Chapter 20
Application of Microbial Fuel Cell as a Biosensor

Ramya Veerubhotla and Debabrata Das

20.1 Introduction

Microbial fuel cells (MFCs) are being studied as a biosensor for a wide gamut of applications. Currently, since the power generated by an MFC is too low to power any practical devices, it is pertinent to use MFC as a sensor for the accurate and simple measurement of various analytes. The present chapter deals with the various upcoming applications of the MFC technology in the field of sensing with an emphasis on Biochemical Oxygen Demand (BOD) bio-sensing. The application of MFCs as BOD biosensor is one of the most widely studied fields followed by the volatile fatty acid sensing and toxicity sensing. Figure 20.1 depicts various novel applications of MFC as biosensor from the literature.

20.2 Microbial Biosensors

Biosensors are devices which combine a bio-receptor, usually an enzyme, microbe or a biologically active molecule that responds to an analyte or change in its concentration and a transducer that converts the response into a measurable output (D'Souza 2001; Su et al. 2011). Occasionally, an amplifier is also employed which multiplies the signal for implicit applications. The transducer is usually chosen depending upon the analyte as well as the biological sensing element. It is desirable to have biosensors that are portable, reproducible and those which provide rapid and accurate results of the parameters to be measured (Lim et al. 2015). Depending

R. Veerubhotla (✉) • D. Das
Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur 721302, India
e-mail: vramya.90@gmail.com

