A microbial fuel cell-based biosensor for the detection of toxic components in water

Nienke Elisabeth Stein

Promotion committee

Thesis supervisors:

Prof dr. Ir. G van Straten

Systems and Control Group

Wageningen University

Prof dr. ir. C.J.N. Buisman

Subdepartment of Environmental Technolgy

Wageningen University

Thesis co-supervisors

Dr ir. K.J. Keesman

Systems and Control Group

Wageningen University

Dr. ir. H.V.M. Hamelers

Subdepartment of Environmental Technolgy

Wageningen University

Other members

Prof. dr. M.A. Cohen Stuart, Wageningen University

Prof. Dr. F Barriere, Univesite de Rennes

Prof. dr. ir. M.H.G Verhaegen, Delft University of Technology

Dr. ir. P. van der Maas, WLN, Glimmen

A microbial fuel cell-based biosensor for the detection of toxic components in water

Nienke Stein

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Chapter 1

1. General introduction to the thesis

1.1 Water quality

Every person needs safe drinking water to survive. To guard the safety and quality of drinking water, governments have established regulations about the allowed concentrations of chemical and biological components in drinking water and in surface water. The US Environmental Protection Agency (EPA) found that up to 25% of rivers used for drinking water supply and up to 18% of lakes, ponds and reservoirs used for drinking water supply were contaminated to such extend that it was not suitable for use as drinking water source. The main causes of impairment included pathogens, mercury, other metals and polychlorinated biphenyls (PCBs) [1]. In Europe the 'Water Framework Directive' [2] sets the standards for the allowed concentrations of chemical and biological components. Water companies have to meet these standards before delivering water to their clients. Water quality may change due to, amongst others, leakage within the distribution system, contamination of the used source or through terrorist attacks. Monitoring water quality in the system is thus needed. The U.S. Environmental Protection Agency has even started a water security program to identify possible threats regarding drinking water quality and to protect the US water and wastewater systems [3].

To monitor the presence of chemical components in water, many tests are performed regularly. Commonly, samples are taken from drinking water sources and in the distribution network that are analyzed for the presence of chemical and biological components. The analysis of these samples is done off-line and for chemicals advanced equipment that can measure very low concentrations is used. These measurements, however, can take several hours to days, are offline, discrete and very specific. Only specific components that are looked for will be measured. Unknown chemical components such as degradation products or reaction products, when several components are present, cannot be detected easily [4-6]. To overcome some of these disadvantages online measurements of chemical toxic components are performed as well. Biosensors can be used for online measurements.

1.2 Generic biological toxicity sensor

Biosensors are sensors that use a biological sensing element such as DNA [7, 8] enzymes or living organisms [6, 9, 10] combined with a physical transducer, which converts a biological signal into a measurable signal such as light intensity or an electrical signal. Biosensors using whole living organisms can react to unknown chemical components and to cumulative effects when several components are present together. The ability to give a fast response to bio-available species makes them suitable to apply in early warning systems that provide early detection and awareness of toxicity. Measures in the water intake can be taken before the chemical species are identified. Water companies like to use several types of sensors to broaden the range of components that can be measured. A perfect biosensor should meet the following requirements: It has to be selective if one component has to be monitored or detect a wide range of components if it is used in an early warning system. It has to be stable, has a short response time, be reproducible and accurate in detection. It should need no external substrate, be low in costs and maintenance, simple in operation, and use little energy. Furthermore, it needs to have a long lifespan, be able to monitor continuously, and the detection should be independent of the evolution or growth of the organisms and false positive/ false negative signals should be limited [11, 12]. Currently there is no single biosensor that meets all these requirements but many biosensors using whole organisms are available and still suitable for the use of water quality monitoring. Examples of currently employed biosensors for the detection of toxic components are shown in Table 1.1 and include the deployment of microorganisms, invertebrates and vertebrates. Biosensors using enzymes as biological sensor elements are also widely available [21, 22] but are mostly specific. The biosensors exhibit several different types of biological signals, e.g. change of movement pattern or change in light intensity. Biosensors that use an electrochemical signal such as current or potential are called electrochemical biosensors. Electrochemical biosensors are widely used for the detection of specific components but also for the detection of multiple species [10, 13, 14]. They can be used in turbid media and their sensitivity is usually good [15].

