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Stabilizing the baseline current of a microbial fuel cell-based biosensor through overpotential control under non-toxic conditions

Nienke E. Stein [a,b], Hubertus V.M. Hamelers [a,*], Cees N.J. Buisman [a,b]

[a] Wetsus, Centre of Excellence for Sustainable Water Technology, PO Box 1113, 8900CC Leeuwarden, The Netherlands
[b] Subdepartment of Environmental Technology, Wageningen University, P.O. box 8129, 6700 EV Wageningen, The Netherlands

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ABSTRACT

A MFC-based biosensor can act as online toxicity sensor. Electrical current is a direct linear measure for metabolic activity of electrochemically active microorganisms. Microorganisms gain energy from anodic overpotential and current strongly depends on anodic overpotential. Therefore control of anodic overpotential is necessary to detect toxic events and prevent false positive alarms. Anodic overpotential and thus current is influenced by anode potential, pH, substrate and bicarbonate concentrations. In terms of overpotential all factor showed a comparable effect, anode potential 1.2% change in current density per mV, pH 0.43%/mV, bicarbonate 0.75%/mV and acetate 0.8%/mV. At acetate saturation the maximum acetate conversion rate is reached and with that a constant bicarbonate concentration. Control of acetate and bicarbonate concentration can be less strict than control of anode potential and pH. Current density changes due to changing anode potential and pH are in the same order of magnitude as changes due to toxicity. Strict control of pH and anode potential in a small range is required.

The importance of anodic overpotential control for detection of toxic compounds is shown. To reach a stable baseline current under nontoxic conditions a MFC-based biosensor should be operated at controlled anode potential, controlled pH and saturated substrate concentrations.

1. Introduction

The microbial fuel cell (MFC) is a device in which microorganisms convert chemical energy into electrical energy [1]. Electrochemically active microorganisms oxidize organic matter anaerobically and transfer the electrons to the anode. For these microorganisms organic matter serves as electron donor and the electrode as electron acceptor. Electrical current in a MFC is thus a direct linear measure for metabolic activity of the electrochemically active microorganisms. As current can be monitored easily on line, a MFC can thus act as an online biosensor for the metabolic activity of the microorganisms. Such an online MFC-based biosensor does not need a transducer to read the signal and convert it to an electrical signal because the measured signal is already an electrical current. This is a major advantage over many other types of online biosensors that often need complex non-linear transducers [2–4].

Toxic substances have an inhibitory effect on the metabolism of microorganisms and in case of electrochemically active microorganisms, an inhibitory effect on the transfer rate of electrons to the electrode. Presence of toxic substances can thus decrease the current compared to a situation with no toxic compounds present. This means that the microbial fuel cell can be used as an online sensor for the presence of toxic compounds in water.

An online sensor is typically used to guard the water quality of water flows like wastewater, drinking water or process water. Detection of toxic compounds is needed to protect consumers and downstream processes from the negative effects of the toxic compounds. In most cases, the presence of a toxic compound will be an exceptional event.

The water to be investigated is continuously fed to the anode of the MFC based biosensor and when a toxic substance is present, this is indicated by the sensor as a decrease in current [5]. If this current drop is detected, an alarm is given and measures can be taken to protect customers and/or downstream processes.

Ideally there should only be a change in current, when there is a toxic compound present in the water fed to anode of the MFC-based biosensor. If another factor than a toxic compound causes a current drop this will give a so-called false positive result, the current drop is wrongly interpreted as the presence of a toxic compound. Such a false positive outcome must be prevented as it leads to an unnecessary alarm with sometimes costly measures. Too frequent occurrence of false positive results may lead users to ignore the alarm completely making online control ineffective. This means that it is crucial to assure that under non-toxic conditions the current remains stable to prevent false positive alarms [6,7]. This is especially important as the non-toxic conditions are the rule and toxic events are the exception.

* Tel.: +31 317483447; fax: +31 317 482108.
E-mail addresses: nienke.stein@wetsus.nl (N.E. Stein), bert.hamelers@wur.nl (H.V.M. Hamelers), cees.buisman@wetsus.nl (C.N.J. Buisman).

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In this paper we will show that control of anode overpotential is a prerequisite for obtaining the desired stable current under non-toxic conditions for the online sensor.