D. Das (ed.), *Microbial Fuel Cell*,
https://doi.org/10.1007/978-3-319-66793-5_20

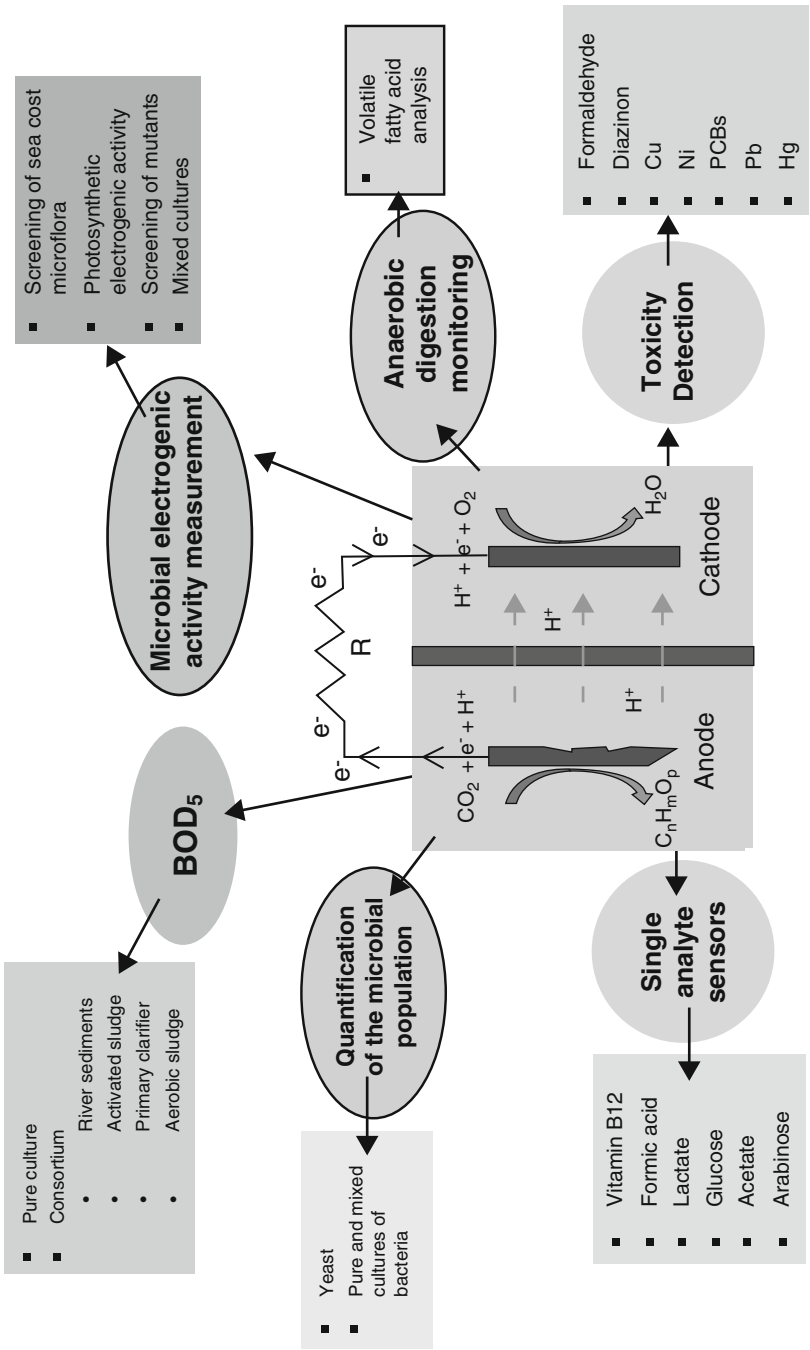

Fig. 20.1 Typical applications of MFC as sensor

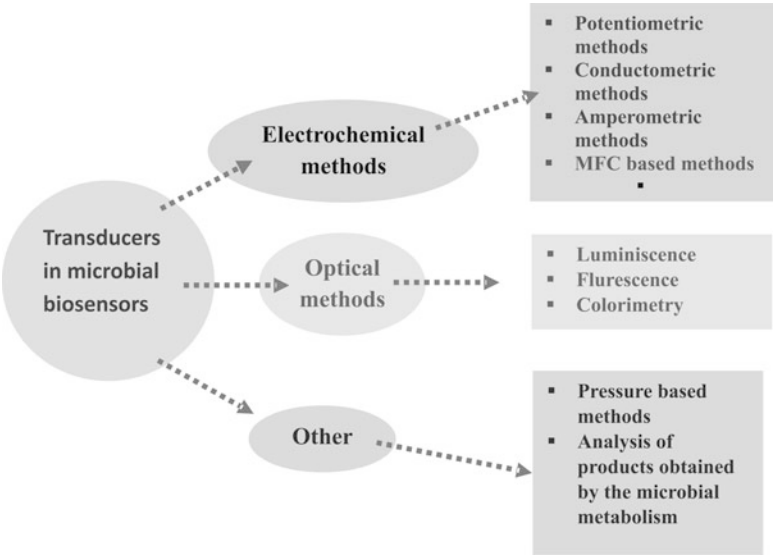

Fig. 20.2 Classification of the transducers of various microbial biosensors

on the type of the transducer, microbial biosensors are classified as depicted in Fig. 20.2.

Microbial biosensors in general pose various advantages as microorganisms are easy to grow and maintain, can work independently, and are easy to adapt and regenerate. Unlike use of the enzyme-based biosensors which require stringent micro-environment to sustain the activity, microbial biosensors can work with the help of a wide variety of substrates and environments depending on the culture conditions. Since purification steps are absent in these sensors, use of microbes is quite inexpensive as compared to enzymatic biosensors. The same microbes can be utilized to monitor various analytes depending on the transducer used. Microbes also offer unique advantages in diverse immobilization methods possible to entrap them while retaining the activity. Further, genetic engineering techniques offer new avenues and applications in the field of microbial biosensors. Among the different transduction methods (Fig. 20.2), electrochemical method of transduction is the most convenient method for the detection of analytes. The output signal is easy to read and process as it does not require sophisticated equipment unlike fluorescence or bioluminescence. MFCs use various techniques of electrochemistry such as amperometry, voltammetry, cyclic or conductometry for the quantification of the BOD.

20.3 Principle of MFC as a Biosensor

An MFC consists of anaerobic anode chamber where electroactive microbes act as biocatalysts utilizing the nutrients added. The electrons generated by the metabolism of the nutrients present in the medium is proportional to the concentration of

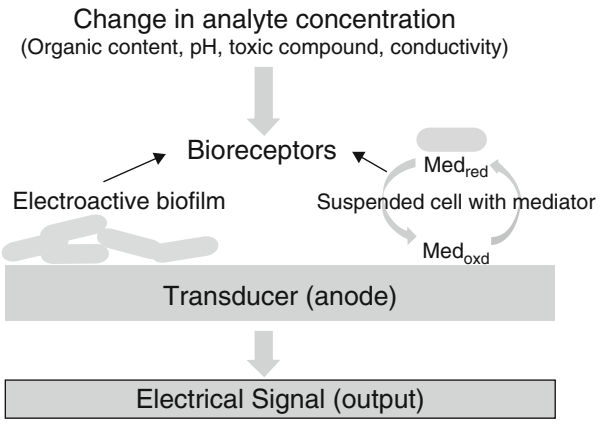

Fig. 20.3 Principle of MFC as a sensor

the nutrient solution in the anode chamber. These electrons travel through the external circuit to the cathode where the oxygen reduction takes place. Both anode and the cathode chambers are separated by a proton exchange membrane that selectively allows the movement of protons to oxygen reduction. The change in the analyte concentration can be precisely determined by the bioreceptors (electroactive bacteria) which is usually immobilized on the transducer (anode) itself. Typically the electrons generated by the metabolism in the anode chamber are measured which acts as the signal for sensing as represented in Fig. 20.3.