Recently, a new biosensor has been proposed for the detection of toxic components or for BOD measurements in water and is based on the microbial fuel cell (MFC) [16-20]. The

MFC-based biosensor is an electrochemical biosensor. In an MFC-based biosensor, bacteria growing on the anode catalyze the oxidation of organic material present in the inflow and electrons are produced at the anode. When toxic components affect the bacteria this can be detected from the electrochemical signal. An MFC meets many requirements of the perfect sensor: It can be used as an early warning signal because it is sensitive to many components. It can be low in cost, simple in operation and can monitor continuously. Bacteria grow on the anode and the signal produced is an electrical signal. This means that no transducer is needed to translate the signal to a readable signal. It is a one-step process from the sensing element to the measurable signal. Many other biosensors need a transducer and one of the advantages of an MFC is that a transducer is not needed. Another advantage is the use of naturally available microorganisms. No genetically modified bacteria are needed as is the case in some other types of biosensors [6, 9].

Table 1.1: Examples of biosensors. Part A: examples of commercially available biosensors using whole organisms for toxicity detection, part B: electrochemical biosensors

Name [reference]	*Organism*	*Signal*	*Type of toxicity*	**Online/ offline**
A: **commercially available biosensors**				
Microtox [4, 6]	*Photobacterium phosphoreum* (bacteria)	Luminescence	Inorganics, organics, no metal	Offline/ semi-online
Vibrio harveyi [6]	*Vibrio Harveyi* (bacteria)	Luminescence	Organic, compounds, no metals, no less inert organics such as anilins	Offline
Selenastrum microplate [6]	*Selenastrum capricomutum* (algae)	Cell count	Metals, herbicides	Offline
Rotoxkit F [6]	*Brachionus*	Mortality	Specific toxicant	Offline

	plicatilis (rotifers)		mainly drugs and pesticides.	
Daphnia toximeter [23]	Daphnia magna (water flea)	Speed, swimming height, size, number of daphnids speed class index, height class index, width class index, distance, speed variation	pesticides, neurotoxins, respiratory toxins, insecticides	Online
Fish toximeter [23, 24]	Fish: tiger barb, or other (local) fish depending on water temperature at site	Speed, swimming height, size, number of fish, speed class index, height class index, width class index, distance, speed variation	pesticides, neurotoxins, respiratory toxins	Online
Musselmonitor [24-26]	mussel	Open/ closing of shell	Heavy metals and organochlorides.	Online

B **electrochemical biosensors**

[27, 28]	Organophosphate hydrolase (enzyme)	Potential	Organophosphate compounds	Offline
Mitoscan [29]	submitochondrial particles	NADH reduction activity	general cellular toxicants	Offline
[14, 30-32]	*Pseudomonas putida* JS444 (bacterium)	Current	Synthetic organophosphorous components, phenol	Offline
[33]	*Pseudomonas aeruginosa*	Potential	Cephalosporin antibiotics	Offline

[13]	(bacterium) Rhodococcus sp. (bacterium)	Potential	Chlorinated and brominated hydrocarbons	Offline

1.3 Microbial fuel cell

Already in the beginning of the twentieth century people became aware that bacteria can donate electrons to a solid surface [34-37] and are thus electrochemically active. However, it took until the end of the century before the potential of this was realized. When research on electrochemically active bacteria really took off in 2003 it focused on electricity production by bacteria in a microbial fuel cell (MFC). In an MFC bacteria catalyze the degradation of organic component. Under anaerobic conditions they donate the electrons to a solid electrode. The emphasis of MFC- research was and still is on improving the electricity production and minimizing the energetic losses in the cell [38-40]. When research continued it was realized that more could be done with the microbial fuel cell. For example, it can also be used as an electrolysis cell. By adding a little extra energy to the electrical energy produced by the bacteria, hydrogen can be produced at the cathode [41, 42]. More applications of using the MFC were also started. Not only the degradation of organic components was important but also the use of microorganisms as catalyst for bioelectrochemical production of chemicals such as methane [43], hydrogenperoxide [44] and ethanol [45]. Other processes such as biological denitrification [46] or ammonium recovery from urine [47] at the cathode were investigated and even the combination of microbial fuel cells with plants to produce electrical energy from light is investigated [48].

Another possible application is the use of the microbial fuel cell as a sensor, both as a sensor for biological oxygen demand (BOD) [16, 18, 49, 50] or as toxicity sensor [17, 20, 51, 52]. When the organic component concentration is constant, bacteria will produce a constant electrical current. However, when their metabolism is affected by the presence of toxic components, the substrate consumption rate will decrease and with that the

electrical current. In this way, at constant substrate concentration, the microbial fuel cell can act as a sensor for toxic components in water.

