Microorganisms have evolved to respond to environmental factors allowing adaptions to changing conditions and minimisation of potential harm. Microbes have the ability to sense a wide range of biotic and abiotic factors including nutrient levels, analytes, temperature, contaminants, community quorum, and metabolic activity. Due to this ability, the use of whole-cell microbes as biosensors is attractive as it can provide real-time in situ information on biologically relevant factors through qualitative and quantitative outputs. Interestingly, many of the environments where these biosensors will be of most use lack oxygen; and as such the use of anaerobic microorganisms to sense environmental factors with easy to use outputs is essential. Furthermore, sensing of contaminants can be linked with bioremediation of known contaminated environments, allowing a flexible, multiplexed device.

Traditionally, a whole-cell microbial biosensor is described as an analytic device consisting of microorganisms that produce a measurable output in response to a particular stimulus. In the current literature, microbial biosensors are developed in laboratory based E. coli strains, consisting of a cloned gene pathway incorporating regulatory and reporter genes, e.g. gfp expression under regulation by an arsenic sensitive regulatory protein. Microbial fuel cell (MFC) technology however, is now being incorporated with both naturally existing and synthetic microorganisms to produce novel biosensing devices, for detection of environmentally relevant compounds (Table 1). Initially MFCs were developed for energy production via the oxidation of organic matter, utilising anaerobic bacteria such as Geobacter, Shewanella, Desulfuromonas, Rhodopseudomonas, and Desulfovibrio. Species from these genera are essential in microbial community driven oxidation-reduction reactions, necessary for biochemical cycling, and transformation of elements, such as carbon (C), nitrogen (N), sulfur (S) and Iron (Fe), through the biosphere. MFC designs typically consist of electrodes that act as electron acceptors and donors, allowing redox reactions to occur at a distance while creating an electrical current (Figure 1). In the environment, MFCs take advantage of the microorganisms associated with biogeochemical cycles that are able to utilise electrodes as electron acceptors or donors. The electrode-associated microbes are able to extracellularly transfer electrons directly from their central metabolism to an electrode surface through a network of shuttles, cytochromes, and electrical conductive nanowires. Since the electrons come directly from central metabolism, the current produced is a direct measurement of metabolic activity of the electrode associated community. However, such processes can be limited by factors, such as O2 availability, pH levels, available substrates and electron donor availability. Nevertheless, there has been interest in utilising naturally existing anaerobic microbial communities as biosensors in the environment where a correlation can be determined between available substrates and current production.

Electrically integrated microbial biosensor

Organic matter content in an environmental system is normally evaluated in terms of the biochemical oxygen demand (BOD). BOD is a measurement of the required dissolved oxygen to completely degrade the organic matter. MFCs allow the use of the electrode for anaerobic respiration to gain similar information about organic oxidation rates in real-time. A single-chamber MFC containing a mixed anaerobic community has been used as a biosensor for the
Table 1. Examples of microbial fuel cell based biosensors that utilise current as the output.