The MFC system can be utilized for the principle of biosensing in two possible ways:

1. The concentration of the organic component (analyte) or a specific growth molecule is proportional to the electrons generated by the system which can be measured by the electron flow (current, voltage, coloumbic efficiency). Examples of such sensors include BOD biosensors, single analyte sensors for the detection of glucose, vitamins etc., volatile fatty acid monitoring sensors etc.
2. In other cases, the MFC-based system can also be utilized to monitor the toxic contaminants or pollutants where the concentration or titre of the pollutant added hampers the activity of immobilized electroactive bacteria (Abrevaya et al. 2015). In such cases, the concentration of the toxic compound is inversely proportional to the electrons generated by the bacteria and hence the signal. Typical examples of such toxicity sensors include detection and quantification of formaldehyde, heavy metals etc.

In MFC-based biosensors, the electrons generated by the bacteria can be diverted to anode via two well known mechanisms: (1) Mediator-driven and (2) Mediator-less as demonstrated in Fig. 20.3 (Abrevaya et al. 2015). In mediator-driven systems, a soluble mediator either added externally or produced by microbe itself can shuttle the electrons between the cells and electrodes. Various mediators are known to enhance the electron transfer which are described in the previous chapters. Such systems can operate even with non-electroactive bacteria

and the concentration and diffusion of the mediator greatly affects the output signal. On the other hand, mediatorless systems have bacteria immobilized to the electrode. Electrons generated at the anode can result in the generation of the signal either by the outer membrane complex or with the help of bacterial nanowires. In most cases, a biofilm formation is induced on the surface of the anode with time. Such a mechanism does not involve any external addition of the mediator and is hence more suitable for practical applications.

20.4 Advantages of MFC as a Sensor

Use of an MFC as biosensor eliminates the need for a separate transducer by acting as a biofilm anode. Besides, the microbial population expands itself by the reproduction of the microbes in the provided volumes inducing a self-regeneration capability. Thus, an MFC can possess self-start property and work as a standalone biosensor. Depending upon the volume of the anode chamber and the bacteria used, the range of current output from the cells is in the range of mA-μA. Thus, the output signal from the MFC (usually in the form of current or voltage) is simple to read and interpret. Further, the fabrication of the devices does not need expensive probes, construction methods and stringent maintenance. The aforementioned unique properties of MFC make it one of the ideal platforms for the sensing as well as quantification of various analytes. The key to accurate sensing with high reproducibility is a stable/consistent biofilm with a defined microbial population at the anode which is resistant to environmental fluctuations. Environmental parameters such as pH, temperature, conductivity, electron acceptors, toxicants, composition of wastewater added will greatly influence the measurement (Abrevaya et al. 2015). Further, inherent system parameters such as ion transport across the membranes used, effective cathode reduction reaction, and electron transport rate, configuration of the device, volume of the device and mode of operation will define the net output signal and its stability with time.

20.5 BOD and Its Importance

BOD is the amount of dissolved oxygen needed by aerobic biological organisms to break down organic material present in a given wastewater sample at certain temperature over a specific time period. Measuring the BOD of water bodies is of immense environmental significance. BOD quantifies how the biodegradable organic content of a water body can affect the oxygen concentration in it. The pollution load of effluents from industries is reflected by means of BOD and is critical as an agreeable amount of dissolved oxygen is necessary for a healthy aquatic life. Hence, there are strict limits as to what level of BOD containing wastewater should be discharged in to the nearby water bodies depending on the

Pollution Control Board of different countries. Furthermore, the wastewater/effluent treatment method adopted and various engineering aspects of wastewater treatment involve an accurate measurement of BOD. Thus, a rapid quantification of BOD is essential for the assessment of water quality (Jouanneau et al. 2014).