1.4 MFC as toxicity sensor

When a microbial fuel cell is used as a sensor for toxicity it needs to meet the requirements set by the European Water Framework Directives or other applicable regulations. This means that the detection limit of the sensor has to be lower than the maximum concentration of toxic components that is allowed by the Directives. To be a reliable sensor it has to be both sensitive to the component to be detected and robust against other disturbances in the system and it should be reproducible.

A toxicity sensor using pure cultures may be reliable and reproducible. Pure cultures, however, will be specific. Alternatively, also mixed cultures can be used that are sensitive to a broad range of components. In this way the sensor can be used as a generic sensor which is a prerequisite for the use of an early warning system.

So far the focus of MFC research has mainly been on energy, chemicals production and on nutrient recovery. Hence, many question regarding the MFC as toxicity sensor are still left. More specific research is necessary regarding design, control, and operation and the effect of these issues on sensitivity, robustness and reproducibility of the sensor. Research on microbial fuel cell-based biosensors describes mostly the use of batch tests [18, 20, 51]. However, online detection may be quicker and have more applications. The possibilities to use the sensor online have to be investigated. The research described in this thesis focuses on the use of an online microbial fuel cell based biosensor for the detection of toxic components in water and addresses the issues mentioned.

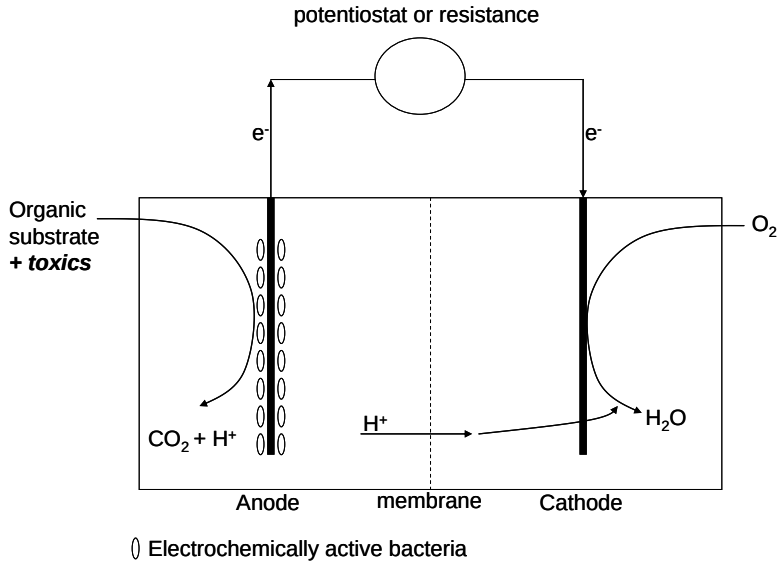

Figure 1.1: schematic set-up of a microbial fuel cell-based biosensor

1.5 MFC Theory

A microbial fuel cell (MFC) is a device in which bacteria convert chemical energy into electrical energy. Figure 1.1 gives a schematic set-up of an MFC. The MFC contains two compartments separated by an ion exchange membrane. The first compartment contains an electrode on which bacteria grow. The bacteria oxidize organic matter anaerobically and donate the electrons to the electrode that acts as an anode. The anode is connected to another electrode in the second compartment where an electron acceptor such as oxygen is present. This electron acceptor is reduced at the electrode which thus acts as a cathode. In an MFC-based biosensor the anode is the electron acceptor while the substrate, e.g. acetate, is the electron donor. The oxidation of acetate taking place at the anode is:

$C_2H_3O_2^- + 4\ H_2O \rightarrow 2\ HCO_3^- + 9\ H^+ + 8\ e^-$

Protons are released during the oxidation reaction and to balance the charge, cations move towards the cathodic side of the MFC through the ion exchange membrane or anions can move towards the anodic side.

A possible reduction reaction at the cathode is the reduction of oxygen following

$O_2 + 4\ H^+ + 4e^- \rightarrow 2\ H_2O$

Microorganisms gain energy from the potential difference between electron acceptor and electron donor [39, 53].

The theoretical value of the electron donor potential at zero-current can be determined by the Nernst equation.

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

In which

$E_{electron\_donor}$ = 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})

For acetate E_0 = 0.187 V vs. normal hydrogen electrode (NHE).