<table>
<thead>
<tr>
<th>Microorganism(s) used as sensor unit</th>
<th>Inputs and outputs</th>
<th>Detection limits</th>
<th>Reactor type</th>
<th>Lab or field based?</th>
<th>Time for detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed community from artificial wastewater</td>
<td>BOD</td>
<td>20-100 mg L(^{-1}) BOD</td>
<td>Two chamber MFC</td>
<td>Lab</td>
<td>60 min</td>
<td>Chang et al.(^1)</td>
</tr>
<tr>
<td>Mixed community from artificial wastewater</td>
<td>BOD</td>
<td>~100-350 mg L(^{-1}) BOD</td>
<td>Single chamber MFC</td>
<td>Lab</td>
<td>40 min</td>
<td>Di Lorenzo et al.(^1)</td>
</tr>
<tr>
<td><em>Shewanella oneidensis</em> MR-1</td>
<td>Fumarate</td>
<td>10 mM operational concentration 0.83 (\mu)M LOD</td>
<td>Single chamber MFC</td>
<td>Lab</td>
<td>100 (\mu)M detectable after 1 min</td>
<td>Si et al.(^1)</td>
</tr>
<tr>
<td>Mixed microbial communities (3 soil sites used with known Fe(III) reducers, sulfate reducers or methane producers)</td>
<td>Acetate metabolism, correlated to either Fe(III) or sulfate reduction or methane production</td>
<td>10 (\mu)M LOD</td>
<td>Single chamber MFC</td>
<td>Lab</td>
<td>Not reported</td>
<td>Wardman et al.(^6)</td>
</tr>
<tr>
<td>Mixed microbial community including Geobacter species</td>
<td>Sodium acetate, correlating to U (VI) reduction</td>
<td>5 mM acetate resulting in (\leq50) mA/m(^2)</td>
<td>Embedded electrodes in soil</td>
<td>Field (Rifle site, Colorado, USA)</td>
<td>Not reported</td>
<td>Williams et al.(^7)</td>
</tr>
<tr>
<td><em>Shewanella oneidensis</em> MR-1 D(^{mtrA})</td>
<td>First input: IPTG Second input: 3-oxo-C6-HSL Activates: MtrA</td>
<td>Not reported</td>
<td>Two chambered MFC</td>
<td>Lab</td>
<td>Not reported</td>
<td>Hu et al.(^8)</td>
</tr>
<tr>
<td><em>Shewanella oneidensis</em> MR-1 (\Delta)cyaAC, (\Delta)cyaA and (\Delta)cyaC</td>
<td>DMSO</td>
<td>8.3 mM operational concentration</td>
<td>Cathodic MFC</td>
<td>Lab</td>
<td>(&gt;45) h</td>
<td>Aragula et al.(^9)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (\Delta)lacI(^{rhI})</td>
<td>First input: 3-oxo-C12-HSL Second input: C4-HSL Activates: phz operon</td>
<td>15 (\mu)M operational concentration</td>
<td>MFC</td>
<td>Lab</td>
<td>Not reported</td>
<td>Li et al.(^10)</td>
</tr>
</tbody>
</table>

BOD, biological oxygen demand; LOD, limit of detection; MFC, microbial fuel cell; operational concentration, concentration for optimal current output.

Figure 1. Current possible applications of microbial fuel cell technology. (a) Soil samples can be tested *ex situ* in single or double chamber (pictured) MFCs. Reduction of organics can be by either the resident or transplanted microbial community, producing a current output. Synthetically derived microbes may also be incorporated in this design. (b) Electrodes can be implanted into sub-soil zones where the naturally existing anaerobic community can reduce any present compounds, resulting in a detectable current. These designs can use organic (e.g. acetate) or inorganic (electrode) electron acceptors to drive bioremediation of toxic contaminants such as the reduction of U(VI) to U(IV). (c) MFCs may be implanted into the sub soil and supplied with microbes expressing synthetic sensing pathways. Gene pathways may be simple or based on Boolean logic gate design (pictured) to detect specific compounds which may diffuse into the anodic chamber via a proton exchange membrane.
quantification of glucose. The sensor response was linear for concentration of glucose between 0.025 g L\(^{-1}\) up to 25 g L\(^{-1}\). Unfortunately, such sensors are difficult to implement outside of laboratory conditions due to issues with the reliability of the biosensors, as a new anaerobic community is required for each sample tested\(^{15}\).

Investigation into developing a cost-effective MFC biosensor has resulted in a Subsurface Microbial Activity in Real Time (SMART) system, which allows constant monitoring of anaerobic microbial activity in soils\(^6\). Three sediment samples were tested with known anaerobic communities responsible for either Fe(III) reduction, sulfate reduction, or methane production. Electrical current production was correlated with the degradation of tracer acetate [uniformly \(^{14}\)C labelled] with Fe (III), sulfate, or methane acting as a terminal electron acceptor\(^2\). These lab-based MFC biosensors thus allow the \textit{ex situ} monitoring of organic compound degradation from added sediment samples (Figure 1\(a\)). When placed \textit{in situ}, these devices will primarily be useful for monitoring relative changes in microbial activity in response to environmental perturbations. These microbial systems are sensitive to environmental variations, such as temperature and seasonal changes, and may not be ideal to be implemented as \textit{in situ} standalone devices. However, investigation of current production in these anode-resistor-cathode systems provides insight into microbial activity in sediments, and may allow monitoring of the microbial activity of communities responsible for the transformations of important organic compounds in anoxic environments.

