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Mini Recap on Electricigens Biosensor and Their Applicability

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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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.

1. 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

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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 sensingapplicability in biotechnology, later discussed in this mini review. This paper provides a quick recap regarding microbial fuel cell (MFC) biosensors andrelevant terms connected with it.

2. 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.

2.1 Metabolism of Electricity Generating Microbes

Generally, a bacterium has its own choice for oxidizing asort 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 monthsthe electricity production increased 7 times [2].

Substrate level phosphorylationand 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 yieldenergy.While latter involves ETC dependent ATP synthesis where oxidation occurs [both inorganic ions (in chemo-lithotrophs) and organic compounds (in organo-heterotrophs)] oflesser redox potential associated with reduction of electron accepting electrode of higher (more positive) redox potential. However, inphototrophs 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 dissolvablecompounds like (or iron/ manganese oxides) depending on aerobic and anaerobic breathing of cell resp [3].

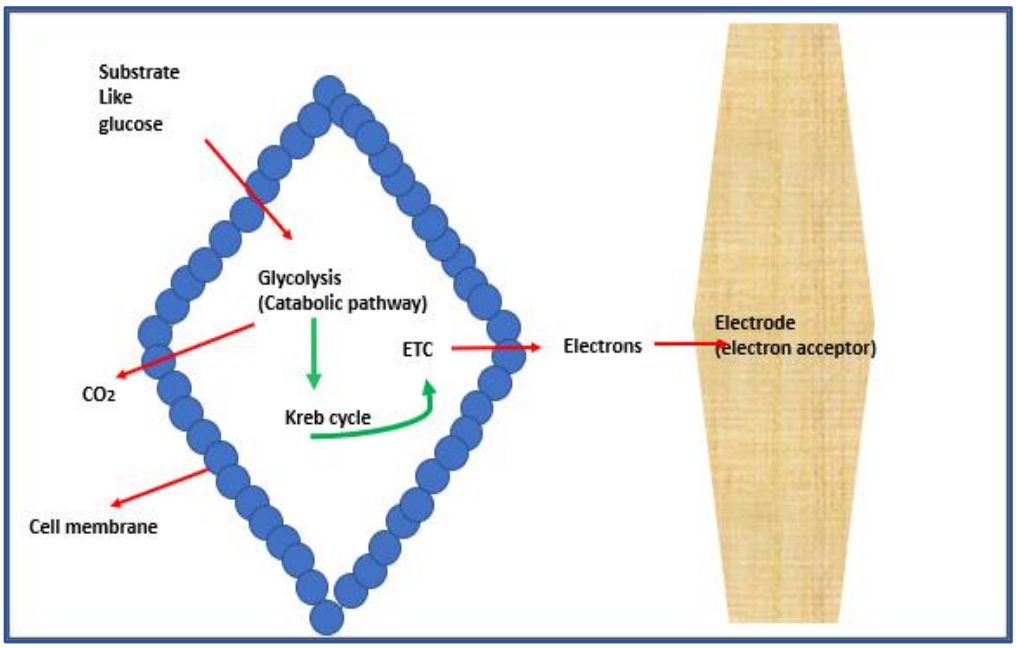

Fig. 1. This is a general Electron Transport Chain pathwayof how exoelectrogens regulate catabolism and cellular respiration

2.2 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:

2.3 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].

2.4 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.

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.

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

Exoelectrogens	Pathway name	Pathway carriers	Ref.
G. sulfurreducens	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	[4-7]
T. potens	Short range	MHC's(c-cyts) are involved in electron transportation across gram positive cell envelop.	[8]
Pseudomonas aeruginosa	Electron shuttle	Pyocyanine and phenzamine-1-carboxamide- self-secreted electron shuttles	[9]
Shewanella. sp	Electron shuttle	Produce flavin and riboflavin	[10]
Klebsiella pneumoniae	Electron shuttle	2,6-di-tert-butyl-p-benzoquinon	[11]
G. metallireducens	Long range	The fliC (Flagellin protein) and type 4 Structural protein pili (pilA) deletion resulted in declineof electron transfer toelectrode.	[12]
S. oneidensis	Long range	Electrically conductive- MR-1nanowires	[13]