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)

Anode potential can be measured or controlled using a potentiostat while electron donor potential depends on the type and concentration of substrate. Energy released during the anodic reaction directly depends on the overpotential following U=η*Q with U=energy (J), η=overpotential (V), Q=charge (C). Overpotential drives electrochemical reactions and

within a certain range one expects that with increasing overpotential the reaction rate will increase [54]. This relationship between overpotential and current produced by microorganisms in an MFC has been observed for bioanodic reactions [55-57].

The electrical signal produced by the bacteria, measured as current or anode potential, depends on several environmental factors that bacteria are exposed to such as pH, substrate availability and toxin concentration [58, 59]. Therefore the signal produced by bacteria can be used as a measure for water quality. A change in water quality can be measured as a change in electrical current or potential. By passing the water to be tested through the anodic compartment it is brought in contact with the bacteria. In this way a continuous online, on-site monitor can be developed. pH can easily be measured and changes in current or potential caused by changing pH can be correlated to and corrected for this changing pH. Substrate concentration can be measured or predicted. When changes in current or potential are not caused by changes in pH or substrate, a changing signal can be attributed to the presence of toxic components affecting the bacteria.

1.5.1 Measurement methods

Several electrochemical detection modes are used in electrochemical biosensors. The mostly used detection modes are amperometry, potentiometry, voltammetry or electrochemical impedance spectroscopy [10, 15, 60]. The used methods and the settings used in the methods affect several parameters in a sensing system such as reaction rates and energy level of the organisms. It is not clear yet what the best method is to use in an MFC-based biosensor to reach a stable system that is both sensitive and robust. The setting that is most optimal for these factors also has to be investigated.

1.6 Thesis scope and outline

The aim of this thesis is to investigate the application of the MFC-based biosensor for toxicity detection and to present methods to obtain a sensitive and robust sensor for the online detection of chemical toxicity.

This thesis concerns the following research questions:

- What conditions are needed to be able to measure changes in water conditions?

- What is the best measurement method and setting to obtain a sensitive sensor?
- How is the relation between overpotential and electrical current in an MFC described?
- Is it theoretically possible to differentiate between different types of kinetic inhibitions using the MFC-based biosensor?
- Using amperometric measurements, at which potential should the working electrode be set to obtain a sensor that is both sensitive for toxic components and robust regarding recovery and non-toxic changes in the water?
- Is it possible to use the microbial fuel cell as an online sensor for toxicity detection?
- Can the theoretical model describing current in the sensor be fit to experimental data and is it possible to differentiate between different kinetic inhibitions?
- Which parameters have an effect on the sensitivity and how should they be set to increase sensitivity?

To answer these questions the thesis comprises results of experimental data combined with kinetic/ electrochemical modeling and provides a protocol to monitor toxicity online using an MFC-based biosensor.

In **chapter 2** the proof of principle is demonstrated and the conditions are investigated that are needed to get a stable background signal. This is needed to be able to measure changes in water quality.

In **chapter 3** three different methods of control are investigated for electrochemical signal detection. The anode potential is an important parameter for the sensitivity. The anode potential can be controlled potentiostatically, it can be influenced by setting the current or the external resistance in such a way that it determines the anode potential.

A polarization curve shows the relation between overpotential and electrical current and is a way to characterize the MFC. The **Intermezzo** describes a model for polarization curves for microbial fuel cells. The Butler-Volmer-Monod (BVM) model is based on enzyme kinetics combined with electrochemical kinetics under steady state conditions.

In **chapter 4** the Butler-Volmer-Monod-model is extended to describe toxicity and relate the type of kinetic inhibition to the parameters in the system.

The sensor will be applied as an online sensor. It is therefore important to detect changes in time. **Chapter 5** describes the parameters that can be estimated online and changes of these can be an indication of the presence of toxic components. A theoretical framework describes the estimation of the parameter values of the models. It also gives a protocol for operating the sensor.

Chapter 6 describes experimental data of polarization curves. These data are fit to the extended BVM-model to distinguish what type of kinetic inhibition the toxic component causes.

Toxic components entering the sensor can diffuse and migrate into different directions in the sensor. These fluxes are affected by cell voltage and current density, especially for charged components. **Chapter 7** investigates the influence of membrane type, current density, and overpotential on the sensitivity of the sensor.

Chapter 8 puts the achievements of the research in the light of the requirements of a sensor in an early warning system and discusses the points of improvement that are needed to apply the sensor. It also discusses the possible applications of the sensor and the use of the described methods for other types of online sensors.

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Chapter 2

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

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

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 term 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.

Keywords: Biosensor, MFC, overpotential control

2.1 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. 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.