Electrochemical organisms are able to grow when supplied with micro and macro-nutrients and a suitable energy source. Other environmental factors like pH, temperature and salinity should also be in a favorable range to allow growth. Keeping these factors constant and optimal will prevent these factors from influencing the current and thus prevent false positive results. Most growth factors can be made constant by optimizing the anolyte by addition of nutrients, substrate and buffer and by keeping the temperature constant. The situation is more complicated for the energy supply of the micro-organisms.

Microorganisms gain energy from the potential difference between electron acceptor and electron donor [1,8]. In a MFC-based biosensor the anode is the electron acceptor while the substrate, in this case acetate, is the electron donor. The reaction taking place at the anode is: $C_2H_3O_2^- + 4\ H_2O \rightarrow 2\ HCO_3^- + 9\ H^+ + 8\ e^-$.

The theoretical value of the electron donor potential can be determined by the Nernst equation.

$$E_{\text{electron\_donor}} = E_0 + \frac{RT}{nF}\ln\frac{[HCO_3^-]^2[H^+]^9}{[\text{Acetate}]} \quad (1)$$

in which

$E_{\text{electron\_donor}}$ = Electron donor potential under the present conditions (V)
E_0 = Standard reduction potential of half reaction (V)
R = Gas constant (8.314 J mol^{-1} K^{-1})
T = Temperature (K)
n = Number of electron involved in the half reaction (–)
F = Faradays constant (96485.3 C mol^{-1})
E_0 = 0.187 V vs NHE for acetate.

The anodic overpotential is defined as the difference between anode potential and electron donor potential and equals:

$$\eta = E_{an} - E_{electron\_donor} \quad (2)$$

η = anodic overpotential (V)
E_{an} = anode potential (V).

The energy released per electron during the anodic reaction directly depends on the overpotential. The overpotential drives electrochemical reactions and within a certain range one expects that with increasing overpotential the reaction rate will increase [9]. This relationship between overpotential and current produced by the microorganisms has also been observed for bioanodic reactions [10–12].

To keep the baseline current constant it is thus important to keep the anodic overpotential constant to assure a constant energy supply. As Eq. (1) shows, overpotential control implies control of anode potential, the concentration and type of substrate, bicarbonate concentration, pH and temperature.

At low anode potential microorganisms gain little energy and will produce a low current. When the anode potential is too low, below $E_{\text{electron\_donor}}$, microorganisms will gain more energy from anaerobic fermentation than from electron donation to the anode and they will switch to this different metabolism if the substrate allows fermentation [1]. No current can then be observed and presence of toxic compounds cannot be detected. The anode potential thus needs to be controlled at a potential higher than $E_{\text{electron\_donor}}$.

In this paper we investigate the influence of anode potential, substrate concentration and pH on the overpotential and the electrical current in a MFC-based biosensor. The influence of these factors is compared to the change in current due to a toxic event. Although temperature will also affect the current, it can be controlled easily and therefore influence of temperature is not investigated. In the experiments described in this paper, a potentiostat was used to control the anode potential. This is novel compared to recent research on MF-based biosensors in which an external resistor was used to measure the cell voltage and calculate the current from that [5,13–19].

2. Materials and methods

2.1. MFC construction

A fuel cell was designed with two flat graphite electrodes. The anode and cathode chamber were separated by a proton exchange membrane (Fumasep FKS, Fumatech Gmbh, Germany). A reference electrode (Ag/AgCl, (+0.2 V vs. NHE, ProSense, The Netherlands) was present in the anode chamber with the tip at 4.85 mm distance from the anode. The anode and cathode chambers had a volume of 33.0 ml each and an electrode surface area of $2.20 \cdot 10^{-3}$ m^2. The anode potential was controlled by a potentiostat (Bank Elektronic Gmbh, Germany and Ivium Technologies, The Netherlands). A recirculation vessel was attached and the pH was measured in here to avoid interference of the pH measurement with the electrode potential control and measurements.