20.6 Methods of Assessing BOD

The standard BOD test takes a period of 5 days and it involves the incubation of sludge in specific BOD bottles in dark for a time period of 5 days. Microbes in the wastewater use the dissolved oxygen to oxidize the organic content for deriving energy for metabolism. The dissolved oxygen is measured before and after the stipulated time using a Clark electrode (Jouanneau et al. 2014). Although both COD and BOD measure the organic content of a sample, BOD exclusively measures only the biodegradable content. A standard testing of BOD is essential for any organic body before the disposal of wastewater. However, certain problems associated with the standard method such as need to maintain the probes, variability of the inoculum and a narrow range of detection has encouraged researchers to innovate new methods for a quick estimation of the BOD. The standard method is not a feasible option for the on-site BOD monitoring as it requires a lot of working space and bulky apparatus such as incubators. Since various environmental applications are dependant on the BOD values, it is crucial to have a portable, rapid and inexpensive method for the quantification with minimum deviation from the BOD_5 value. BOD biosensors based on bioluminescence, photometric, manometric methods, modified standard methods and bioreactor-based sensors are already demonstrated in this regard. The following section of this chapter will discuss in detail the merits and demerits of using MFC as an alternate sensor for BOD providing valuable insights on the challenges and the scope of improvement.

20.7 Application of MFC as a BOD Sensor

The key advantage of using an MFC for BOD monitoring is that the time consumed to give a preliminary estimate of the sample BOD is much less as compared to the conventional standard method. Since the reactor volume can be customized by the user, MFC-based sensors enable quick estimation of BOD even from minimal sample volumes. Typically, the response times of the devices are in the range of few minutes to hours. Depending upon the mode of operation and the configuration, it is possible to operate the system with a minimal maintenance. Unlike the standard method of BOD measurement which deploys the dissolved oxygen monitoring using probes, MFCs employ anaerobic chambers enabling an indirect current measurement. The electrons obtained by the degradation or oxidation of the organic content in an anaerobic environment is proportional to the concentration of the

organic matter or BOD. The electrochemically active bacteria can metabolize the organic content present in the anaerobic chambers with or without the use of mediators and the generated electrons can be diverted to the anode. This unique ability of the electrochemically active bacteria to divert electrons only takes place in the absence of any readily available electron acceptors in the environment the microbes are placed. The very advantage of the use of an MFC is the fact that this flow of electrons can be detected using a simple amperometer in a precise way eliminating the need for the use of DO probes. However, both current and charge output are monitored and corelated with the organic content depending upon the range of the BOD.

Diverse groups of exoelectrogens can be colonized to the anode depending on the acclimatization approach. Hence, this method can not only have a broad range of measurement but also provides a more reliable BOD measurement depending on the nature, diversity, quantity of microbial population present and the configuration of the system in use. Further, reports suggest that online monitoring of the BOD is possible using a continuously operated MFC system with minimum operational requirements. Such a system is demonstrated by Zhang et al. using a submersible MFC system. Another added advantage of an MFC system is the reduced time spans in obtaining the BOD values. Depending on the maturity of the biofilm, the time requirement for measurement can range from a few minutes to hours (Table 20.1). While some MFCs use continuous systems with an acclimatized biofilm, some systems adopt a batch operation where bacterial colonization on the electrodes need to be induced and further measurement of the organic content is attempted. These characteristics suggest the application of MFC for both field applications as well as lab scale rapid analysis of the BOD. The configuration of the MFC adopted also has a profound role in deciding the response time as well as the accuracy of the system.

20.7.1 MFC as a BOD Biosensor—State of Art

The concept that an MFC can act as an amperometric biosensor for BOD estimation is first introduced by Karube et al. (1977). By using an MFC containing whole cells of *Clostridium butylicum* on the platinum electrode, they could estimate the BOD with a relative error of 10%. As the cells metabolized the organic substrates, hydrogen and formate formed by the cells reacted at the electrodes and resulted in the electricity generation. The steady state current linearly varied with the input strength of organic substrate. This led to the measurement of the BOD within 30–40 min which was much less than the time consumed by conventional methods (Karube et al. 1977). Following this, a continuously operated MFC containing a mixed consortia (obtained from a sewage plant) is proposed (Kim et al. 2003). A practical field application is also demonstrated using the set-up for the online monitoring of the real time samples from a wastewater treatment plant. The results showed encouraging results with a good stability in the current generation. The