**Bioremediation of contaminants**

Direct or mediated electron transfer between microbes and an electrode may allow degradation or transformation of pollutants in bioremediation processes\(^{16}\). Compounds such as metals are unable to be degraded, but can have their solubility reduced. This is essential in preventing their spread and contamination\(^{16,17}\). For example, \textit{Geobacter sulfurreducens} is capable of dissimilatory metal reduction, a process where energy is conserved through oxidizing organic or inorganic electron donors while reducing a metal or metalloid\(^{18}\). \textit{Geobacter} species capable of dissimilatory metal reduction have been applied in a practical on-site \textit{in situ} MFC biosensor, using acetate, an intermediate compound produced during metabolic processes\(^7\), to drive reduction of soluble U(VI) to less-soluble U(IV). Anodes were installed down-gradient of the site of bioremediation and a cathode was embedded on the soil surface (Figure 1\(b\)). This allowed a correlation to be determined between the injections of acetate at the site of contamination, with a significant increase in current\(^7\). This spike in activity also allowed monitoring of changing subsurface water flow through the use of microbial activity measurements, in a grid pattern well set up. Thus this multiplexed sensor allows for monitoring of uranium reduction, while acting as an on-site bioreactor, removing the need for continuous sampling and off-site testing\(^7\). The use of a cathode alone can provide the redox potential required for promoting microbial activity, while simplifying the system. In this case, the cathode acts as an electron donor and provides metabolic energy to the subsurface microbial population. These devices can be solar powered, left in remote locations indefinitely, and not require any further input. It is important to note that in subsoil systems undergoing bioremediation, a good understanding of electron donor or acceptor limitations, metabolic activity and community function are essential for efficient remediation.

**Synthetically derived electric biosensors**

Currently, anaerobic biosensors rely on naturally existing microbial communities to degrade organics, however there has been investigation into incorporating synthetically derived biosensing pathways into MFC devices. Such systems have utilised Boolean logic gate ideas, which are based on modular computer based decision circuits, such as AND, NOT and OR gates\(^{10,19}\). For example, for an AND gate, two inputs must be present for a target gene to be transcribed. A synthetic biosensor has been produced that allows incorporation of a range of known regulator genes, and is based on a quorum sensing system. In a demonstration system, IPTG and quorum sensing modules were created through the use of \textit{lacI} and \textit{luxR}\(^6\). In the presence of both IPTG and 3-oxo-C6-HSL inducers, an output module would be activated. To integrate this reporter system to an electrode, MtrA was utilised as an output in a \textit{S. oneidensis} \textit{ΔmtrA} mutant strain. This mutant is unable to produce an electrical current due to disruption of the Mtr extracellular electron transfer (EET) system\(^9\). Thus, to return this pathway back to functionality, both IPTG and 3-oxo-C6-HSL need to be present to activate the logic gate and MtrA production. This synthetic biosensor demonstrates the potential to clone alternate input/output systems, in particular regulator elements with a high specificity for dangerous contaminants in place of LacI and LuxR, with the detection as an electrical output\(^9\). However, problems can arise through mutations to the sensors through random mutation events, or selection via the contaminants present, which may affect functionality. Further research is also necessary to incorporate safety mechanisms into the synthetic microbes to deter fears of accidental release of genetically modified microorganisms. However with further research synthetic microbes may effectively be utilised in \textit{ex situ} (Figure 1\(a\)) or \textit{in situ} (Figure 1\(c\)) MFC biosensing systems.
**Future directions and conclusions**

Anaerobic bacteria may provide value for biosensing technologies to monitor anoxic zones\(^6\)\(^2\)\(^9\). Their ability to naturally cycle contaminants, along with survivability in contaminated soils and waterways is allowing incorporation into both biosensing devices and bioreactors. Many anaerobic biosensors developed are generally reliant on MFC type designs because the ability to utilise an external electron acceptor provides an easy-to-monitor output, in the form of current. Furthermore, these designs have already been linked to degradation of organics, or immobilisation of metals. However, such outputs are subject to environmental fluctuations and as thus may not always be a reliable detection method. Further investigation by monitoring the metabolism of microbes associated with anodes\(^2\)\(^1\) is also still necessary. Currently, there is promise in incorporating synthetic microbes into the anode compartment to produce a biosensing device for a range of contaminants. Hence, whole-cell microbial biosensors based in anaerobic microbes may provide a cost effective means of detection and bio remediation, allowing long-term monitoring that may be deployed in a variety of environments.

**References**


**Biographies**

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