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

In which

$E_{electron\_donor}$ = 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 equation 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 $E_{electron\_donor}$, 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 $E_{electron\_donor}$.

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].

2.2 Materials and methods

2.2.1 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, (+0.2V 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*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.

2.2.2 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 $MgSO_4*7H_2O$, 0.1g/l$CaCl_2*2H_2O$ 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 Ohm 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.

2.2.3 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 hour at the set anode potential, then adding 10 ml medium containing 0.28g/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 -0.45V, increased to -0.15V and decreased to -0.45 V with steps of 0.025V. 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 (+0.2V 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 hours and the current was stable for at least 5 hours (>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.

2.3 Results and discussion

Experiments were carried out to investigate the effect of a dose of nickel 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.

2.3.1 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. Figure 2.1 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 -0.4V the current changed 69.8%, at -0.35V it changed 44.4% and at -0.2V the current changed 15.8%. The initial copper concentration was 85mg/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.

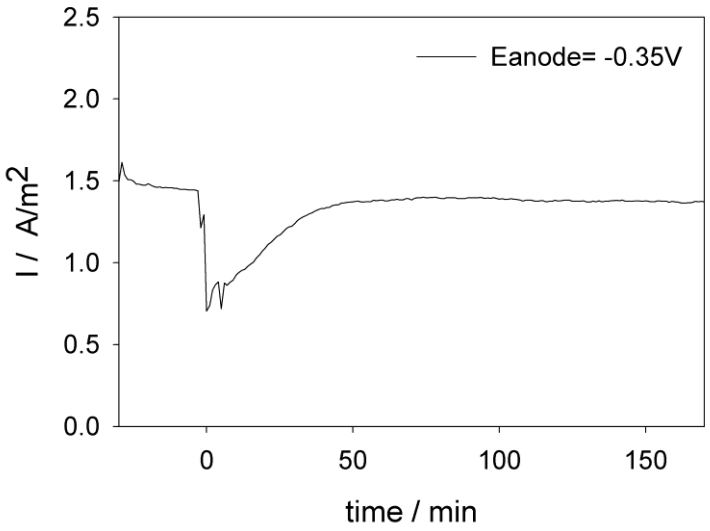

Figure 2.1: Decrease in current density when copper was dosed. Current density for anode potential= -0.35V. Copper was dosed at t=0 min. Current was measured every minute.

The recovery of the current indicates that, as expected, there is a dose-response relationship, meaning that at a lower copper concentration 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 equation 1 it is to be expected that that pH has a larger effect on the current than bicarbonate concentration and this has again a larger effect than the substrate concentration.

2.3.2 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. Figure 2.2 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 $A*m^{-2}*V^{-1}$ until -0.323V. 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 -0.3V. 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 -0.4V this means that it should be controlled at -0.4V +/- 0.007V to keep the current-change less than 10%. When the anode potential is controlled at -0.35V,

it should be controlled at -0.35V +/- 0.017V. When the anode potential is controlled at -0.2V the current changes less than 10% even if the anode potential would change 0.1V.
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.

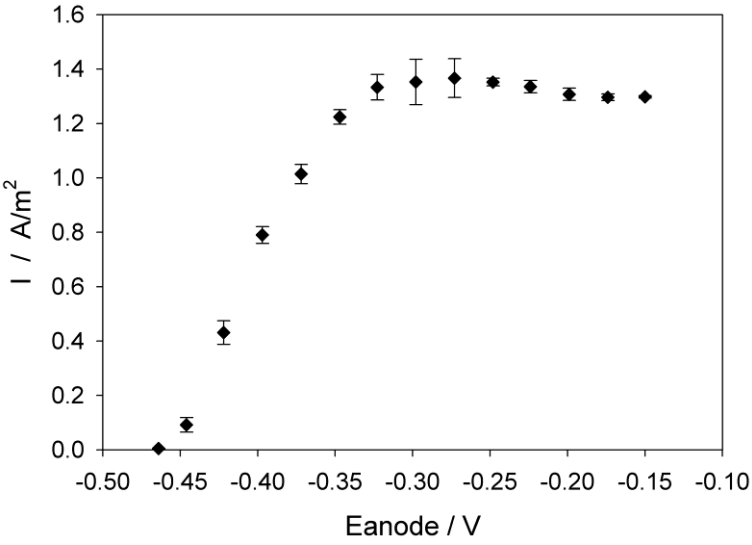

Figure 2.2: Influence of anode potential on current density. Error bars are +/- standard deviations. K3FeCN6 was used as electron acceptor, V vs. Ag/AgCl

2.3.3 pH

Figure 2.3A 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 67 mV. In the tested pH range, the current density therefore

increased linearly with pH with a factor 0.049 $A*m^{-2}*V^{-1}$. In Figure 2.3B 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.

