduced the concern of overpotential development and ion exchange membrane removal solved the PH shifts issue (50). Diffusion of higher redox potential compounds (in wastewater) like nitrates and oxygen in anode section from ion exchange membrane is another disadvantage. To overcome the unwanted effect, azide and cyanide (respiratory inhibitors) inhibited the oxidase and nitrate reductase by lessening the negative effect of electron transfer and current production (51). A new attempt to enhance sensitive response for BOD detection was usage of sulfonated polyether ether ketone membrane that showed 62.5% enhanced sensitivity than Nafion membrane (52).

Bio sensing is also influenced by variables like temperature and electrolyte (53). Maximum signal was received when 25mM PBS was used with 50mM NaCl in cathode at 37<sup>0</sup>C where temperature and electrolyte strength provided ideal conditions for bacteria to grow and biosensor showed 25mg/L glucose sensitivity limit within 5 minutes that further indicated this sensor design to be more appropriate for BOD measurements. There are numerous factors that regulate the activity of a biosensor such as PH, temperature, electrolyte conductivity, response time, oxygen diffusion etc. Below table contains types and little description of few BOD sensors.

<b>MICROBIAL FUEL CELL (MFC) sensors</b>	<b>BOD sensing</b>	<b>Ref</b>
<b>MICROBIAL FUEL CELL (MFC) BOD sensor with no mediator</b>	Mediator less MICROBIAL FUEL CELL (MFC) was used as the conventional way of determining BOD. Agro, dairy, distillery and municipal waste water were the substrates utilized that exhibited BOD concentrations of 200.270,420,140 mg/l resp.	<b>54</b>
<b>Single Chamber MICROBIAL FUEL CELL (MFC) BOD sensor</b>	Glucose glutamic acid, ethanol and acetate were the substrates used. For acetate with integration time 20-60 hours maximum BOD was 1280mg/l resp. GGA with integration time 15-40 hours gave maximum BOD of 800-1000mg/l. Ethanol from 5-20-hour integration time exhibited 320mg/l.BOD	<b>55</b>
<b>Dual chambered</b>	Anaerobic phase of A2/O from treatment plant was used for inoculated sludge. External regulation for optimal conditions was established by adding 500-ohm resistance, phosphate buffer, 7pH and L-cysteine as oxygen absorbent BOD range detected was 10-100mg/L in ten hours	<b>56</b>
<b>Miniature MICROBIAL FUEL CELL (MFC)</b>	Change in BOD was observed within nineteen minutes in this 68μL volumed single chamber MICROBIAL FUEL CELL (MFC) with acetate as a fuel source.	<b>57</b>
<b>Submercible MICROBIAL FUEL CELL (MFC) BOD sensors</b>	Biofilm colonized anode had the decisive power towards the application of this sensor. Groundwater was the substrate with biofilm grown at anode BOD detected was 250mg/L	<b>58</b>
<b>Oligotrophic MICROBIAL FUEL CELL (MFC) BOD sensor</b>	Surface water enriched MICROBIAL FUEL CELL (MFC) showed 6mg/L BOD with 2.6ml/min for 30 minutes feeding rate. They provide reproducibility, stability and long-term operation with low BOD detection limit.	<b>59</b>

**Table 3: MICROBIAL FUEL CELL (MFC) BOD based sensors**

## **5.2 Toxicity sensors**

A sensor that depicts presence of harmful and poisonous compounds in a medium is termed as a toxic sensor. Due to high cost involved in on-site toxic level monitoring, the standard conventional toxicity detection protocol including chemical tools like GC, GC-MC, LC-MS, and HPLC is not preferred (60). MICROBIAL FUEL CELL (MFC)s here fit in the gap as they help in acquiring a basic device i.e., small size, fast, cheap along with good sensitivity characteristic that further aims to provide toxicant biosensing application. Toxicants are present in surroundings both chemically (heavy metals and complex organic compounds) and biologically (metabolic products of some pathogens or physical compounds like coal dust, heavy particles that affect the metabolic activity). Therefore, toxicity monitoring is of utmost importance from public health safety point of view for which microbial fuel cell (MFC)s can be considered as potential contaminant analyzing candidates. In presence of toxicants, microbes change their metabolic activities which is the principle reason on which mechanism of these biosensors rely making the microbial fuel cell (MFC) approach for real time monitoring and detection of toxicants successful for treating the wastewater.

### **A Heavy Metals:**

Heavy metals are difficult to be reduced or removed by certain microbes as they possess long half life time of ten to hundred years. Although some are beneficial to humans, but exceeding a certain concentration while accumulating may give rise to many severe health problems. They level up the scale of toxicity and accumulate in humans by interfering in food chain cycle causing serious health issues like organ damage. Multiple organ or single organ failure by heavy metals is governed by various factors such as its dosage, chemical species, exposure route, gender, age, genetics and individual's nutritional status. Arsenic, cadmium, lead, mercury are some of the most hazardous heavy metals stated as known or probable carcinogens by US Environmental Protection Agency and International Agency For Research On Cancer.(61). Heavy metal ions in a MICROBIAL FUEL CELL (MFC) inhibit exoelectrogen's respiration activities affecting the resulting current output. According to (yu et al), six metal ions were examined out of which  $Hg^{2+}$  showed highest inhibition ratio 13.99% (62). In another study as a replacement for weak sensitivity and less stable sensors, a flat membrane Microbial Fuel Cell (MFC) was developed that detected chromium and nickel heavy ions. These sensors due to high membrane hydrophilicity resulted in bacteria's adhesion with reduced time of acclimation which is why are regarded as best fit for wastewater shocks instead of monitoring drinking water or for measuring the pollutants with COD and BOD(63). Some heavy metals under anaerobic conditions behave as terminal electron acceptor in a Microbial Fuel Cell (MFC) by giving a tough competition to anode and as consequence lesser electrons are transferred to cathode responsible for limiting output voltage.

Such microbial fuel cell (MFC)'s help in specific target compound, an example for it is hexavalent chromium that is reduced by anaerobes to  $\text{Cr}^{3+}$  (which is less toxic) and serves as electron acceptor that leads to expected voltage reduction(64). Other examples with similar concept-based functioning are *Ochrobactrum anthropic* and *Exiguobacterium aesturii* in microbial fuel cell (MFC) for  $\text{Cr}^{6+}$  detection and linear response was shown for detected range of 0.125-5mg/L and 2.5-60mg/L resp. (65,66). Contrastingly iron oxidizing bacteria showed negative results in the presence of such anaerobic consortium, iron ions behaved as electron donors in the anolyte further giving a linear voltage response to  $\text{Fe}^{+2}$  concentration range 3-20mM(67). Cathode sensing elements also showed good progress in  $\text{Cr}^{+6}$  and  $\text{Cu}^{+2}$  detection. For Cr (VI), sediment microbial fuel cell (MFC) was developed which depicted linearity between increasing voltage and metal ions concentration (0.2-0.7mg/L) accompanied with good sensitivity and specificity even in presence of other ions(68). Similarly in case of  $\text{Cu}^{+2}$  a microbial fuel cell (MFC) was developed where copper ions deposition was on cathode surface as they acted as electron acceptor and linear responsive ranged between 5-160 mg/L (69).

### **B. Antibiotics as toxicants:**

The capability to fight various bacterial infections although led to the terminology of being called as wonder drugs, but antibiotics can also show misleading effects when they are mishandled and exposed to environment and that's why emphasizing on the need for tracing and managing their discharge is extremely essential. Therefore, the real time analytical technology like microbial fuel cell (MFC)'s can be considered as detectors in the fields(70).

A single chamber microbial fuel cell (MFC) carrying hydrophilic carbon anode was developed by Wu et al for detecting antibiotic tobramycin, system showed significant drop in voltage when reached a value greater than or equivalent to 0.93g/L tobramycin concentration. Such sensors demonstrated recovery of exoelectrogenic biofilm microbes after hundred hours that indicated the strength attained due to self-healing microbial properties(71).

Schneider et al tested ten Beta lactam antibiotics against *E. coli* and *Staphylococcus aureus* at different concentrations ranging from 1 to 75  $\mu\text{g}/\text{ml}$  with a panel system which was created by joining miniaturized microbial fuel cell (MFC)'s together and concluded this as a faster approach because measurements due to change in output voltage were recorded within four hours, which is contrasting to the conventional disc diffusion technique that takes one to two days for exhibiting results (72).

Examination of another single chambered microbial fuel cell (MFC) for analysing an antibiotic named levofloxacin illustrated a detection limit up to 1000  $\mu\text{g}/\text{L}$ . Ferric phosphate nanoparticles were used as cathode catalyst that helped in detection of varied concentrations within five minutes. For LEV concentration range 0.1-

100 µg/L a linear interval response was noted. The advantage of long-term usage was also seen as it produced steady state of electricity for about two years (73).