Table 20.1 MFC as a BOD biosensor – present state of art

Source of the biocatalyst (Microbes)	Mediator	Measurement time	Range of detection (mg L^{-1})	Mode of operation	Volume (mL)	References
Clostridium butyricum	No	70 min	10–300	Batch	100	Karube et al. (1977)
Consortium (river sediments)	No		5–10	Batch	25	Kang et al. (2003)
Consortium (anaerobic sludge)	No	40 min–2 h	50–400	Continuous	50	Di Lorenzo et al. (2009)
Consortium (primary clarifier)	No	40 min	10–250	Fed batch	500	Zhang and Angelidaki (2011)
Escherichia coli	Poly-neutral red		50–1000			Liu et al. (2012)
Consortium (anaerobic and aerobic sludge)	No	5–20 h	32–1280	Batch	11.8	Modin and Wilén (2012)
Mixed consortia	No	5–1 min.	20–200	Continuous	5	Moon et al. (2004)
Geobacter-dominated biofilms	No	17.5 h	174 to 1200	Batch mode	1	Commault et al. (2016)
Anaerobic sludge	No	3–5 min	0.025 to 25	Batch	100	Kumlanghan et al. (2007)

work also noted the significance of recalibration for long time use of the set-up. Various attempts by the researchers in this regard along with their respective BOD ranges is tabulated in Table 20.1.

Further, Moon et al. (2004) adopted a step change strategy for the quantification of the input fuel stream concentration. They observed that the response time of the devices are directly proportional to the anode chamber volumes. They achieved a drastic reduction in the response time (upto 5 min) using a small chamber volume (5 mL) (Moon et al. 2004). In order to measure the BOD in the lower range, the use of oligotrophic microbes (which are electroactive) in an MFC is first suggested by Kang et al. (2003). Their research suggested that use of a smaller membrane and a highly active cathode can effectively prevent the oxygen diffusion from the cathode to anode. The leakage of the oxygen is thus minimized into the anode chamber in order to maximize the coloumbic efficiency which resulted in obtaining a good reproducibility inspite of using low nutrient concentration. Kumlanghan et al. (2007) used an MFC-based BOD biosensor by integrating the system with an anaerobic digester. The consortium for each batch reaction is taken from the reactor itself. With a response time of 5 min, the system showed good correspondence with the input feed concentration (Kang et al. 2003). Their study also optimized the electrolyte concentration as the ion transport plays an important role in the overall performance (Kumlanghan et al. 2007).

While all the above mentioned preliminary studies used an MFC of double chambered configuration, Di Lorenzo et al. put forth the use of a single chambered MFC system in a continuous mode as a BOD biosensor. A strong correlation between the hydraulic retention time (HRT) and the response time of the MFC is shown successfully (Di Lorenzo et al. 2009). Another interesting approach for the *in situ* monitoring of the BOD content of groundwater is suggested by Zhang and Angelidaki (2011). A submersible MFC is developed by their team with a systematic study of the effect of temperature, pH, conductivity and the input feed concentration on the sensitivity of the system. They suggested that their endeavours can further open doors to monitor the bioremediation of toxic compounds in a non-invasive and simple way (Zhang and Angelidaki 2011).

On the other hand, use of mediator-based BOD biosensor in conjunction with nanoparticle aided pretreatment (TiO_2) is demonstrated by Liu et al. Neutral red, the mediator used in the study, is co-immobilized along with *E. coli* cells on a glassy carbon anode. The authors recommended that photocatalysis of the organic molecules prior to addition into the anodic chamber can substantially cause pronounced signals as compared to the untreated ones (Liu et al. 2012). A novel BOD sensor which works on the principle similar to that of an electrolysis cell was proposed by Modin and Wilén (2012) where external input of 0.2 V is supplied to anode to aid the microbial degradation of the organic compounds. With acetate as the source of carbon, the system could measure a very high range of BOD, probably due to the external voltage supplied. The system also avoided the use of an ion exchange membrane which eliminated the demerits of using membranes such as pH splitting (Modin and Wilén 2012). Upcoming applications demonstrated an MFC for measuring the BOD of cow milk unlike others who measure the organic content

of wastewaters (Commault et al. 2016). Selectively adopted *Geobacter* dominated biofilm is utilized in their study by using a new strategy of ethanol acclimatization (Commault et al. 2016).

One such BOD biosensor which works with the help of electrochemically active bacteria is already marketed by KORBI Co. Ltd. as BOD analyzer, HABS Series.

20.7.2 Challenges of MFC-Based BOD Biosensors

One of the potential problems using MFCs as biosensors is the significant diffusion of oxygen from cathode chambers to the anode. Microbes then aerobically respire instead of utilizing the anode causing a drop in the coloumbic efficiency and thus interfere with the measurement (Moon et al. 2005). This can lead to improper results as it becomes difficult to accurately estimate the BOD due to interference from the oxygen, especially when the BOD of low nutrient concentration is being measured. Hence, sparging the influent organic fuel to remove the inherent oxygen content while minimizing the oxygen leakage into anode chamber can improve the coloumbic yield. An endeavour in this direction is attempted by Ayyaru and Dharmalingam (2014) where the use of a sulphonated poly ether ketone membrane to improve the response is suggested. Besides, the use of membrane can itself result in problems such as pH splitting, substrate transfer across the membrane etc. (Christgen et al. 2015). Hence there is a need for the development of membraneless systems or highly efficient membrane cathode assembly units to overcome such effects.