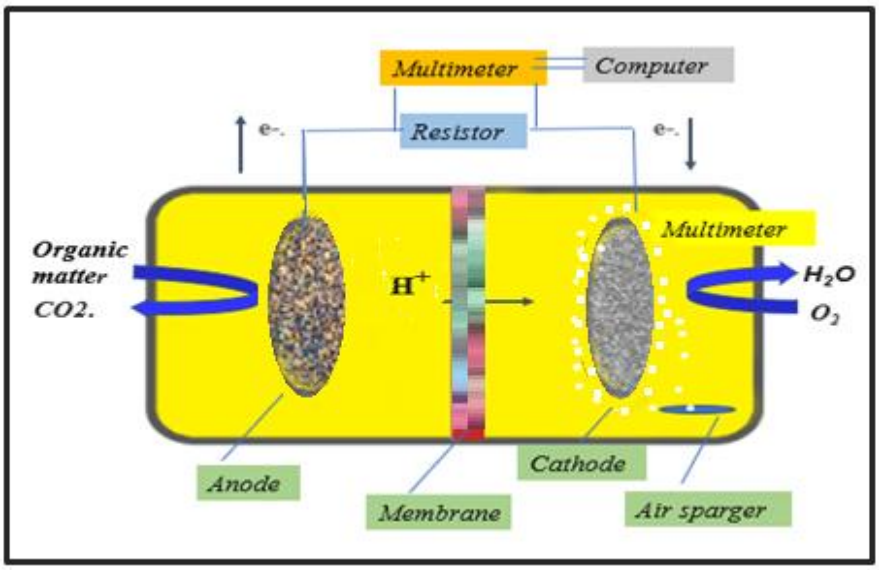

Fig. 2. It is a pictorial representation of a Microbial fuel cell activity

3.1 Fundamentals of Biosensors

Biosensors predominantly comprise three constitents- 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].

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_2 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 bioluminiscence sensors, fluorescence and even as colorimetric sensors. Other type is peizoelectric or gravimetric biosensor that detects the resonating frequency change of peizoelectric material based on surface molecules adsorption or desorption [19]. Table 1 illustrates few examples of biosensors.

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 transducerthat 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 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 0.025 g/L [30]. Wall jet microbial fuel cell (MFC) was used as sensor in order to detect volatile fatty acid and gas contents [31]. First BOD detected in microbial fuel cell (MFC) was till100mg/ml limit; later a packed bed microbial fuel cell (MFC) with carbon cloth anode detected enhanced BOD range up to 350 mg/ml [32]. Components of microbial fuel cell (MFC) biosensors can also be seen in Fig. 4.

Table 2. Different types of biosensors with their important examples

Biosensors	Features with Examples	Ref.
Amperometric	..Holds great potential to be used in food industry ..Monitors Ethanol, glucose and lactate in wine.	[20]
Potentiometric	..Ion selective electrodes for electrical output. ..Initially used ammonia for urea detection. ..Also determines carbon, sugars, and pesticides.	[21]
Conductimetric	..Thin film electrodes as cost effective approach towards miniaturization. ..No reference electrodes and light sensitive transducers needed. ..Detects protein markers and heavy metals	[22]
Microbial fuel cell	Self-powered portable devices capable for good online monitoring of environment (BOD, chemical pollutants) ex-Cr^{+6} was sensed in 1-8mg/L range within 74 mins.	[23]
Bioluminescence	..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]
Immunosensors	..Immunochemical reactions assisted with transducers. ..Antibodies bind to their specific antigens and interact with pathogens toxins. Ex-PfHRP2 biomarker for malarial detection	[25]
Fluorescence	..Molecular fluroscence is very beneficial as it can detect a single molecule also, no damage to host system, both fluroscence intensity and decay can help in measurements. Ex. Concanavalin A, ..Glucose oxidase and dehydrogenase are few fluroscence based glucose sensors.	[26]
Baroxymeter	..They are microbial pressure sensors. ..Pressure drop in close vessel due to microbial intake of oxygen is termed asBacterial Respirometry which is the basis for toxicity assessment in wastewater.	[27]
Biodefense	..Biological attack, in detecting biowarfare agents by recognizing chemical markers. Ex- HPV can be detected by nucleic acid biosensors	[28]
Impedimetric biosensors	..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]