### **C. Organic contaminants:**

Organic nitrogen, phosphate and polychlorinated biphenyl are some of the most commonly available compounds present in water bodies that promote eutrophication and as a result public health is compromised. A dual chambered microbial fuel cell was utilized for measuring PBS and an organophosphorus compound detection, the inhibition ratios indicated by Kim et al for diazinon and PCB were 61% and 38% resp. (74).

A single micro sized microbial fuel cell with optimized anode (0.2 V) against a reference electrode was constructed for formaldehyde detection in water. Fast current signals were sensed depicting high sensitivity for pollutant concentration ranging from 0.001 to 0.1%. This miniaturized approach is special as it uses three electrode configuration that provide enhanced sensitivity and reliability and an air bubble shield that uplifted microbial biofilm growth and the transfer of electrons (75).

Paper microbial fuel cell (MFC) is one of recent discoveries for detecting toxicants (chemicals) in water phase that showed significant voltage drop on 0.1% formaldehyde addition. The carbon (biodegradable) was printed on sheet paper for electrode preparation; The anode was kept in liquid phase with cathode exposed to air. The paper basement worked as a separator between electrodes that allowed easy mass transfer possibly due to the capillary action of the paper. In addition to this, two microbial fuel cell (MFC) can be grouped together in a parallel order providing a better sensitivity for formaldehyde with complete inhibition in 115 minutes which is 60 minutes more than what is seen in single paper MICROBIAL FUEL CELL (MFC) (76). A contrasting example in terms of inhibition effects is a dual chambered MICROBIAL FUEL CELL (MFC) developed by Chen et al that showed increase in voltage output instead when p-nitrophenol (PNP) was added as a substrate against aerobic strain *Pseudomonas monteilii* LZU-3 in an anode chamber under optimal conditions of external resistance, pH and temperature (77).

### **D. Acid toxicants:**

Detection and online monitoring of acidic toxicity in a water body is of prime importance as it can destabilize the aquatic ecosystem. Toxicity present in wastewater (like mine drainage) have a tendency to lower the water pH that further reduces important microbial activity, affects the aquatic life forms and mitigates the self-purification attribute of water bodies, cumulatively all these drawbacks deteriorate the water quality. Microbial fuel cell (MFC)'s can be used as warning signals in advance where toxic inflow into waste water plants can be detected when a toxic incident is created through HCL addition for changing the pH. A batch mode fed single

chamber air cathode microbial fuel cell (MFC) was designed by Shen et al where externally HCL was added in the electrolyte chamber to modify the pH.

At pH 3-4, voltage reduction was seen which was recovered after no further HCL addition. But there was irreversible damage by biofilm at pH 2 so significant output voltage drop was observed in such a case of strong acidic condition(78).Cathode share sensor by Jiang et al was another important design as it neglected the variations occurring in cathode and thereby guaranteed detection. These sensors have a potential to detect pH in water i.e., based on interruption in microbial fuel cell (MFC) voltage. Toxic shock by acidic anolyte was applied and immediate fall in voltage from 200mV to 0 was observed when pH was decreased from 6-4 (79).Reporting of damage by Acid rain is another impressive feature made possible. Rhizosphere microbes have a potential to generate current by degrading organic extracts of rhizodeposits in a plant microbial fuel cell (MFC) which clearly stated that any change in current occurred must have got influenced by the change in concentration of bioavailable substrate(80). In a plant microbial fuel cell (MFC) concentrated H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> solution stimulated acid rain and therefore its application ends up in reducing rice plant's photosynthetic activity. There was repeated voltage drop observed in every two minutes when artificial rain was sprayed on plant leaves which is also relatable to electrochemical behaviour seen by rhizosphere microbes (81)

Shock sensors	Toxicity sensing	Ref.														
<b>Potassium Ferricyanide mediated MICROBIAL FUEL CELL (MFC)</b>	Heavy metals (2mg/L) and their corresponding inhibition ratios tested by this biosensor. <table border="1"> <thead> <tr> <th>Heavy metals</th> <th>Inhibition ratios</th> </tr> </thead> <tbody> <tr> <td>Cu<sup>2+</sup></td> <td>12.56%</td> </tr> <tr> <td>Hg<sup>2+</sup></td> <td>13.99%</td> </tr> <tr> <td>Zn<sup>2+</sup></td> <td>8.81%</td> </tr> <tr> <td>Cd<sup>2+</sup></td> <td>9.29%</td> </tr> <tr> <td>Pb<sup>2+</sup></td> <td>5.59%</td> </tr> <tr> <td>Cr<sup>3+</sup></td> <td>1.95%</td> </tr> </tbody> </table>	Heavy metals	Inhibition ratios	Cu <sup>2+</sup>	12.56%	Hg <sup>2+</sup>	13.99%	Zn <sup>2+</sup>	8.81%	Cd <sup>2+</sup>	9.29%	Pb <sup>2+</sup>	5.59%	Cr <sup>3+</sup>	1.95%	<b>82</b>
Heavy metals	Inhibition ratios															
Cu <sup>2+</sup>	12.56%															
Hg <sup>2+</sup>	13.99%															
Zn <sup>2+</sup>	8.81%															
Cd <sup>2+</sup>	9.29%															
Pb <sup>2+</sup>	5.59%															
Cr <sup>3+</sup>	1.95%															
<b>Prussia blue cathode MICROBIAL FUEL CELL (MFC)</b>	Sensitive detection with no extra power supply 1mg/L metals like Co <sup>2+</sup> , Cd <sup>2+</sup> , Pb <sup>2+</sup> ,Cu <sup>2+</sup> showed inhibition absorbance 28.4% 11% 33.8% 66.6% resp.. Cu <sup>2+</sup> >Pb <sup>2+</sup> >Cd <sup>2+</sup> > Co is the toxicity order detected	<b>83</b>														
<b>Soil microbe (K<sub>3</sub>FeCn<sub>6</sub>) MICROBIAL FUEL CELL (MFC)</b>	Cadmium toxicity to soil can be measured by electrical signals. Soil microbes were used to generate electricity. Startup time of the MICROBIAL FUEL CELL (MFC) and the coulomb generated showed linear response to Cd <sup>2+</sup> concentration range(10-100mg/kg).	<b>84</b>														
<b>Photo based biosensor</b>	First plant MICROBIAL FUEL CELL (MFC) used as water sensor Microalgae from wastewater plant is the inoculant Detected formaldehyde sensitively and quickly (69.2±16.7% <sup>-1</sup> cm <sup>2</sup> )	<b>85</b>														
<b>Mediator based sensor</b>	Methylene blue is used as mediator. Detected even low limit arsenic (arsenate 46µM arsenite 4.4µM)	<b>86</b>														

<b>SDS MICROBIAL FUEL CELL (MFC)</b>	Dual chambered MICROBIAL FUEL CELL (MFC) with mixed microflora. Sensor response to sodium dodecyl analyte: Higher external resistance 1000ohms showed lesser recovery time 2.5hrs but for 100 ohms exceeded 3.2 hrs	<b>87</b>
<b>Single chamber air cathode</b>	Inhibition ratios for Cu (II) at flow rate 1.3ml/min with response time 120 minutes 5mg/L-60 7mg/L-85	<b>88</b>

**Table 4: List of few MICROBIAL FUEL CELL (MFC) toxicity Sensors**

### 5.3 Different sensory applications:

Microbial Fuel Cell (MFC)'s can also contribute in detection of air quality and carbon monoxide monitoring is one such example. It is very harmful gas which works on the principle of inhibiting anode activity in a microbial fuel cell (MFC) resulting in reduction of power production. The proportionality observed due to voltage drop on CO addition helped in its analysis. The range detected was 10 to 70 % and drop in voltage was within 0.8-24mv, with response time of nearly 60 minutes (89). For detecting formaldehyde, biocathode used as a sensing element is a novel gas diffusion-based construction done by Zhang, et al. This gas diffusion-based sensor worked both in aerobic and anaerobic water and directly detected formaldehyde sensitively ranging from 0.0005-0.005%. With electrochemical study the reason for response of this sensor was stated as inhibition of microbe toxically for the oxygen reduction also called cathodic reactions (90). With the increase in land requirements, actions to combat emission of greenhouse gasses must be investigated and therefore, this methane emission flux analysis is very needful. Another novel application of microbial fuel cell (MFC)'s as sensors deals with methane gas emission (91) that states a positive correlation between exoelectrogenic and methanogenic activity in abundance in the paddy fields of china. This sensor is an alternate to principal that only on artificial enrichment (i.e with oxidants for long time microbial fuel cell (MFC) operation) exoelectrogens can suppress methanogenic property. Exoelectrogens abundance, voltage signals were directly correlated with methane emission flux and mrcA gene quantity. These sensors showed a quick response time of ten minutes. Better, correct and quick detection of H<sub>2</sub>O<sub>2</sub> is much required in environmental, physiological and industrial applications and microbial fuel cell (MFC) has made this possible (92). For this two sensory configurations were employed with anionic exchange and cationic exchange membrane resp. out of which CEM proved to be superior as it showed higher sensitivity for H<sub>2</sub>O<sub>2</sub> 11.0  $\mu$ A mM<sup>-1</sup> and fast response of five seconds. Through ANN technology incorporation, the peak areas and height for fermentable substrate like glucose starch can be differentiated with non-fermentable substrates like acetate and butyrate, this is also very useful application of water testing for different substrates as determined by Feng et al., 2013 (93) where non fermentable substrates gave higher peaks.