2.2. Microorganisms and medium

The anode chamber of the fuel cell was continuously fed with medium at a rate of 0.7 ml/min. The medium consisted of deionized water containing 0.74 g/l KCl, 0.58 g/l NaCl, 1.36 g/l KH_2PO_4, 1.74 g/l K_2HPO_4, 0.28 g/l NH_4Cl, 0.01 g/l $MgSO_4 \cdot 7H_2O$, 0.1 g/l$CaCl_2 \cdot 2H_2O$ and 1 ml/l of trace element mixture [20]. The medium was kept anaerobic by continuously flushing with nitrogen gas. Acetate was used as substrate for the microorganisms in known influent concentrations between 20 mM and 0.5 mM. The anode chamber was inoculated with 20 ml of anolyte taken from an active microbial fuel cell [21] and operated across a resistor of 1000 Ω without potential control until a stable system was established. The anode potential was then controlled by a potentiostat at the set potential. The cathode chamber was recycled with a 1 liter solution of 30 mM $K_3Fe(CN)_6$ and 30 mM $K_4Fe(CN)_6$ and 10 mM phosphate buffer (pH7) or was recycled with 10 mM phosphate buffer and replaced regularly with a fresh solution to ensure enough electron acceptor. The redox potential of the catholyte was measured continuously.

2.3. Experimental conditions

The experiments were temperature controlled at 303 ± 1 K. Acetate concentrations of the influent and effluent were measured by an ion chromatograph (Metrohm 761 Compact IC) equipped with a conductivity detector and an ion exclusion column (Metrosep Organic Acids 6.1005.200).

The influence of copper was measured by controlling the anode potential for at least 1 h at the set anode potential, then adding 10 ml medium containing 0.28 g/l $CuCl_2$ and adding another 10 ml clean medium immediately afterwards.

To investigate the influence of anode potential on current, microorganisms were cultivated in a MFC with a set anode potential of −0.4 V vs Ag/AgCl for several weeks. Then a polarization curve was made in triplicate. The anode potential was decreased from −0.4 V to −0.45 V, increased to −0.15 V and decreased to −0.45 V with steps of 0.025 V. Each potential was kept constant for 1800 sec. Average current of the last 900 sec was taken for the calculations as it takes a few minutes for the current to stabilize after anode potential is changed. All reported potentials are relative to Ag/AgCl (+0.2 V vs. NHE).

pH was controlled and logged continuously with a pH-controller (Endress and Hauser) and 100 mM NaOH was dosed to keep the pH at the set value automatically. The pH was controlled at pH 6.97 during

the experiments testing the influence of anode potential and substrate concentration.

The influent acetate concentration was kept constant for at least 48 h and the current was stable for at least 5 h (>1 HRT) before changing the acetate concentration at the same anode potential. To investigate the influence of bicarbonate, 0.84 g/l of $NaHCO_3$ was added for these experiments.

3. Results and discussion

Experiments were carried out to investigate the effect of a dose of copper on the current density. Furthermore the effect of anode potential, pH, acetate and bicarbonate concentration on the current density was examined. These effects were compared with the effect of the presence of a toxic component to get an idea about how strict to control the environmental factors in order to be able to detect the presence of a toxic component.

3.1. Toxic event

A dose of copper was added to the anolyte to obtain an indication of the size of current drop that can be observed in the sensor. For three different anode potentials, copper was dosed into the anolyte of the sensor. Fig. 1 shows a typical response of the sensor. The sensor reacts directly on the presence of the copper and shows a large drop. The response of the sensor depends on the applied anode potential, at an anode potential of −0.4 V the current changed 69.8%, at −0.35 V it changed 44.4% and at −0.2 V the current changed 15.8%. The initial copper concentration was 85 mg/l at the moment the pulse was added. After dosing the copper concentration decreases quickly in the anode because of the mixing with non-toxic medium. This decreasing concentration is accompanied with an increasing current and when the copper is completely washed out of the sensor, the current recovers to nearly the initial baseline value under non-toxic conditions.

The recovery of the current indicates that, as expected, there is a dose–response relationship, meaning that at a lower copper concentrations a smaller current drop is to be expected. This means that selection of the size of the current drop that would lead to an alarm depends on the toxicant level to be determined. As an example, the maximum concentration of copper allowed in drinking water for human consumption is 2 mg/l in the Netherlands [22], this would mean a substantially smaller drop should be detected than measured here.

To control the anodic overpotential both the anode potential and the electron donor potential need to be controlled. The anode potential can be controlled with a potentiostat, while the electron donor potential is determined by the pH, bicarbonate concentration and the acetate concentration. From Eq. (1) it is to be expected that pH has a larger effect on the current than bicarbonate concentration and this has again a larger effect than the substrate concentration.