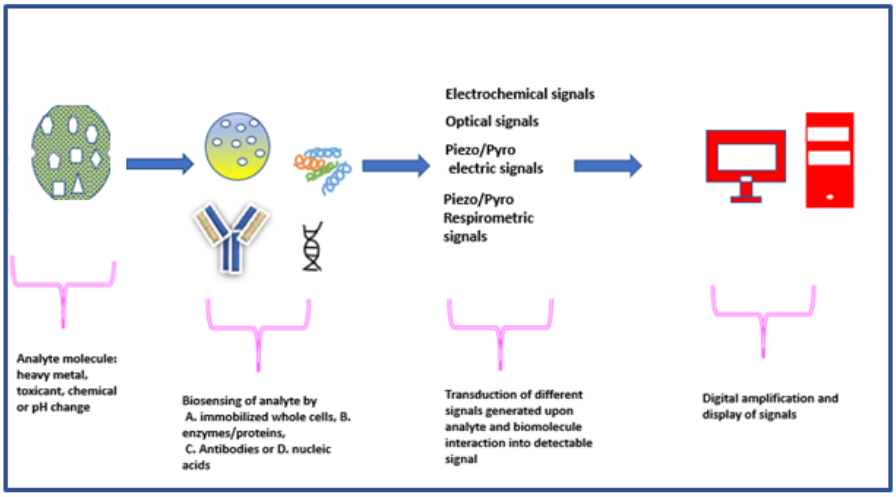

Fig. 3. Stepwise pictorial description of working of a biosensor

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 beappropriate 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 growthis supported by Kong et al who used a novel niobium dobed electrode (lanthum 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 $FePO_4$ in place of carbon or platinum have shown promising results [39]. Electrolyte pH, system sensitivity, anode and cathodetype 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.

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 byanalyzing 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 PublicHealth Association, the sample incubation for 5-7 days with seed microbegrown at roughly 20℃ in the dark before DO (dissolved oxygen) measurement isthe 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 generatedpeak current and the fed organic loadallows 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 waysforBODcalculation 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 andunstable 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)sused 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)