### A. VFA biosensors

Knowing the volatile fatty acid concentration is crucial in microbial fuel cell (MFC)'s. VFA adds a benefit of monitoring biogas generation which is much required as unstable anaerobic digestion of biogas is a limitation for it to completely replace fossil fuels as an alternate in spite of having many benefits (94). Jin et al developed a three chambered system in which anaerobic effluent in middle chamber moved to anodic chamber (through AEM) where ironized VFA's produced electrons abundantly and oxygen reduction reaction was carried out at cathode. Broader range 170-3405mg/L was detectable as microbial community was separated with bulk solution (95).

Kaur et al proposed microbial fuel cell (MFC) sensor array which with voltage/ current correlation with VFA quantity sensitively detected acetate, propionate and butyrate in range 5-40mg/L microbial fuel cell (MFC) biosensors allow voltage and current correlation with VFA quantity as depicted by kaur et al (96).

### **B. Dissolve oxygen detection:**

Dissolved oxygen measurement plays a vital role for water quality control as changes in its level reflects the presence of organic pollutants in a water body. Its real time analysis leads to easy understanding of aquatic ecosystems (97, 98). Clark type oxygen sensors are the conventional ones replaced by microbial fuel cell (MFC)s as they easily get affected by environmental parameter like pressure, temperature. The alternative allows in-field monitoring and is comparatively much stable for longer time. For D.O measuring microbial fuel cell (MFC)'s, the working foundation bases on the cathode behavior (97). Although cathodic efficiency challenges the microbial fuel cell (MFC) systems performance, but oxygen behaving as electron acceptor helps in determining the cathodic reactions of oxygen reduction through voltage output. DO monitoring can serve as an alerting signal before time the risks of dead zone formation appear due to periodic oxygen stratification in some enriched freshwater bodies take place (99,100). A Microbial Fuel Cell (MFC) system was developed containing several cathodes placed at differential depths to online monitor the lakes. With an external voltage of 1000 ohms, DO range 0-9mg/L showed linear response to voltage current. On further optimizing the setup, high voltages and good correlation with DO concentration was seen in the span of 67 days (101).

### **C. MIC biofilm sensors:**

Corroded biofilm can easily lead to microbiologically influenced corrosion also termed as biocorrosion which is a major issue in oil, gas and water industry and according to Flemming is also the cause for twenty percent corrosion losses amounting to 50 billion dollar each year in United States (102). Two key causes for corrosion in a MICROBIAL FUEL CELL (MFC) biofilm: Fermentative bacteria release corrosive organic acids in which the low pH established makes this biocorrosion type easily detectable. Anaerobes like sulphate reducing bacteria in the anaerobic environment of oil pipeline, anaerobes like sulphate reducing easily diverts to

elemental  $F^0$  (electron donors) for replacing carbon during starvation period that causes corrosion. This method is more difficult to detect in comparison to first one (103). The extracellular electrons in the reaction process needs to be transported to SRB's cytoplasm for sulphate reduction. This transfer is only possible because of electrigenic biofilm. Detecting corrosive biofilm is important in aspect of decision making for to use what that can mitigate and combat the negative effects of corroded biofilm.

Mechanical ways to detect biofilm includes infra-red absorbance, fibre optical and other electrochemical devices (104). The non-mechanical way involves usage of certain chemicals like biocide (105). The existing biofilm detectors carry a big demerit i.e., need of additional power to detect the resistance of biofilm that eventually interferes with the metabolism of biofilm. Gu, 2012 stated that exoelectrogenic behaviour and response to metal attack can be used to depict the biofilm analysis. In a MICROBIAL FUEL CELL (MFC) biofilm sensor solid state anode feeds cathodic biofilm with electrons and if anaerobes like SRB attaches to biofilm then electrogenic biofilm will transfer SRB biofilm in cytoplasm of sessile cells for sulphate reduction and open circuit voltage after calibration can be used to detect sulfate or nitrate reduction. Measurement of electrogenic biofilms can detect the corrosive biofilm. (106).

#### **D. Monitoring microbial activities:**

Electron input to the anode can help in demonstrating the microbial activities (M.A) and the biomass quantity. This current and microbe relationship here for monitoring is used in two ways. First method is detecting MA with microbial respiration measurement. The respiration is covered by the anode so Microbial Fuel Cell (MFC) current was correlated with biofilm activity but had a drawback as it was restricted to only anaerobic biofilm. Another limitation was: also assessing this microbial biofilm activity as a reflection for elsewhere monitoring site leading to inaccuracies in measurement (107). The Second method: by using relevant parameters to replace biomass as a symbol of active microbial concentration. In one example ATP concentration was selected to know the presence of MA which was correlated with current density and linearity was observed (108). As this method proved to be faster and more accurate therefore is considered better. Another specific example is Ecoli enumeration with thionine as an electron acceptor in Microbial Fuel Cell (MFC) where steady state current was related with ecoli cells number. Along with being rapid it also showed good sensitivity of  $10^5$  cells per ml (109).

M.a was also monitored in arsenate utilizing microbes like *Bacillus selenitireducens* that are found in hyper saline Mono lake and salt saturated Searles lake. These microbes were grown in Microbial Fuel Cell (MFC) with Microbial Fuel Cell (MFC) anode as electron acceptor where growth was independent of arsenate and simultaneously oxidized lactate. When arsenate was added to this, there was decrease in current generation as it increased competence against anode electron acceptor already present in the MICROBIAL FUEL CELL (MFC) (110).

## E. Powering other sensors externally:

There are different examples like coupling of Microbial Fuel Cell (MFC)'s for monitoring anaerobic digester. Liu et al. designed a system with Up flow anaerobic fixed bed, liquid gas separator along with wall jet microbial fuel cell, where due to external circulation the two MICROBIAL FUEL CELL (MFC)s were coupled with the separator and potential data would help monitor the digester. Although capacitor and convertor were externally needed to increase the output, microbial fuel cell (MFC) powered a wireless sensor that could detect environmental parameters such as temperature and humidity (111). One microbial fuel cell (MFC) was attached to a power management cell where detection of humidity, temperature, pH. CO<sub>2</sub> was attained with a similar approach as discussed above (112). A power management unit was created to power an autonomous temperature sensor with DC convertor and capacitors. The coordination of two microbial fuel cell (MFC)'s in a unit was as such that supplied power by one microbial fuel cell (MFC) was for two days and meanwhile other microbial fuel cell (MFC) recovered. This way they supported the sensor for 20 days (113). The biggest advantage of having Microbial Fuel Cell (MFC) biosensors is it demands low maintenance and can operate for longer duration due to self-power machinery unlike battery-based sensors.

## 5. MICROBIAL FUEL CELL (MFC) Improvements for sensing:

These powerful strong sensors still face objections in real world applications, this concern increases the need of bringing modifications in the design, optimization protocols, substrate type and the electrogenicity by biofilm for better and diverse exposure of such analytical featuring technology. Integration of miniaturization approach with nanotech is responsible in modernization for improved microbial fuel cell (MFC)'s to enhance biosensing applicability which is explained with examples in next section. Given below is a table that entails a few examples where modifications done to a microbial fuel cell (MFC) ended up giving a better result.