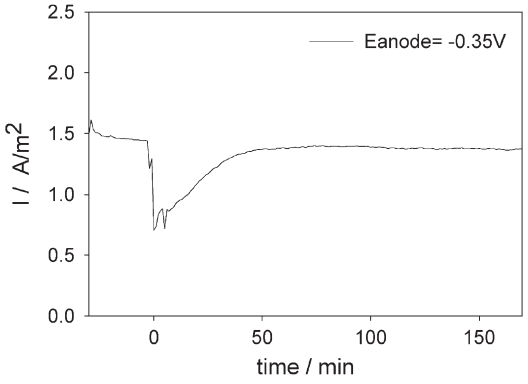

Fig. 1. Decrease in current density when copper was dosed. Current density for anodepotential = −0.35 V. Copper was dosed at t = 0 min. Current was measured every minute.

3.2. Anode potential

After inoculation and growth of microorganisms at a controlled anode potential of −0.4 V vs. Ag/AgCl until a stable current was achieved, the anode potential was varied stepwise. Fig. 2 shows the current density as function of the anode potential. From a zero current potential of −0.466 V, the current density increases linearly with potential with a slope of 10.18 $Am^{-2}V^{-1}$ until −0.323 V. From this potential on current density increased further but with a decreasing slope, until a maximum current density of 1.367 A/m^2 is reached at an anode potential of −0.273 V. Current density slowly decreased to 1.298 A/m^2 when the anode potential was increased to −0.150 V. A maximum current density was also observed by others in a similar system [10,23].

The current of the MFC-based biosensor can be expected to be more stable at anode potentials in the flattened area of the graph, so at anode potentials higher than −0.3 V. In this range a change in the anode potential leads to a negligible change in current. To detect a toxic event an appropriate noise level should be kept. When a change in current of e.g. 10% or more should be detected, the anode potential should be controlled well enough to keep changes in current due to changes in anode potential lower than 10%. At an anode potential of −0.4 V this means that it should be controlled at −0.4 V ± 0.007 V to keep the current-change less than 10%. When the anode potential is controlled at −0.35 V, it should be controlled at −0.35 V ± 0.017 V. When the anode potential is controlled at −0.2 V the current changes less than 10% even if the anode potential would change 0.1 V.

The slope of the polarization curve at these high anode potentials is so small that a change in potential does not lead to a significant change in current density. Of course is the choice of the anode potential not only determined by its effect on the baseline current density but also by consideration of the sensitivity of the sensor, as the relative response was larger at lower anode potentials. These data show that in order to keep the baseline current density stable a good control of the anode potential is needed and that an appropriate level of the anode potential should be chosen to secure sensitivity.

3.3. pH

Fig. 3A shows the dependency of the current density on the pH of the anolyte. When pH was increased from 6.9 to 7.6, current density increased 20% from 0.20 A/m^2 to 0.24 A/m^2 while the anode potential was kept constant at −0.40 V. pH was only changed over a small range as larger pH changes can have a toxic influence on the metabolism of microorganisms [8]. It can be calculated from Eq. (1) that an increase of 1 pH unit gives an increase in overpotential of

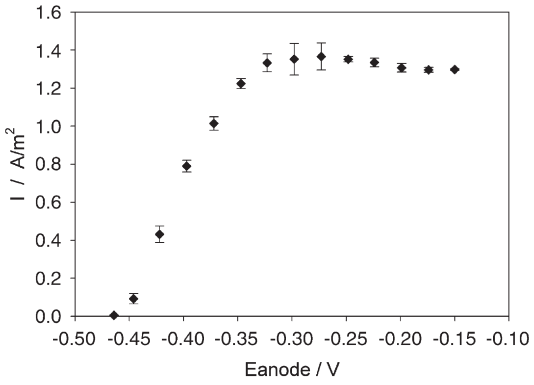

Fig. 2. Influence of anode potential on current density. Error bars are +/− standard deviations. K_3FeCN_6 was used as electron acceptor, V vs Ag/AgCl.