BOD measurement of the real-time wastewater samples using MFCs, however, still remains highly challenging due to the drastic effect of the indigenous properties of the wastewater such as pH, conductivity, presence of external mediators, inhibitors or ready electron acceptors for microbial respiration. This variation in the internal factors also makes the *in situ* monitoring of the organic pollutants impractical. Several studies highlighted the prominence of the pH and conductivity of the anolyte (wastewater) on the performance of an MFC (Zhang and Angelidaki 2011; Kim et al. 2009). Possible approaches to reduce the dependancy of pH on the bioelectric output are the use of membraneless design and to provide an appropriate buffering in the system.

Detecting the presence of inherent electron acceptors in the wastewater samples and quantifying them is tedious and impractical. Furthermore, presence of toxic inhibitors in the wastewaters can alter biofilm structure, microbial community leading to decrease the bacterial viability. Researchers attempted to solve this by the addition of certain inhibitors such as azide and cyanide (Chang et al. 2005). Although such an addition in minute quantities is claimed to not affect the performance of the system in the absence of nitrate or other electron acceptors. It is, however, not encouraged as it can affect the bacterial community of the anodic biofilm. Viability of the biofilm and its sensitivity towards environmental parameters is to be thus considered for stability of the biofilm as well as the overall

performance of the sensor. Further, efforts are needed to maintain a uniform electrochemical environment in the cells to eliminate batch to batch variation and ensure accurate measurement. Significant progress is needed in the pretreatment methods to bring down the BOD level to the desirable range and to eliminate unwanted compounds. This can further make the MFC-based BOD biosensors superior as compared to the standard BOD measurement methods in terms of accuracy.

20.8 Upcoming Applications of MFC in the Field of Sensing

In the recent years, there is a growing interest in the novel applications of MFCs as sensors for various applications as depicted in Fig. 20.4.

20.8.1 Screening of Electroactive Microbes

Since electroactive microbes form the basis of various bioelectrochemical systems such as MFCs, electrolysis cells, microbial desalination cells, microbial carbon capture cells, microbial electrosynthesis cells etc., finding appropriate microbes

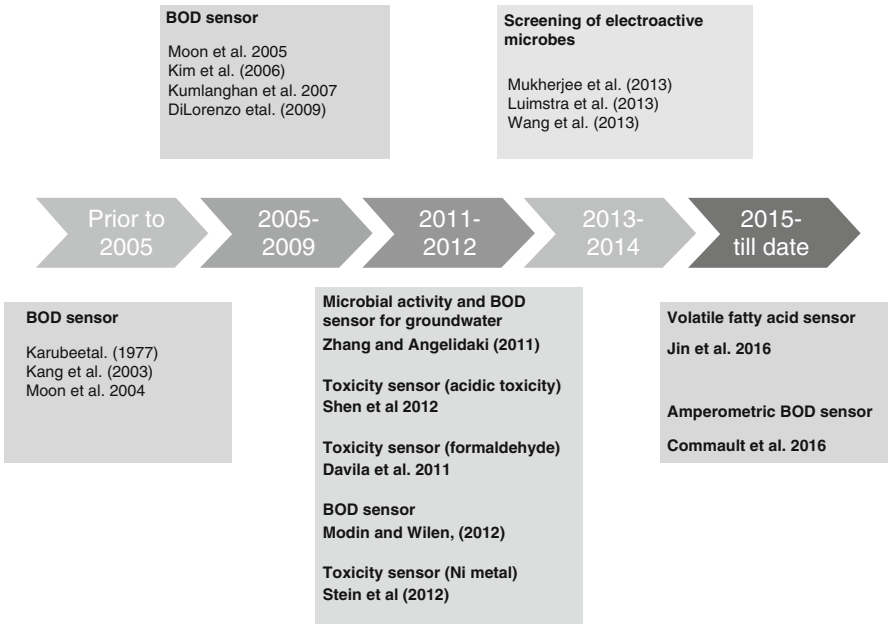

Fig. 20.4 Timeline of the various research works in the field of application of MFC as a sensor

suitable for the system becomes most crucial for the improvement of MFCs. Portable microfluidic platforms are reported for providing a quick assessment of the electrogenic potential of such microbes (Mukherjee et al. 2013). Such platforms are successful in preliminary screening of bioelectric potential of multiple strains simultaneously with tiny sample volumes (Hou et al. 2011). Such techniques are particularly useful for the evaluation of the performance of genetically modified strains. Choi et al. (2015) deviced a paper based disposable and inexpensive 48-well MFC array on a paper platform for practical real world applications.