MICROBIAL FUEL CELL (MFC) sensors	Improvements	Ref.
<b>CNT based biosensing</b>	Carbon nanotube are the anode modifiers used. Simultaneous addition of <i>G. sulfureducens</i> with CNT enabled composite biofilm formation at anode. Reduced startup and stable power generation. Reduced anodic resistance(180ohm) with increased voltage(650mV) maintained. Found to be better than no CNT added MICROBIAL FUEL CELL (MFC)'s	<b>114</b>
<b>MnO<sub>2</sub> sensor</b>	Used as a cathode catalyst for ORR reactions at cathode Both catalyst crystalline forms were compared beta and gamma βMnO <sub>2</sub> gave a better performance and system worked for 1.5 years	<b>115</b>
<b>PB/PANI</b>	Prussian blue polyalanine is modified oxygen reduction cathode.	<b>116</b>

	<p>Showed good cathode potential. Efficiently effective replacement for platinum electrodes.</p>	
<b>Optimized sensor</b>	<p>This sensor was optimized for water toxicity Carbon cloth showed better results than layered corrugated carbon NaAC 0.5mg/ml Internal and external resistance were kept same 0.1mg Cd<sup>2+</sup> and 1 mg Cu<sup>2+</sup> detected on waste water</p>	<b>117</b>
<b>2D anode sensor</b>	<p>Carbon felt and indium tin oxide anodes were compared for Pb<sup>2+</sup> toxicant detection. ITO showed better sensitivity due to its conductive metal oxide nature.</p>	<b>118</b>
<b>Two channel bacteria based sensor</b>	<p>Used to simplify the configuration of device. Two 90 µL single chamber MICROBIAL FUEL CELL (MFC) were involved. For calibrating surrounding changes like temp, pH unwanted binding, reference channel was used. This sensor effectively reduced internal resistance, increased transferring of mass and gave better sensitivity.</p>	<b>119</b>
<b>SPEEK sensor</b>	<p>Study focused on single chamber MICROBIAL FUEL CELL (MFC) where sulfonated poly ether ether ketone membrane was utilized for analysing BOD Compared with Nafion it showed better sensitivity by 62.5% and 28 ohms lesser internal resistance</p>	<b>120</b>
<b>Multi anode paper based sensor</b>	<p>Multilayered carbon cloth anode i.e., flexible in nature for microbial adhesion. 28.4µ/cm<sup>2</sup> power density was produced. Efficient source of obtaining power from bacterial metabolism</p>	<b>121</b>
<b>Quorum sensing sensor</b>	<p>Intensified QS in systems responded linearly to Pb<sup>2+</sup> sensing. Showed faster recovery to copper ion shock.. With AHL's addition full recovery observed for copper target.</p>	<b>122</b>
<b>Data mining sensor</b>	<p>Combats the biggest limitation of target specificity detection Genomic data of microbial colony used for detecting substrate High accuracies and sensitivity observed for family and phyla</p>	<b>123</b>

**Table 5: Depicts improvements done for better sensing applicability**

## **6. Biosensing through size minimizing approach:**

To limit various constraints like overpotentials, ohmic losses, and increased response time, scaled down approach started gaining attention and microbial fuel cell (MFC)'s of small sizes are constructed. The high mass flux density per unit area with reduced ohmic losses in a bioreactor is the most essential advantage of such mini or micro microbial fuel cell (MFC). The miniaturization encodes high surface area to volume ratio leading to successful mass transfer ensuring well organized substrate utilization along with less cost of micro fabrication processes involved.(124, 125). Another important advantage envisioned is the ease it brings when multiple smaller units are to be stacked/ joined replacing the need for creating a huge single reactor. The well-maintained

microenvironment, high sensitivity, very easy portability demands miniaturization approach to be the need of the hour. There are very few examples of miniaturized microbial fuel cell (MFC)s as sensors. The very first attempt for creating such sensor was a dual reservoir sensor with silicon plates and 144 $\mu$ L working volume detecting 0.1% formaldehyde through a sharp voltage drop supporting irreversible biofilm activation (126). Another great discovery was an Air trap bubble microbial fuel cell (MFC) (127) that with same volume detected same toxicant (formaldehyde from 0.1%-0.001%) with even more steep voltage drop. The integration of nanotechnology is an asset to minimized size sensors. For example: Combination of Nano/microfabrication technique to electrochemical microbial biosensors can help in high throughput screening (128). A partial gold coated disposable chip was used to intergrate eight sensory cells each with independent operation. Each chamber of the fabricated chip consisted of gold working, counter and reference electrode. Potentiostatic signal was measured from the chip and presence of ethanol and phenol was determined (129): Polydimethyl siloxane and gold fabricated electrodes were used in a laminar flow microfluidic bioelectrochemical two electrode system. Pure culture of *Geobacter sulfurreducens*, short hydraulic retention and response time allowed to monitor quantitative analysis of anthraquinone disulphide (AQDS) in a solution (130).

Nanomaterials also have a significant role in bioelectro chemical cells. Though carbon nanotubes is most popular example but silk derived carbon mat is also a good example for it shows high sensitivity in detecting fenamiphos (organo phosphate pesticides). This mat is a blend of amino, pyridine, carbonyl functional groups that helps Au@Pt nanoparticles self-immobilize on carbon surface and provide a compatible microenvironment for *E. coli* (131).

## **7. Conclusion, Challenges and Future perspective:**

The online in situ monitoring, cost effective outlook, simple operation, easy portability, green approach and better stability (compared to conventional methods like for BOD) are the essential characteristics that base the need for investing time in understanding, exploring, and attempting to compensate the drawbacks associated in such sensors for making them become approved standard methods. Till date data have illustrated noteworthy potential of self-powered MICROBIAL FUEL CELL (MFC)'s as they are utilized not only for BOD analysis, certain toxic components detection, DO detection, microbial activity analysis but also as power source for other sensors. Although the analytical technology has been used for two decades now but still encompasses limitations with it. First, stability for longer duration use is affected when the standard conditions suitable to exoelectrogens change resulting in weakening of important parameters like sensitivity, reproducibility and selectivity. Secondly, Poor detection limit for analytes particularly toxicants by these sensors is another cause of worry as it does not match with water quality standard by WHO and hence is needed to be lowered with considerable amount for meeting requirements. Thirdly, quality of water also has an impact on output current;

Variation in the amount of BOD can weaken the toxicant sensors output signal. The combined shock due to variation in BOD and toxicity agent concentration (that can occur when receiving waterbody is in close proximity with waste streams like animal farm effluence, rayon industry extracts, and petroleum factory pollutants) was studied with minimum two operating microbial fuel cell (MFC) sensors. There are four kinds of combined shocks [high organic matter concentration (O.M)/low Toxic agent (T.A) concentration, low OM/high T.A, low O.M/ low T.A, high O.M/ high (T.A)] that affect signal output adversely especially in aquatic environment monitoring. A pre made response chart of the four kinds of combined shock cases by sudden changes in BOD and toxicity helped in analysing the distinctions quantitatively in this study and also concluded fixing of oversaturation organic matter (acetate here) concentration eradicated the combined shock signal interference of toxic (copper here)agent monitoring (132). Another novel invention of utilizing Biocathode (ORR-oxygen reduction reaction) as a sensing element satisfactorily showed positive results for removing combined shock complications where organic matter once fixed with oversaturation concentration would not affect the toxicity monitoring.

Biocathode has showed benefits like low cost, long stability with no degradation in performance and can be used as ORR sensing element for monitoring fumarate, extensively found in food products. Biocathode catalyses reduction of substrates like protons,  $O_2$ ,  $CO_2$ , short chain fatty acids etc. It showed high sensitivity with detection limit 0.0005% formaldehyde while anode sensing was applicable for higher concentrations  $>0.0025\%$ .

This is great example from future perspective as it is just one setup with anode and biocathode as the two electrodes for oxidation and reduction (133). Although the biggest drawback most microbial fuel cell (MFC) biosensors have is to quantify specific toxicant, but with small steps definite changes can be welcomed through integration of genetic engineering and molecular sciences ex- certain microbes like *Pseudomonas monteilii* LZU-3 have shown contribution in detecting PNP (134). HATOX-2000 is a Korean based company technology that is already present in the markets for toxicity monitoring. Also, scale down approach to limit the losses has potential to do wonders for real world applications.

With all the examples and advantages seen so far, the main objectives to be completely fulfilled in near future are remote location bio sensing, considerable increase in the self-power generated by these microbial fuel cell (MFC)'s and minimizing the contamination chances for improved efficiency; looking after all this will help in dealing with current struggles and challenges and make these small sensors a great contribution to the mankind.

## 7. References:

1 Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W. and Rabaey, K., 2006. Microbial fuel cells: methodology and technology. *Environmental science & technology*, 40(17), pp.5181-5192.