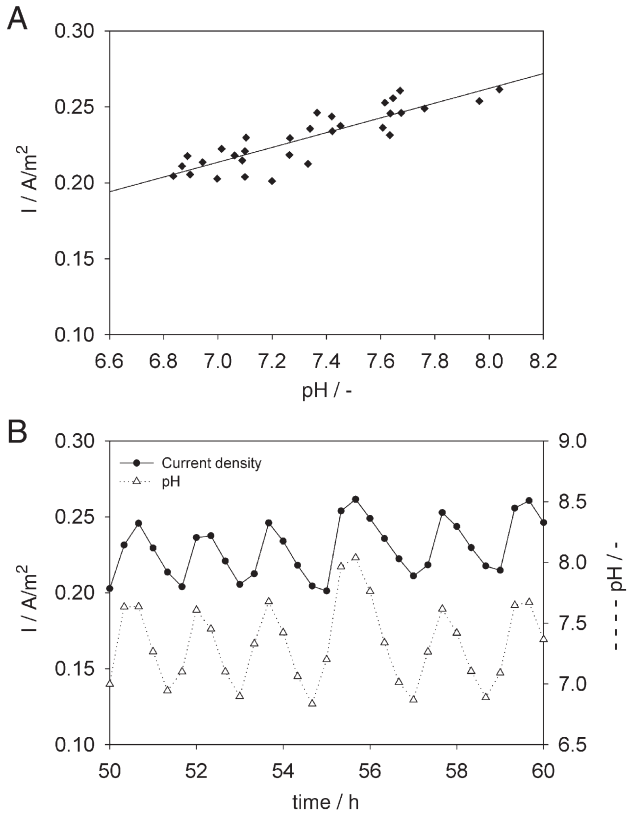

Fig. 3. A: Current density vs. pH B: Current density (●) and pH (Δ) in time. Anode potential controlled at −0.4 V vs Ag/AgCl.

67 mV. In the tested pH range, the current density therefore increased linearly with pH with a factor 0.73 $A m^{-2} V^{-1}$. In Fig. 3B the change in current density is shown to react immediately with change in pH. A sudden change in pH can thus easily give the same kind of quick current density drop as observed for copper dosing.

A current density drop of 10% can thus be brought about by a change in pH of only 0.35 pH units. It is thus important to keep pH stable within less than 0.1 pH unit to keep the baseline current stable.

3.4. Bicarbonate and substrate

According to Eq. (1) the overpotential increases 7 mV per 10 fold increase in acetate concentration. The square term in Eq. (1) indicates that the bicarbonate concentration plays a larger role than the substrate concentration. A 10 fold increase in bicarbonate leads to a 15 mV lower overpotential. The microorganisms consume acetate as substrate while bicarbonate is formed. Acetate and bicarbonate concentration are therefore closely linked to each other. The overall effect of acetate consumption is thus that overpotential decreases as a result of the lowered acetate concentration and the increased bicarbonate concentration. As a change in overpotential causes a change in current, changing concentrations would lead to a changing baseline current. Such a change in baseline current would then wrongly be described as a toxic event.

With a fixed influent concentration and a constant conversion, the concentration of acetate in the anolyte will be stable. Above a certain acetate concentration the growth of microorganisms will be substrate saturated, meaning that the only effect of a further increase of the substrate concentration will be on the overpotential. Above acetate concentrations of 1 mM only small changes in current were observed. When acetate concentration is increased from 2 mM to 6 mM the current density changes only 4 % at an anode potential of −0.4 V. Going from 2 to 6 mM acetate is equivalent to an increase in overpotential of 5 mV.

Fig. 4 shows the effect of an addition of bicarbonate on the polarization curve. Bicarbonate concentration was increased to10 mM, equivalent to a 3.3 fold increase. At anode potential = −0.4 V the decrease in current density is 0.02 A/m^2 or 6%. The overpotential decreases 8 mV due to the 3.3-fold increase in bicarbonate concentration.

To get a stable overpotential with respect to acetate and bicarbonate it is important to have stable conversion rate, which can be achieved once the conversion is substrate saturated. In this case a minimum concentration of 1 mM would be needed. Once a stable conversion rate has been achieved the bicarbonate concentration will be stable too. During the experiments an average bicarbonate concentration of 3 mM was formed.

It is further advised to use certain background concentration of bicarbonate in the anolyte. Relative changes as a result of changes in consumption rate are damped out this way. These outcomes confirm that the experiments testing influence of pH and anode potential were indeed performed under substrate saturation. Control of specific acetate and bicarbonate concentrations are not necessary as long as the bacteria are substrate saturated. A MFC-based biosensor should thus be operated under saturated substrate concentration to get a stable baseline current under non-toxic conditions.