20.8.2 Toxicity Sensing

MFCs are being applied as potential toxicity sensing elements owing to the change in the bioelectric output in the presence of a toxic compound. Since conventional methods of estimating the toxic elements in the soil, water and sediments are usually time-consuming, and require a lot of equipment, skilled personnel to interpret the results, it is beneficial to use MFC, because the biofilm of the system is highly sensitive to certain toxic compounds. Arsenic biosensor by genetically modified *Shewanella* sp. (Webster et al. 2014); nickel detection by Stein et al. (2012); online biomonitoring of Pb, Hg and PCBs using MFCs (Kim et al. 2007); and microfabricated formaldehyde biosensor (Dávila et al. 2011) are some of the works in the field of toxicity biosensing by MFCs. However, the research is still in a native state and there is a need to precisely detect the pollutants and obtain reproducible results.

20.8.3 VFA Sensing

Instead of measuring the overall BOD or the strength of wastewater, MFCs are now emerging as the biosensors for the volatile fatty acid (VFA) monitoring of the anaerobic digestion process. MFCs have been shown to have a precise correlation with the concentrations of single analyte species and can thus form promising VFA biosensors. This can avoid use of instruments like gas chromatography, tedious sample preparation methods to detect the analytes and enable an accurate and facile detection of the VFAs. Notable attempts in this regard are made by several researchers (Kaur et al. 2013; Jin et al. 2016).

20.9 Conclusion and Future Perspectives

MFC have the potential of being developed into promising biosensor devices that can be marketed for practical applications. To exploit the inherent unique advantages of the system, an interdisciplinary approach with collective efforts from

researchers of various fields is needed. Improvement of the sensitivity and reproducibility of the system enhances the practical applicability of the MFC-based biosensors. Miniaturization and multiplexing can also provide rapid and preliminary estimation of various useful analytes in a simple manner.

References

Abrevaya, X. C., Sacco, N. J., Bonetto, M. C., Hilding-Ohlsson, A., & Cortón, E. (2015). Analytical applications of MFCs. Part I: Biochemical oxygen demand. *Biosensors & Bioelectronics, 63*, 580–590.

Ayyaru, S., & Dharmalingam, S. (2014). Enhanced response of MFC using sulfonated poly ether ether ketone membrane as a biochemical oxygen demand sensor. *Analytica Chimica Acta, 818*, 15–22.

Chang, I. S., Moon, H., Jang, J. K., & Kim, B. H. (2005). Improvement of a MFC performance as a BOD sensor using respiratory inhibitors. *Biosensors & Bioelectronics, 20*, 1856–1859.

Choi, G., Hassett, D. J., & Choi, S. (2015). A paper-based MFC array for rapid and high-throughput screening of electricity-producing bacteria. *Analyst, 140*(12), 4277–4283.

Christgen, B., Scott, K., Dolfing, J., Head, I. M., & Curtis, T. P. (2015). An evaluation of the performance and economics of membranes and separators in single chamber MFCs treating domestic wastewater. *PloS One, 10*, 1–13.

Commault, A. S., Lear, G., Bouvier, S., Feiler, L., Karacs, J., & Weld, R. J. (2016). *Geobacter*-dominated biofilms used as amperometric BOD sensors. *Biochemical Engineering Journal, 109*, 88–95.

Dávila, D., Esquivel, J. P., Sabat, N., & Mas, J. (2011). Silicon-based microfabricated MFC toxicity sensor. *Biosensors & Bioelectronics, 26*, 2426–2430.

Di Lorenzo, M., Curtis, T. P., Head, I. M., & Scott, K. (2009). A single-chamber MFC as a biosensor for wastewaters. *Water Research, 43*, 3145–3154.

D'Souza, S. F. (2001). Microbial biosensors. *Biosensors & Bioelectronics, 16*, 337–353.

Hou, H., Li, L., De Figueiredo, P., & Han, A. (2011). Microfluidic MFC Array for multiplexed long-term parallel analysis of microbial activities. In *15th international conference on miniaturized systems for chemistry and life sciences*, Seattle, Washington.

Jin, X., Angelidaki, I., & Zhang, Y. (2016). Microbial electrochemical monitoring of volatile fatty acids during anaerobic digestion. *Environmental Science & Technology, 50*(8), 4422–4429.