- 2 Rabaey, K. and Verstraete, W., 2005. Microbial fuel cells: novel biotechnology for energy generation. *TRENDS in Biotechnology*, 23(6), pp.291-298.
- 3 Guang, L., Koomson, D.A., Jingyu, H., Ewusi-Mensah, D. and Miwornunyuie, N., 2020. Performance of exoelectrogenic bacteria used in microbial desalination cell technology. *International journal of environmental research and public health*, 17(3), p.1121.
- 4 Voordeckers, J.W., Kim, B.C., Izallalen, M. and Lovley, D.R., 2010. Role of *Geobacter sulfurreducens* outer surface c-type cytochromes in reduction of soil humic acid and anthraquinone-2, 6-disulfonate. *Applied and environmental microbiology*, 76(7), pp.2371-2375.
- 5 Leang, C., Coppi, M.V. and Lovley, D.R., 2003. OmcB, ac-type polyheme cytochrome, involved in Fe (III) reduction in *Geobacter sulfurreducens*. *Journal of Bacteriology*, 185(7), pp.2096-2103.
- 6 Inoue, K., Leang, C., Franks, A.E., Woodard, T.L., Nevin, K.P. and Lovley, D.R., 2011. Specific localization of the c- type cytochrome OmcZ at the anode surface in current- producing biofilms of *Geobacter sulfurreducens*. *Environmental Microbiology Reports*, 3(2), pp.211-217.
- 7 Kim, B.C., Postier, B.L., DiDonato, R.J., Chaudhuri, S.K., Nevin, K.P. and Lovley, D.R., 2008. Insights into genes involved in electricity generation in *Geobacter sulfurreducens* via whole genome microarray analysis of the OmcF-deficient mutant. *Bioelectrochemistry*, 73(1), pp.70-75.
- 8 Wrighton, K.C., Thrash, J.C., Melnyk, R.A., Bigi, J.P., Byrne-Bailey, K.G., Remis, J.P., Schichnes, D., Auer, M., Chang, C.J. and Coates, J.D., 2011. Evidence for direct electron transfer by a Gram-positive bacterium isolated from a microbial fuel cell. *Applied and environmental microbiology*, 77(21), pp.7633-7639.
- 9 Rabaey, K., Boon, N., Höfte, M. and Verstraete, W., 2005. Microbial phenazine production enhances electron transfer in biofuel cells. *Environmental science & technology*, 39(9), pp.3401-3408.
- 10 Marsili, E., Baron, D.B., Shikhare, I.D., Coursolle, D., Gralnick, J.A. and Bond, D.R., 2008. *Shewanella* secretes flavins that mediate extracellular electron transfer. *Proceedings of the National Academy of Sciences*, 105(10), pp.3968-3973.
- 11 Deng, L., Li, F., Zhou, S., Huang, D. and Ni, J., 2010. A study of electron-shuttle mechanism in *Klebsiella pneumoniae* based-microbial fuel cells. *Chinese Science Bulletin*, 55(1), pp.99-104
- 12 Tremblay, P.L., Aklujkar, M., Leang, C., Nevin, K.P. and Lovley, D., 2012. A genetic system for *Geobacter metallireducens*: role of the flagellin and pilin in the reduction of Fe (III) oxide. *Environmental microbiology reports*, 4(1), pp.82-88.
- 13 Leung, K.M., Wanger, G., El-Naggar, M.Y., Gorby, Y., Southam, G., Lau, W.M. and Yang, J., 2013. *Shewanella oneidensis* MR-1 bacterial nanowires exhibit p-type, tunable electronic behavior. *Nanoletters*, 13(6), pp.2407-2411.
- 14 Liu, F., Rotaru, A.E., Shrestha, P.M., Malvankar, N.S., Nevin, K.P. and Lovley, D.R., 2012. Promoting direct interspecies electron transfer with activated carbon. *Energy & Environmental Science*, 5(10), pp.8982-8989.
- 15 Logan, B.E. and Regan, J.M., 2006. Electricity-producing bacterial communities in microbial fuel cells. *TRENDS in Microbiology*, 14(12), pp.512-518.
- 16 . Turner, A.P., 2013. Biosensors: sense and sensibility. *Chemical Society Reviews*, 42(8), pp.3184-3196
- 17 1 J. D. Newman and A. P. F. Turner, *Biosens. Bioelectron.*, 2005, 20, 2435–2453 and references therein.
- 18 Mohanty, S.P. and Kougiannos, E., 2006. Biosensors: A tutorial review. *Ieee Potentials*, 25(2), pp.35-40.
- 19 Parkhey, P. and Mohan, S.V., 2019. Biosensing applications of microbial fuel cell: Approach toward miniaturization. In *Microbial Electrochemical Technology* (pp. 977-997). Elsevier.
- 20 Goriushkina, T.B., Soldatkin, A.P. and Dzyadevych, S.V., 2009. Application of amperometric biosensors for analysis of ethanol,

glucose, and lactate in wine. *Journal of Agricultural and Food chemistry*, 57(15), pp.6528-6535.

21 Trivedi, U.B., Lakshminarayana, D., Kothari, I.L., Patel, N.G., Kapse, H.N., Makhija, K.K., Patel, P.B. and Panchal, C.J., 2009. Potentiometric biosensor for urea determination in milk. *Sensors and Actuators B: Chemical*, 140(1), pp.260-266.

22 Jaffrezic-Renault, N. and Dzyadevych, S.V., 2008. Conductometric microbiosensors for environmental monitoring. *Sensors*, 8(4), pp.2569-2588.

23 Liu, B., Lei, Y. and Li, B., 2014. A batch-mode cube microbial fuel cell based “shock” biosensor for wastewater quality monitoring. *Biosensors and Bioelectronics*, 62, pp.308-314.

24 Jia, K., Eltzov, E., Toury, T., Marks, R.S. and Ionescu, R.E., 2012. A lower limit of detection for atrazine was obtained using bioluminescent reporter bacteria via a lower incubation temperature. *Ecotoxicology and environmental safety*, 84, pp.221-226.

25 Hemben, A., Ashley, J. and Tothill, I.E., 2017. Development of an immunosensor for PfHRP 2 as a biomarker for malaria detection. *Biosensors*, 7(3), p.28

26 Pickup, J.C., Hussain, F., Evans, N.D., Rolinski, O.J. and Birch, D.J., 2005. Fluorescence-based glucose sensors. *Biosensors and Bioelectronics*, 20(12), pp.2555-2565.

27 Tzoris, A., Fernandez-Perez, V. and Hall, E.A., 2005. Direct toxicity assessment with a mini portable respirometer. *Sensors and Actuators B: Chemical*, 105(1), pp.39-49.

28 Nikoleli, G.P., Karapetis, S., Bratakou, S., Nikolelis, D.P., Tzamtzis, N., Psychoyios, V.N. and Psaroudakis, N., 2016. Biosensors for security and bioterrorism: Definitions, history, types of agents, new trends and applications. *Biosensors for Security and Bioterrorism Applications*, pp.1-13.

29 Guan, J.G., Miao, Y.Q. and Zhang, Q.J., 2004. Impedimetric biosensors. *Journal of bioscience and bioengineering*, 97(4), pp.219-226.

30 Kumlanghan, A., Liu, J., Thavarungkul, P., Kanatharana, P. and Mattiasson, B., 2007. Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter. *Biosensors and bioelectronics*, 22(12), pp.2939-2944.

31 Liu, Z., Liu, J., Zhang, S., Xing, X.H. and Su, Z., 2011. Microbial fuel cell based biosensor for in situ monitoring of anaerobic digestion process. *Bioresource technology*, 102(22), pp.10221-10229.

32 Di Lorenzo, M., Curtis, T.P., Head, I.M. and Scott, K., 2009. A single-chamber microbial fuel cell as a biosensor for wastewaters. *Water research*, 43(13), pp.3145-3154.

33 Liu, Y., Ding, M., Ling, W., Yang, Y., Zhou, X., Li, B.Z., Chen, T., Nie, Y., Wang, M., Zeng, B. and Li, X., 2017. A three-species microbial consortium for power generation. *Energy & Environmental Science*, 10(7), pp.1600-1609.

34 Yong, Y.C.; Cai, Z.; Yu, Y.Y.; Chen, P.; Jiang, R.; Cao, B.; Sun, J.Z.; Wang, J.Y.; Song, H. Increase of riboflavin biosynthesis underlies enhancement of extracellular electron transfer of *Shewanella* in alkaline microbial fuel cells. *Bioresour. Technol.* 2013, 130, 763–768

35 Kong, X., Zhou, X., Tian, Y., Wu, X., Zhang, J. and Zuo, W., 2016. Niobium doped lanthanum calcium ferrite perovskite as a novel electrode material for symmetrical solid oxide fuel cells. *Journal of Power Sources*, 326, pp.35-42.

36 Choudhury, P., Prasad Uday, U.S., Bandyopadhyay, T.K., Ray, R.N. and Bhunia, B., 2017. Performance improvement of microbial fuel cell (MICROBIAL FUEL CELL (MFC)) using suitable electrode and Bioengineered organisms: A review. *Bioengineered*, 8(5), pp.471-487..

37 Stein, N.E., Hamelers, H.V. and Buisman, C.N., 2010. Stabilizing the baseline current of a microbial fuel cell-based biosensor through overpotential control under non-toxic conditions. *Bioelectrochemistry*, 78(1), pp.87-91.

38 Chen, Z., Niu, Y., Zhao, S., Khan, A., Ling, Z., Chen, Y., Liu, P. and Li, X., 2016. A novel biosensor for p-nitrophenol based on an aerobic anode microbial fuel cell. *Biosensors and Bioelectronics*, 85, pp.860-868.