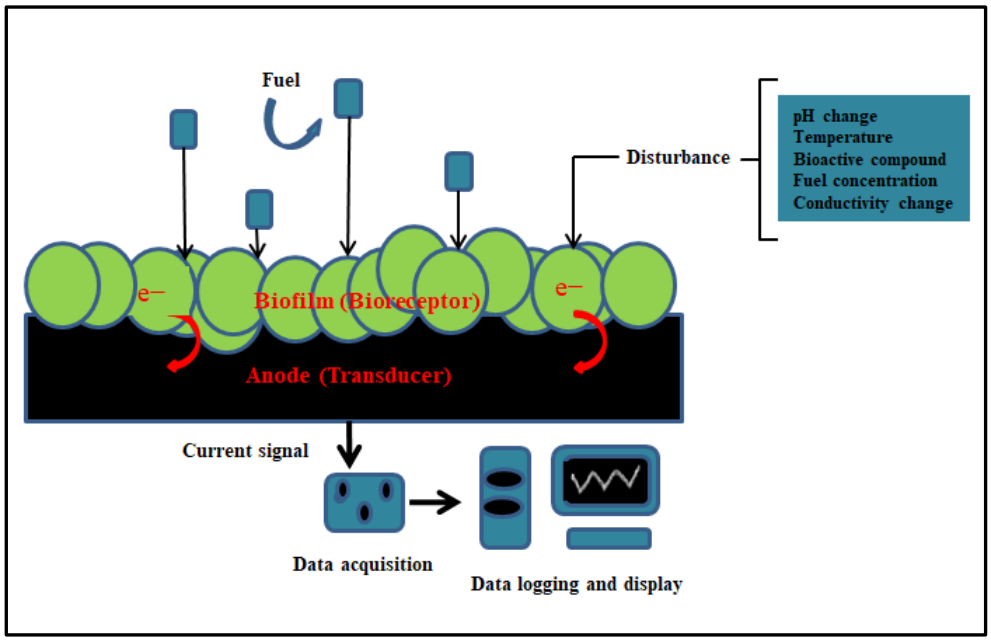

Fig. 4. Is a pictorial representation of Microbial Fuel Cell (MFC) biosensors

showed stability in its performancefor 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_2/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 redoxpotential 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 sulfonatedpolyether 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 NaClin cathode at 37^0C 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.

5.2 Toxicity Sensors

A sensor that depicts presence of harmful and poisonous compounds in a medium is termed as a toxic sensor. Due tohigh cost involved in on-site toxic level monitoring, the standard conventionaltoxicity 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 theyhelp in acquiringa basic device i.e.,small size, fast, cheapalong 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 frompublic 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 successfulfor treating the wastewater.

5.2.1 Heavy metals

Heavy metals are difficult to be reduced or removed by certain microbesas 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 manysevere 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 failureby heavy metals is governed by various factors such as its dosage, chemical species, exposure root, 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 Hg2+ 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 acclimationwhich is why are regarded as best fit for wastewater shocks instead of monitoring drinking water or for measuring the pollutants withCOD 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 transferredto 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 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 Cr^{6+} detection and linear response was shown for detected range of 0125-5mg/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 fe^{+2} concentration range 3-20 mM [67]. Cathode sensing elements also showed good progress in Cr^{+6} and 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 Cu^{+2}an microbial fuel cell (MFC) was developed where copper ions deposition was on cathode surface as they acted as electron acceptor and linear responsive rangelied between 5-160 mg/L [69].

Table 3. Microbial Fuel Cell (MFC) BOD based sensors

Microbial Fuel Cell (MFC) sensors	BOD sensing	Ref
MICROBIAL FUEL CELL (MFC) BOD sensor with no mediator	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.	[54]
Single Chamber MICROBIAL FUEL CELL (MFC) BOD sensor	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 320 mg/l.BOD	[55]
Dual chambered	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	[56]
Miniature MICROBIAL FUEL CELL (MFC)	Change in BOD was observed within nineteen minutes in this 68µL volumed single chamber MICROBIAL FUEL CELL (MFC) with acetate as a fuel source.	[57]
Submercible MICROBIAL FUEL CELL (MFC) BOD sensors	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	[58]
Oligotrophic MICROBIAL FUEL CELL (MFC) BOD sensor	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.	[59]

5.2.2 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 tenBeta lactam antibiotics against E. coli and Staphylococcus aureus at different concentrationsranging from 1 to 75 µg/ml with a panel system which was created by joining miniatured 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 illustrateda detection limitup to 1000 µg/L. Ferric phosphate nanoparticles were used as cathode catalyst that helped indetection of varied concentrations within five minutes. For LEV concentration range 0.1-100 µg/L a linearinterval response was noted. The advantage of long-term usage was also seen as it produced steady state of electricity for about two years [73].

5.2.3 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 anorganophosphorus 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 constructedfor 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 then 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 my 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-3in an anode chamberunder optimal conditions of external resistance, pH and temperature [77].

5.2.4 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 waterpH that further reduces important microbial activity, affects the aquatic like 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 wheretoxic inflow into waste water plants can be detected when a toxicincident 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_2SO_4 and HNO_3 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].

5.3 Different Sensory Applications

Microbial Fuel Cell (MFC)'s canalso 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 itsanalysis. 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 sensorsdeals 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_2O_2 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_2O_2 11.0 μA mM-1 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. [93] where non fermentable substrates gave higher peaks.

5.3.1 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 quantitysensitively detected acetate, propionate and butyrate in range 5-40 mg/L microbial fuel cell (MFC) biosensorsallow voltage and current correlation with VFA quantity as depicted by kaur et al [96].