3.5. Comparing the overpotential determining factors

At an anode potential of −0.4 V (vs. Ag/AgCl), a change of 50 mV anode potential led to a 58% change in current density. So change in anode potential leads to a change of 1.2%/mV overpotential. A change in pH of 0.7 changes overpotential 46.9 mV and this led to 20% decrease in current density. pH gives a current density decrease of 0.43%/mV. A 3.3 fold increase in bicarbonate concentration equals a decrease in overpotential of 8 mV and led to a decrease of 6% in current density. Bicarbonate gives a current density change of 0.75%/mV. Acetate increase from 2 to 6 mM gives an increase in overpotential of 5 mV. Current density increases 4% resulting in a change of 0.8%/mV. An overview of the effects is given in Table 1 and shows that in terms of overpotential all factors have similar effect.

From the experiment with copper it has become clear that even small changes in current density in the order of few percent might be the result of a toxic event. It has been shown that changes in environmental conditions can also give current density changes in the same order of magnitude. This shows the necessity of a good control of these variables.

The finding that all factors have a similar effect in terms of overpotential allows a calculation on the allowable range of the different factors. For instance to keep the overpotential change within 5 mV for each factor, the following ranges are acceptable; anode potential

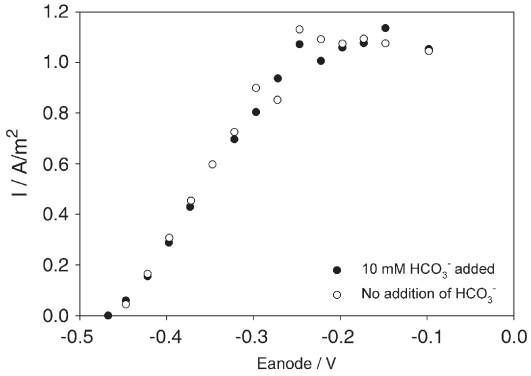

Fig. 4. Polarization curve of a MFC-based biosensor with (●) and without (o) added bicarbonate. The current density is not significantly influenced by the increased level of bicarbonate.

Table 1
Overview of investigated effects on current change.

	Anode potential	pH	Bicarbonate concentration	Acetate concentration
Induced change	50 mV	0.7 pH	4.3 fold increase	Increase from 2 to 6 mM
Effect on potential	50 mV	46.9 mV	8 mV	5 mV
Effect on current density	58%	20%	6%	4%
Change per mV	1.2%/mV	0.43%/mV	0.75%/mV	0.8%/mV

Standard conditions: Vanode = −0.4 V, pH = 7, acetate influent concentration 5 mM.

5 mV, pH 0.08, bicarbonate 2.1 fold increase, acetate 1.7 fold increase (at substrate saturation).

Working under substrate saturated conditions gives a stable acetate concentration and consequently bicarbonate concentration. Especially when a background bicarbonate level is used in the influent, the 5 mV condition is easily met for acetate and bicarbonate.

However without proper control of anode potential, a change in anode potential of 5 mV occurs frequently. For example for an MFC based sensor using a fixed resistor, the anode potential can change as a result of changing membrane properties. If in time the membrane resistance increases, a bigger potential will occur over the membrane and consequently the anode potential will be lowered. Very strict control of the anode potential like in this case with a potentiostat is therefore necessary. The same applies to the pH; this can change easily as result of change in chemical composition of the anolyte.

4. Conclusion

Overpotential control is important to obtain a stable baseline current. A stable baseline current is needed to prevent false positive alarms. It is therefore important to control the anodic potential in a suitable range, as the anodic potential strongly influences the overpotential and changes in current can be in the same order of magnitude as changes due to a toxic event. In the described experiments a potentiostat was used for this.

Strict control of pH is necessary as well. pH is the environmental parameter that has most influence on overpotential and therefore on measured current.

Bicarbonate and substrate have much smaller effect on the current when substrate is in excess and the microorganisms aren't metabolically limited. Control of these factors can be less strict than control of anode potential and pH.

The extent of control of anode potential and pH depends on the application of the sensor and the noise-level that is acceptable for the application. From the data presented in the paper some idea about the needed strictness of control can be formed.