- 39 Zeng, L., Li, X., Shi, Y., Qi, Y., Huang, D., Tadó, M., Wang, S. and Liu, S., 2017. FePO<sub>4</sub> based single chamber air-cathode microbial fuel cell for online monitoring levofloxacin. *Biosensors and Bioelectronics*, 91, pp.367-373.
- 40 Chang, I.S., Jang, J.K., Gil, G.C., Kim, M., Kim, H.J., Cho, B.W. and Kim, B.H., 2004. Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor. *Biosensors and Bioelectronics*, 19(6), pp.607-613.
- 41 35 A. Kumlanghan, J. Liu, P. Thavarungkul, Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter, *Biosens. Bioelectron.* 22 (2007) 2939e2944
- 42 Chang, I.S., Moon, H., Jang, J.K. and Kim, B.H., 2005. Improvement of a microbial fuel cell performance as a BOD sensor using respiratory inhibitors. *Biosensors and Bioelectronics*, 20(9), pp.1856-1859.
- 43 Kim, B.H., Chang, I.S., Gil, G.C., Park, H.S. and Kim, H.J., 2003. Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. *Biotechnology letters*, 25(7), pp.541-545.
- 44 Kim, M., Youn, S.M., Shin, S.H., Jang, J.G., Han, S.H., Hyun, M.S., Gadd, G.M. and Kim, H.J., 2003. Practical field application of a novel BOD monitoring system. *Journal of Environmental Monitoring*, 5(4), pp.640-643.
- 45 Jouanneau, S., Recoules, L., Durand, M.J., Boukabache, A., Picot, V., Primault, Y., Lakel, A., Sengelin, M., Barillon, B. and Thouand, G., 2014. Methods for assessing biochemical oxygen demand (BOD): A review. *Water research*, 49, pp.62-82.
- 46 Di Lorenzo, M., 2016. Use of microbial fuel cells in sensors. *Microbial electrochemical and fuel cells*, pp.341-356.
- 47 41 Kim, B.H., Chang, I.S., Gil, G.C., Park, H.S. and Kim, H.J., 2003. Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. *Biotechnology letters*, 25(7), pp.541-545.
- 48 Kim, M., Youn, S.M., Shin, S.H., Jang, J.G., Han, S.H., Hyun, M.S., Gadd, G.M. and Kim, H.J., 2003. Practical field application of a novel BOD monitoring system. *Journal of Environmental Monitoring*, 5(4), pp.640-643.
- 49 Peixoto, L., Min, B., Martins, G., Brito, A.G., Kroff, P., Parpot, P., Angelidaki, I. and Nogueira, R., 2011. In situ microbial fuel cell-based biosensor for organic carbon. *Bioelectrochemistry*, 81(2), pp.99-103.
- 50 Modin, O. and Wilén, B.M., 2012. A novel bioelectrochemical BOD sensor operating with voltage input. *Water research*, 46(18), pp.6113-6120.
- 51 Chang, I.S., Moon, H., Jang, J.K. and Kim, B.H., 2005. Improvement of a microbial fuel cell performance as a BOD sensor using respiratory inhibitors. *Biosensors and Bioelectronics*, 20(9), pp.1856-1859.
- 52 Ayyaru, S. and Dharmalingam, S., 2014. Enhanced response of microbial fuel cell using sulfonated poly ether ether ketone membrane as a biochemical oxygen demand sensor. *Analytica chimica acta*, 818, pp.15-22.
- 53 Kumlanghan, A., Liu, J., Thavarungkul, P., Kanatharana, P. and Mattiasson, B., 2007. Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter. *Biosensors and bioelectronics*, 22(12), pp.2939-2944
- 54 Parkash, A., 2016. Utilization of different types of wastewater for production of electricity using non mediated microbial fuel cell. *MOJ Proteomics Bioinform*, 4(1), p.00107.
- 55 Wang, Y., Liu, X., Wang, M., Zhang, P., Zong, Y. and Zhang, Q., 2018. A single-chamber microbial fuel cell for rapid determination of biochemical oxygen demand using low-cost activated carbon as cathode catalyst. *Environmental technology*, 39(24), pp.3228-3237.
- 56 Shuai, T., Panyue, Z., Yingmei, L., Duo, Z. and Baohe, W., 2014. Performances of double-chamber microbial fuel cell-based BOD sensor. *Chinese Journal of Environmental Engineering*, 8(6), pp.2626-2632.
- 57 Chouler, J. and Di Lorenzo, M., 2015. Towards miniature microbial fuel cells for water quality monitoring. In *Proceedings of the 6th European Fuel Cell-Piero Lunghi Conference, EFC 2015*.
- 58 Zhang, Y. and Angelidaki, I., 2011. Submersible microbial fuel cell sensor for monitoring microbial activity and BOD in groundwater: focusing on impact of anodic biofilm on sensor applicability. *Biotechnology and bioengineering*, 108(10), pp.2339-

59 Kang, K.H., Jang, J.K., Moon, H., Chang, I.S. and Kim, B.H., 2003. A microbial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor. *Biotechnology letters*, 25(16), pp.1357-1361.

60 Choi, S.H. and Gu, M.B., 2003. Toxicity biomonitoring of degradation byproducts using freeze-dried recombinant bioluminescent bacteria. *Analytica Chimica Acta*, 481(2), pp.229-238.

61 Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. and Sutton, D.J., 2012. Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology*, pp.133-164.

62 Yu, D., Bai, L., Zhai, J., Wang, Y. and Dong, S., 2017. Toxicity detection in water containing heavy metal ions with a self-powered microbial fuel cell-based biosensor. *Talanta*, 168, pp.210-216.

63 Xu, Z., Liu, B., Dong, Q., Lei, Y., Li, Y., Ren, J., Mccutcheon, J. and Li, B., 2015. Flat microliter membrane-based microbial fuel cell as “on-line sticker sensor” for self-supported in situ monitoring of wastewater shocks. *Bioresource technology*, 197, pp.244-251.

64 Cheung, K.H. and Gu, J.D., 2007. Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: a review. *International Biodeterioration & Biodegradation*, 59(1), pp.8-15.

65 Wang, G.H., Cheng, C.Y., Liu, M.H., Chen, T.Y., Hsieh, M.C. and Chung, Y.C., 2016. Utility of *Ochrobactrum anthropi* YC152 in a microbial fuel cell as an early warning device for hexavalent chromium determination. *Sensors*, 16(8), p.1272.

66 Wu, L.C., Tsai, T.H., Liu, M.H., Kuo, J.L., Chang, Y.C. and Chung, Y.C., 2017. A green microbial fuel cell-based biosensor for in situ chromium (VI) measurement in electroplating wastewater. *Sensors*, 17(11), p.2461.

67 Tran, P.H.N., Luong, T.T.T., Nguyen, T.T.T., Nguyen, H.Q., Van Duong, H. and Kim, B.H., 2015. Possibility of using a lithotrophic iron-oxidizing microbial fuel cell as a biosensor for detecting iron and manganese in water samples. *Environmental Science: Processes & Impacts*, 17(10), pp.1806-1815.

68 Zhao, S., Liu, P., Niu, Y., Chen, Z., Khan, A., Zhang, P. and Li, X., 2018. A novel early warning system based on a sediment microbial fuel cell for in situ and real time hexavalent chromium detection in industrial wastewater. *Sensors*, 18(2), p.642.

69 Wu, S., Deng, H., Han, C., Liu, L. and Zhong, W., 2018. A Novel Sediment Microbial Fuel Cell Based Sensor for On- Line and in situ Monitoring Copper Shock in Water. *Electroanalysis*, 30(11), pp.2668-2675.

70 Gothwal, R. and Shashidhar, T., 2015. Antibiotic pollution in the environment: a review. *Clean Soil Air Water* 43: 479-489.

71 Wu, W., Lesnik, K.L., Xu, S., Wang, L. and Liu, H., 2014. Impact of tobramycin on the performance of microbial fuel cell. *Microbial cell factories*, 13(1), pp.1-7.

72 Schneider, G., Czeller, M., Rostás, V. and Kovács, T., 2015. Microbial fuel cell-based diagnostic platform to reveal antibacterial effect of beta-lactam antibiotics. *Enzyme and microbial technology*, 73, pp.59-64.

73 Zeng, L., Li, X., Shi, Y., Qi, Y., Huang, D., Tadó, M., Wang, S. and Liu, S., 2017. FePO<sub>4</sub> based single chamber air-cathode microbial fuel cell for online monitoring levofloxacin. *Biosensors and Bioelectronics*, 91, pp.367-373.

74 Kim, M., Hyun, M.S., Gadd, G.M. and Kim, H.J., 2007. A novel biomonitoring system using microbial fuel cells. *Journal of environmental monitoring*, 9(12), pp.1323-1328.

75 Yang, W., Wei, X., Fraiwan, A., Coogan, C.G., Lee, H. and Choi, S., 2016. Fast and sensitive water quality assessment: a  $\mu$ L-scale microbial fuel cell-based biosensor integrated with an air-bubble trap and electrochemical sensing functionality. *Sensors and Actuators B: Chemical*, 226, pp.191-195.