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

Shock sensors	Toxicity sensing	Ref.
Potassium Ferricyanide mediated MICROBIAL FUEL CELL (MFC)	Heavy metals (2mg/L) and their corresponding inhibition ratios tested by this biosensor. Heavy metals Inhibition ratios Cu^{2}+ 12.56% Hg^{2+} 13.99% Zn^{2+} 8.81% Cd^{2+} 9.29% Pb^{+2} 5.59% Cr^{+3} 1.95%	[82]
Prussia blue cathode MICROBIAL FUEL CELL (MFC)	Sensitive detection with no extra power supply 1mg/L metals like Co^{2+}, Cd^{2+}, $Pb^{2+}Cu^{2+}$showed inhibition absorbance 28.4% 11% 33.8% 66.6% resp.. Cu^{2+} >Pb^{2+} >Cd^{2+} > Co is the toxicity order detected	[83]
Soil microbe (K_3FeCn_{6-}) MICROBIAL FUEL CELL (MFC)	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^{+2}concentration range(10-100mg/kg).	[84]
Photo based biosensor	First plant MICROBIAL FUEL CELL (MFC) used as water sensor Microalgae from wastewater plant is the inoculant Detected formaldehyde sensitively and quickly ($69.2\pm16.7\%^{-1}cm^{-2}$)	[85]
Mediator based sensor	Methylene blue is used as mediator. Detected even low limit arsenic (arsenate 46µM arsenite 4.4µM)	[86]
SDS MICROBIAL FUEL CELL (MFC)	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	[87]
Single chamber air cathode	Inhibition ratios for Cu (II) at flow rate 1.3ml/min with response time 120 minutes 5mg/L-60 7mg/L-85	[88]

5.3.2 Dissolve oxygen detection

Dissolved oxygen measurement plays a vital role for water quality control as changes in its level reflects the presence of organic pollutantsin 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.Omeasuring 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].

5.3.3 MIC biofilm sensors

Corroded biofilm can easily lead to microbiologically influenced corrosion also termed as biocorrosion which isa 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 reducingeasily 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 metalattack 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].

5.3.4 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 correlatedwith 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].

5.3.5 Powering other sensors externally

There are different examples like coupling of Microbial Fuel Cell (MFC)'s for monitoring anaerobic digestor. 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 digestor. 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_2 was attained with a similar approach as discussed above [112]. mA 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.

5.4 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.

Table 5. Depicts improvements done for better sensing applicability

MICROBIAL FUEL CELL (MFC) sensors	Improvements	Ref.
CNT based biosensing	Carbon nanotube are the anode modifiers used. Simultaneous addition of G. sulfureducens with CNT enabled composite biofilm formation at anode. Reduced startup and stable power generation. Reduced anodic resistance(180 ohm) with increased voltage(650mV) maintained. Found to be better than no CNT added MICROBIAL FUEL CELL (MFC)'s	[114]
MnO_2 sensor	Used as a cathode catalyst for ORR reactions at cathode Both catalyst crystalline forms were compared beta and gamma βMnO_2 gave a better performance and system worked for 1.5 years	[115]
PB/PANI	Prussian blue polyalanine is modified oxygen reduction cathode. Showed good cathode potential. Efficiently effective replacement for platinum electrodes.	[116]
Optimized sensor	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 0.1 mg Cd^{+2} and 1 mg Cu^{+2} detected on waste water	[117]
2D anode senor	Carbon felt and indium tin oxide anodes were compared for Pb^{2+} toxicant detection. ITO showed better sensitivity due to its conductive metal oxide nature.	[118]

Two channel bacteria based sensor	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.	[119]
SPEEK sensor	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	[120]
Multi anode paper based sensor	Multilayered carbon cloth anode i.e., flexible in nature for microbial adhesion. 28.4 µ/cm^2 power density was produced. Efficient source of obtaining power from bacterial metabolism	[121]
Quorum sensing sensor	Intensified QS in systems responded linearly to Pb^{2+} sensing. Showed faster recovery to copper ion shock.. With AHL's addition full recovery observed for copper target.	[122]
Data mining sensor	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 phlya	[123]

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 144µL 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.

Biocathodehas 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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