Jouanneau, S., Recoules, L., Durand, M. J., Boukabacheb, A., Picotb, V., Primaultc, Y., Lakeld, A., Sengeline, M., Barillonf, B., & Thouand, G. (2014). Methods for assessing biochemical oxygen demand (BOD): A review. *Water Research, 49*, 62–82.

Kang, K. H., Jang, J. K., Pham, T. H., Moon, H., Chang, I. S., & Kim, B. H. (2003). A MFC with improved cathode reaction as a low biochemical oxygen demand sensor. *Biotechnology Letters, 25*, 1357–1361.

Karube, I., Matsunaga, T., Mitsuda, S., & Suzuki, S. (1977). Microbial electrode BOD sensors. *Biotechnology and Bioengineering, 19*, 1535–1547.

Kaur, A., Kim, J. R., Michie, I., Dinsdale, R. M., Guwy, A. J., & Premier, G. C. (2013). MFC type biosensor for specific volatile fatty acids using acclimated bacterial communities. *Biosensors & Bioelectronics, 47*, 50–55.

Kim, M., Youn, S. M., Shin, S. H., Jang, J. G., Han, S. H., Hyun, M. S., Gadd, G. M., & Kim, H. J. (2003). Practical field application of a novel BOD monitoring system. *Journal of Environmental Monitoring, 5*, 640–643.

Kim, M., Hyun, M. S., Gadd, G. M., & Kim, H. J. (2007). A novel biomonitoring system using MFCs. *Journal of Environmental Monitoring, 9*, 1323–1328.

Kim, M., Hyun, M. S., Gadd, G. M., Kim, G. T., Lee, S. J., & Kim, H. J. (2009). Membrane-electrode assembly enhances performance of a MFC type biological oxygen demand sensor. *Environmental Technology, 30*, 329–336.

Kumlanghan, A., Liu, J., Thavarungkul, P., Kanatharana, P., & Mattiasson, B. (2007). MFC-based biosensor for fast analysis of biodegradable organic matter. *Biosensors & Bioelectronics, 22*, 2939–2944.

Lim, J. W., Ha, D., Lee, J., Lee, S. K., & Kim, T. (2015). Review of micro/nanotechnologies for microbial biosensors. *Frontiers in Bioengineering and Biotechnology, 3*, 1–13.

Liu, L., Zhang, S., Xing, L., Zhao, H., & Dong, S. (2012). A co-immobilized mediator and microorganism mediated method combined pretreatment by TiO_2 nanotubes used for BOD measurement. *Talanta, 93*, 314–319.

Modin, O., & Wilén, B. M. (2012). A novel bioelectrochemical BOD sensor operating with voltage input. *Water Research, 46*, 6113–6120.

Moon, H., Chang, I. S., Kang, K. H., Jang, J. K., & Kim, B. H. (2004). Improving the dynamic response of a mediator-less MFC as a biochemical oxygen demand (BOD) sensor. *Biotechnology Letters, 26*, 1717–1721.

Moon, H., Chang, I. S., Jang, J. K., Kim, K. S., Lee, J., Lovitt, R. W., & Kim, B. H. (2005). On-line monitoring of low biochemical oxygen demand through continuous operation of a mediator-less MFC. *Journal of Microbiology and Biotechnology, 5*, 192–196.

Mukherjee, S., Su, S., Panmanee, W., Irvin, R. T., Hassett, D. J., & Choi, S. (2013). A microliter-scale MFC array for bacterial electrogenic screening. *Sensors and Actuators A: Physical, 201*, 532–537.

Stein, N. E., Hamelers, H. M. V., Van Straten, G., & Keesman, K. J. (2012). On-line detection of toxic components using a MFC-based biosensor. *Journal of Process Control, 22*, 1755–1761.

Su, L., Jia, W., Hou, C., & Lei, Y. (2011). Microbial biosensors: A review. *Biosensors & Bioelectronics, 26*(5), 1788–1799.

Webster, D. P., TerAvest, M. A., Doud, D. F. R., Chakravorty, A., Holmes, E. C., Radens, C. M., Sureka, S. I., Gralnick, J. A., & Angenent, L. T. (2014). An arsenic-specific biosensor with genetically engineered *Shewanella oneidensis* in a bioelectrochemical system. *Biosensors & Bioelectronics, 62*, 320–324.

Zhang, Y., & Angelidaki, I. (2011). Submersible MFC sensor for monitoring microbial activity and BOD in groundwater: Focusing on impact of anodic biofilm on sensor applicability. *Biotechnology and Bioengineering, 108*, 2339–2347.