76 Chouler, J., Cruz-Izquierdo, Á., Rengaraj, S., Scott, J.L. and Di Lorenzo, M., 2018. A screen-printed paper microbial fuel cell biosensor for detection of toxic compounds in water. *Biosensors and Bioelectronics*, 102, pp.49-56.

77 Chen, Z., Niu, Y., Zhao, S., Khan, A., Ling, Z., Chen, Y., Liu, P. and Li, X., 2016. A novel biosensor for p-nitrophenol based on an aerobic anode microbial fuel cell. *Biosensors and Bioelectronics*, 85, pp.860-868.

- 78 Shen, Y.J., Lefebvre, O., Tan, Z. and Ng, H.Y., 2012. Microbial fuel-cell-based toxicity sensor for fast monitoring of acidic toxicity. *Water Science and Technology*, 65(7), pp.1223-1228.
- 79 Jiang, Y., Liang, P., Liu, P., Yan, X., Bian, Y. and Huang, X., 2017. A cathode-shared microbial fuel cell sensor array for water alert system. *International Journal of Hydrogen Energy*, 42(7), pp.4342-4348.
- 80 Schampelaire, L.D., Bossche, L.V.D., Dang, H.S., Höfte, M., Boon, N., Rabaey, K. and Verstraete, W., 2008. Microbial fuel cells generating electricity from rhizodeposits of rice plants. *Environmental Science & Technology*, 42(8), pp.3053-3058.
- 81 Li, T., Wang, X., Zhou, Q., Liao, C., Zhou, L., Wan, L., An, J., Du, Q., Li, N. and Ren, Z.J., 2018. Swift acid rain sensing by synergistic rhizospheric bioelectrochemical responses. *ACS sensors*, 3(7), pp.1424-1430.
- 82 Yu, D., Bai, L., Zhai, J., Wang, Y. and Dong, S., 2017. Toxicity detection in water containing heavy metal ions with a self-powered microbial fuel cell-based biosensor. *Talanta*, 168, pp.210-216.
- 83 Yu, D., Zhang, H., Bai, L., Fang, Y., Liu, C., Zhang, H., Li, T., Han, L., Yu, Y., Yu, H. and Dong, S., 2020. Visual detection of the toxicity of wastewater containing heavy metal ions using a microbial fuel cell biosensor with a Prussian blue cathode. *Sensors and Actuators B: Chemical*, 302, p.127177.
- 84 Jiang, Y.B., Deng, H., Sun, D.M. and Zhong, W.H., 2015. Electrical signals generated by soil microorganisms in microbial fuel cells respond linearly to soil Cd<sup>2+</sup> pollution. *Geoderma*, 255, pp.35-41.
- 85 Chouler, J., Monti, M.D., Morgan, W.J., Cameron, P.J. and Di Lorenzo, M., 2019. A photosynthetic toxicity biosensor for water. *Electrochimica Acta*, 309, pp.392-401.
- 86 Rasmussen, M. and Minter, S.D., 2015. Long-term arsenic monitoring with an *Enterobacter cloacae* microbial fuel cell. *Bioelectrochemistry*, 106, pp.207-212.
- 87 Stein, N.E., Hamelers, H.V. and Buisman, C.N., 2012. The effect of different control mechanisms on the sensitivity and recovery time of a microbial fuel cell based biosensor. *Sensors and Actuators B: Chemical*, 171, pp.816-821.
- 88 Shen, Y., Wang, M., Chang, I.S. and Ng, H.Y., 2013. Effect of shear rate on the response of microbial fuel cell toxicity sensor to Cu (II). *Bioresource technology*, 136, pp.707-710.
- 89 Zhou, S., Huang, S., Li, Y., Zhao, N., Li, H., Angelidaki, I. and Zhang, Y., 2018. Microbial fuel cell-based biosensor for toxic carbon monoxide monitoring. *Talanta*, 186, pp.368-371.
- 90 Jiang, Y., Liang, P., Liu, P., Wang, D., Miao, B. and Huang, X., 2017. A novel microbial fuel cell sensor with biocathode sensing element. *Biosensors and Bioelectronics*, 94, pp.344-350.
- 91 Wu, S.S., Hernández, M., Deng, Y.C., Han, C., Hong, X., Xu, J., Zhong, W.H. and Deng, H., 2019. The voltage signals of microbial fuel cell-based sensors positively correlated with methane emission flux in paddy fields of China. *FEMS microbiology ecology*, 95(3), p.fiz018.
- 92 Liu, W., Yin, L., Jin, Q., Zhu, Y., Zhao, J., Zheng, L., Zhou, Z. and Zhu, B., 2019. Sensing performance of a self-powered electrochemical sensor for H<sub>2</sub>O<sub>2</sub> detection based on microbial fuel cell. *Journal of Electroanalytical Chemistry*, 832, pp.97-104.
- 93 Feng, Y., Barr, W. and Harper Jr, W.F., 2013. Neural network processing of microbial fuel cell signals for the identification of chemicals present in water. *Journal of environmental management*, 120, pp.84-92.
- 94 Falk, H.M., Reichling, P., Andersen, C. and Benz, R., 2015. Online monitoring of concentration and dynamics of volatile fatty acids in anaerobic digestion processes with mid-infrared spectroscopy. *Bioprocess and biosystems engineering*, 38(2), pp.237-249.
- 95 Jin, X., Angelidaki, I. and Zhang, Y., 2016. Microbial electrochemical monitoring of volatile fatty acids during anaerobic digestion. *Environmental science & technology*, 50(8), pp.4422-4429.
- 96 Kaur, A., Kim, J.R., Michie, I., Dinsdale, R.M., Guwy, A.J., Premier, G.C. and Centre, S.E.R., 2013. Microbial fuel cell type biosensor for specific volatile fatty acids using acclimated bacterial communities. *Biosensors and Bioelectronics*, 47, pp.50-55.

- 97 Markfort, C.D. and Hondzo, M., 2009. Dissolved oxygen measurements in aquatic environments: the effects of changing temperature and pressure on three sensor technologies. *Journal of Environmental quality*, 38(4), pp.1766-1774.
- 98 Ansa-Asare, O.D., Marr, I.L. and Cresser, M.S., 2000. Evaluation of modelled and measured patterns of dissolved oxygen in a freshwater lake as an indicator of the presence of biodegradable organic pollution. *Water research*, 34(4), pp.1079-1088.
- 99 Zhao, F., Harnisch, F., Schröder, U., Scholz, F., Bogdanoff, P. and Herrmann, I., 2006. Challenges and constraints of using oxygen cathodes in microbial fuel cells. *Environmental science & technology*, 40(17), pp.5193-5199.
- 100 Oh, S., Min, B. and Logan, B.E., 2004. Cathode performance as a factor in electricity generation in microbial fuel cells. *Environmental science & technology*, 38(18), pp.4900-4904.
- 101 Song, N., Yan, Z., Xu, H., Yao, Z., Wang, C., Chen, M., Zhao, Z., Peng, Z., Wang, C. and Jiang, H.L., 2019. Development of a sediment microbial fuel cell-based biosensor for simultaneous online monitoring of dissolved oxygen concentrations along various depths in lake water. *Science of the total environment*, 673, pp.272-280.
- 102 Flemming, H.C. and Schaule, G., 1994. Microbial deterioration of materials-biofilm and biofouling: Biofouling. *Mikrobielle Werkstoffzerstoerung-Biofilm und Biofouling: Biofouling. Werkstoffe und Korrosion;(Germany)*, 45(1).
- 103 Xu, D. and Gu, T., 2014. Carbon source starvation triggered more aggressive corrosion against carbon steel by the *Desulfovibrio vulgaris* biofilm. *International Biodeterioration & Biodegradation*, 91, pp.74-81.
- 104 George, R.P., Muraleedharan, P., Dayal, R.K. and Khatak, H.S., 2006. Techniques for biofilm monitoring. *Corrosion Reviews*, 24(1-2), pp.123-150.
- 105 Bruijs, M.C.M., Venhuis, L.P., Jenner, H.A., Licina, G.J. and Daniels, D., 2001. Biocide optimisation using an on-line biofilm monitor. *Power Plant Chem*, 3(7), pp.400-405.
- 106 Gu, T., 2012. Methods and devices for the detection of biofilms. World Intellectual Property Organization: Patent WO2012/149487.
- 107 Tront, J.M., Fortner, J.D., Plötze, M., Hughes, J.B. and Puzrin, A.M., 2008. Microbial fuel cell biosensor for in situ assessment of microbial activity. *Biosensors and Bioelectronics*, 24(4), pp.586-590.
- 108 Zhang, Y. and Angelidaki, I., 2011. Submersible microbial fuel cell sensor for monitoring microbial activity and BOD in groundwater: focusing on impact of anodic biofilm on sensor applicability. *Biotechnology and bioengineering*, 108(10), pp.2339-2347.
- 109 Patchett, R.A., Kelly, A.F. and Kroll, R.G., 1988. Use of a microbial fuel cell for the rapid enumeration of bacteria. *Applied microbiology and biotechnology*, 28(1), pp.26-31.
- 110 Miller, L.G. and Oremland, R.S., 2008. Electricity generation by anaerobic bacteria and anoxic sediments from hypersaline soda lakes. *Extremophiles*, 12(6), pp.837-848.
- 111 Liu, Z., Liu, J., Zhang, S., Xing, X.H. and Su, Z., 2011. Microbial fuel cell based biosensor for in situ monitoring of anaerobic digestion process. *Bioresource technology*, 102(22), pp.10221-10229.
- 112 Zheng, Q., Xiong, L., Mo, B., Lu, W., Kim, S. and Wang, Z., 2015. Temperature and humidity sensor powered by an individual microbial fuel cell in a power management system. *Sensors*, 15(9), pp.23126-23144.
- 113 Khaled, F., Ondel, O. and Allard, B., 2016. Microbial fuel cells as power supply of a low-power temperature sensor. *Journal of Power Sources*, 306, pp.354-360.
- 114 Liang, P., Wang, H., Xia, X., Huang, X., Mo, Y., Cao, X. and Fan, M., 2011. Carbon nanotube powders as electrode modifier to enhance the activity of anodic biofilm in microbial fuel cells. *Biosensors and Bioelectronics*, 26(6), pp.3000-3004.
- 115 Kharkwal, S., Tan, Y.C., Lu, M. and Ng, H.Y., 2017. Development and long-term stability of a novel microbial fuel cell BOD sensor with MnO<sub>2</sub> catalyst. *International journal of molecular sciences*, 18(2), p.276.