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References

[1] B.E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstaete, K. Rabaey, Microbial fuel cells: methodology and technology, Environ. Sci. Technol. 40 (2006) 5181–5192.
[2] J. Hasan, D. Goldbloom-Helzner, A. Ichida, T. Rouse, M. Gibson, Technologies and Techniques for Early Warning Systems to Monitor and Evaluate Drinking Water Quality: A State-of-the-Art Review USA Environmental protection agency, 2005, p. 236.
[3] A. Willemsen, M.A. Vaal, D.d. Zwart, Microbiotest as tools for environmental monitoring, RIVM, 1995.
[4] M.W. Toussaint, A comparison of standard acute toxicity tests with rapid-screening toxicity tests, Environ. Toxicol. Chem. 14 (1995) 907–915.
[5] M. Kim, M.S. Hyun, G.M. Gadd, H.J. Kim, A novel biomonitoring system using microbial fuel cells, J. Environ. Monit. 9 (2007) 1323–1328.
[6] R.F. Taylor, J.S. Schultz, Handbook of chemical and biological sensors, Institute of physics publishing, Bristol, UK, 1996.
[7] H.T. Tien, A. Ottova-Leitmannova (Eds.), Planar lipid bilayers (BLMs) and their applications Door, Elsevier, 2003.
[8] M.T. Madigan, J.M. Martinko, J. Parker, Brock biology of microorganisms, Prentice-Hall, Inc, 2000.
[9] A.J. Bard, L.R. Faulkner, electrochemical methods, John Wiley and Sons, 2001.
[10] A.t. Heijne, H.V.M. Hamelers, M. Saakes, C.J.N. Buisman, Performance of non-porous graphite and titanium based anodes in microbial fuel cells, Electrochim. Acta 53 (2008) 5697–5703.
[11] J.R. Kim, S. Cheng, S.E. Oh, B.E. Logan, Power generation using different cation, anion, ultrafiltration membranes in microbial fuel cells, Environ. Sci. Technol. 41 (2007) 1004–1009.
[12] C.I. Torres, A.K. Marcus, B.E. Rittmann, Proton transport inside the biofilm limits electrical current generation by anode-respiring bacteria, Biotechnol. Bioeng. 100 (2008) 872–881.
[13] I.S. Chang, J.K. Jang, G.C. Gil, M. Kim, H.J. Kim, B.W. Cho, B.H. Kim, Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor, Biosens. Bioelectron. 19 (2004) 607–613.
[14] I.S. Chang, H. Moon, J.K. Jang, B.H. Kim, Improvement of a microbial fuel cell performance as a BOD sensor using respiratory inhibitors, Biosens. Bioelectron. 20 (2005) 1856–1859.
[15] G.-C. Gil, I.-S. Chang, B.H. Kim, M. Kim, J.-K. Jang, H.S. Park, H.J. Kim, Operational parameters affecting the performance of a mediator-less microbial fuel cell, Biosens. Bioelectron. 18 (2003) 327.
[16] K.H. Kang, J.K. Jang, T.H. Pham, H. Moon, I.S. Chang, B.H. Kim, A microbial fuel cell with improved cathode reaction as a low biological oxygen demand sensor, Biotechnol. Lett. 25 (2003) 1357–1361.
[17] B.H. Kim, I.S. Chang, G.C. Gil, H.S. Park, H.J. Kim, Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell, Biotechnol. Lett. 25 (2003) 541–545.
[18] A. Kumlanghan, L. Liu, P. Thavarungkul, P. Kanatharana, B. Mattiasson, Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter, Biosens. Bioelectron. 22 (2007) 2939–2944.
[19] H. Moon, I.S. Chang, K.H. Kang, J.K. Jang, B.H. Kim, Improving the dynamic response of a mediator-less microbial fuel cell as a biochemical oxygen demand (BOD) sensor, Biotechnol. Lett. 26 (2004) 1717–1721.
[20] A.J.B. Zehnder, B.A. Huser, T.D. Brock, K. Wuhrmann, Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium, Arch. Microbiol. 124 (1980) 1–11.
[21] A.t. Heijne, B. Hamelers, V.d. Wilde, R.A. Rozendal, C.J.N. Buisman, A bipolar membrane combined with ferric iron reduction as an efficient cathode system in microbial fuel cells, Environ. Sci. Technol. 40 (2006) 5200.
[22] R.O.e.M. Volkshuisvesting, in: Nederland (Ed.), Waterleidingbesluit, 1961, 10-07-2007.
[23] K.Y. Cheng, G. Ho, R. Cord-Ruwisch, Affinity of microbial fuel cell biofilm for the anodic potential, Environ. Sci. Technol. 42 (2008) 3828–3834.