- 116 Fu, L., You, S.J., Zhang, G.Q., Yang, F.L., Fang, X.H. and Gong, Z., 2011. PB/PANI-modified electrode used as a novel oxygen reduction cathode in microbial fuel cell. *Biosensors and Bioelectronics*, 26(5), pp.1975-1979.
- 117 YI, Y., LI, X.Y., JIANG, X.H., XIE, B.Z., LIU, H., LIANG, D.W. and ZHU, Y., 2016. Optimization study of a microbial fuel cell (MICROBIAL FUEL CELL (MFC))-based biosensor. In *Materials Engineering and Environmental Science: Proceedings of the 2015 International Conference on Materials Engineering and Environmental Science (MEES2015)* (pp. 643-652).
- 118 Li, J., Hu, J., Yang, C., Pu, W., Hou, H., Xu, J., Liu, B. and Yang, J., 2019. Enhanced detection of toxicity in wastewater using a 2D smooth anode based microbial fuel cell toxicity sensor. *RSC advances*, 9(15), pp.8700-8706.
- 119 Yang, W., Wei, X. and Choi, S., 2015, November. A two-channel bacteria-based biosensor for water quality monitoring. In *2015 IEEE SENSORS* (pp. 1-4). IEEE.
- 120 Ayyaru, S. and Dharmalingam, S., 2014. Enhanced response of microbial fuel cell using sulfonated poly ether ether ketone membrane as a biochemical oxygen demand sensor. *Analytica chimica acta*, 818, pp.15-22.
- 121 Fraiwan, A., Lee, H. and Choi, S., 2014. A multianode paper-based microbial fuel cell: a potential power source for disposable biosensors. *IEEE Sensors Journal*, 14(10), pp.3385-3390.
- 122 Pan, J., Hu, J., Liu, B., Li, J., Wang, D., Bu, C., Wang, X., Xiao, K., Liang, S., Yang, J. and Hou, H., 2020. Enhanced quorum sensing of anode biofilm for better sensing linearity and recovery capability of microbial fuel cell toxicity sensor. *Environmental research*, 181, p.108906.
- 123** Cai, W., Lesnik, K.L., Wade, M.J., Heidrich, E.S., Wang, Y. and Liu, H., 2019. Incorporating microbial community data with machine learning techniques to predict feed substrates in microbial fuel cells. *Biosensors and Bioelectronics*, 133, pp.64-71.
- 124 ElMekawy, A., Hegab, H.M., Dominguez-Benetton, X. and Pant, D., 2013. Internal resistance of microfluidic microbial fuel cell: challenges and potential opportunities. *Bioresource technology*, 142, pp.672-682.
- 125 Wang, H.Y., Bernarda, A., Huang, C.Y., Lee, D.J. and Chang, J.S., 2011. Micro-sized microbial fuel cell: a mini-review. *Bioresource technology*, 102(1), pp.235-243.
- 126 Dávila, D., Esquivel, J.P., Sabate, N. and Mas, J., 2011. Silicon-based microfabricated microbial fuel cell toxicity sensor. *Biosensors and Bioelectronics*, 26(5), pp.2426-2430.
- 127 Yang, W., Wei, X., Fraiwan, A., Coogan, C.G., Lee, H. and Choi, S., 2016. Fast and sensitive water quality assessment: a  $\mu$ L-scale microbial fuel cell-based biosensor integrated with an air-bubble trap and electrochemical sensing functionality. *Sensors and Actuators B: Chemical*, 226, pp.191-195.
- 128 Scognamiglio, V., 2013. Nanotechnology in glucose monitoring: Advances and challenges in the last 10 years. *Biosensors and Bioelectronics*, 47, pp.12-25.
- 129 Popovtzer, R., Neufeld, T., Biran, D., Ron, E.Z., Rishpon, J. and Shacham-Diamand, Y., 2005. Novel integrated electrochemical nano-biochip for toxicity detection in water. *Nano letters*, 5(6), pp.1023-1027.
- 130 Li, Z., Venkataraman, A., Rosenbaum, M.A. and Angenent, L.T., 2012. A Laminar- Flow Microfluidic Device for Quantitative Analysis of Microbial Electrochemical Activity. *ChemSusChem*, 5(6), pp.1119-1123.131
- 131 Deng, L., Guo, S., Zhou, M., Liu, L., Liu, C. and Dong, S., 2010. A silk derived carbon fiber mat modified with Au@ Pt urchinlike nanoparticles: A new platform as electrochemical microbial biosensor. *Biosensors and Bioelectronics*, 25(10), pp.2189-2193.
- 132 Jiang, Y., Liang, P., Liu, P., Bian, Y., Miao, B., Sun, X., Zhang, H. and Huang, X., 2016. Enhancing signal output and avoiding BOD/toxicity combined shock interference by operating a microbial fuel cell sensor with an optimized background concentration of organic matter. *International journal of molecular sciences*, 17(9), p.1392.
- 133 Jiang, Y., Liang, P., Liu, P., Wang, D., Miao, B. and Huang, X., 2017. A novel microbial fuel cell sensor with biocathode sensing element. *Biosensors and Bioelectronics*, 94, pp.344-350.
- 134 Chen, Z., Niu, Y., Zhao, S., Khan, A., Ling, Z., Chen, Y., Liu, P. and Li, X., 2016. A novel biosensor for p-nitrophenol based on an aerobic anode microbial fuel cell. *Biosensors and Bioelectronics*, 85, pp.860-868.

135 Paret, Dominique. (2016). Introduction to and Recap of the Principles Employed in NFC: Paret/Antenna Designs for NFC Devices. 10.1002/9781119145301.ch2.

136 Aiyer, Kartik & Doyle, Lucinda. (2021). Capturing the signal of weak electric fields: a worthy endeavour. Trends in Biotechnology. 10.1016/j.tibtech.2021.10.002.

137 Madureira Trufen, Carlos Eduardo. (2021). CCP recap 2021.

138 Cocolini, Federico & Cicuttin, Enrico & Cremonini, Camilla & Dario, Tartaglia & Viaggi, Bruno & Kuriyama, Akira & Picetti, Edoardo & Ball, Chad & Abu-Zidan, Fikri & Ceresoli, Marco & Turri, Bruno & Jain, Sumita & Palombo, Carlo & Guirao, Xavier & Gachabayov, Mahir & Machado, Fernando & Eftychios, Litoridis & Kanj, Souha & Sartelli, Massimo. (2021). A pandemic recap: lessons we have learned. World Journal of Emergency Surgery. 16. 10.1186/s13017-021-00393-w.

140 Mestdagh, Eveline. (2021). Recap wetenschappelijk advies.

141 Qiu, Meikang & Qiu, Han & Zeng, Yi. (2021). Critical Remarks and Chapters Recap. 10.1201/9781003139058-12.

142 Ahmed, Jubair. (2020). Electrospinning for the manufacture of biosensor components: A mini- review. Medical Devices & Sensors. 4. 10.1002/mds3.10136.