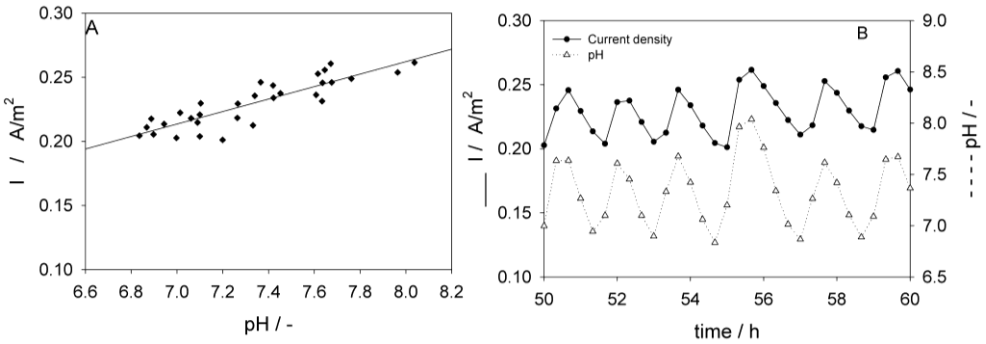

Figure 2.3: A: Current density vs. pH B: Current density (●) and pH (▲) in time. Anode potential controlled at -0.4 V vs. Ag/AgCl.

2.3.4 Bicarbonate and Substrate

According to equation 1 the overpotential increases 7 mV per 10 fold increase in acetate concentration. The square term in equation 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 -0.4V. Going from 2 to 6 mM acetate is equivalent to an increase in overpotential of 5 mV.

Figure 2.4 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 = -0.4V 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.

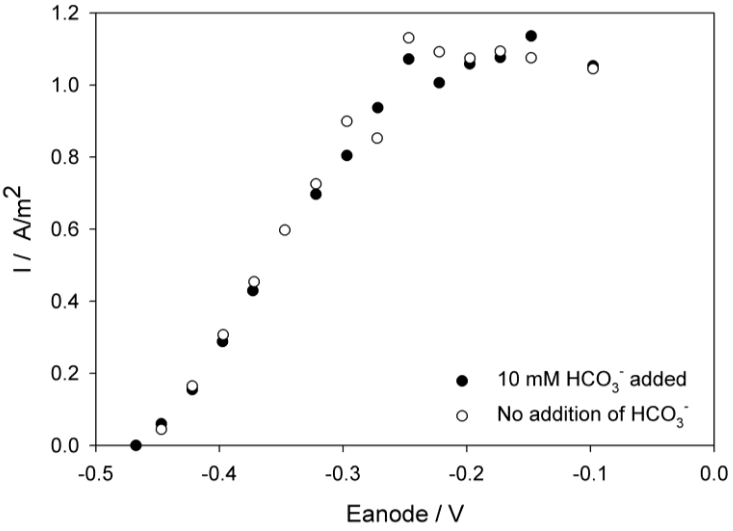

Figure 2.4: Polarization curve of a MFC-based biosensor with (●) and without (o) added bicarbonate. The current density is not significantly influenced by the increased level of bicarbonate

2.3.5 Comparing the overpotential determining factors

At an anode potential of -0.4V (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 8mV 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 5mV. Current density increases 4% resulting in a change of 0.8%/mV. An overview of the effects is given in Table 2.1 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 5 mV, pH 0.08, bicarbonate 2.1 fold increase, acetate 1.7 fold increase (at substrate saturation).

Table 2.1: Overview of investigated effects on current change. Standard conditions: Vanode=-0.4V, pH=7, acetate influent concentration 5 mM.

	Anode potential	*pH*	*Bicarbonate concentration*	*Acetate concentration*
Induced change	50 mV	0.7 pH	4.3 fold increase	Increase from 2-6 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

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.

2.4 Conclusions

Overpotential control is important to obtain a stable base line 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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Chapter 3

Sensitivity and recovery time of a microbial fuel cell based biosensor as influenced by the type of control

This chapter is submitted as:

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3. Sensitivity and recovery time of a microbial fuel cell based biosensor as influenced by the type of control

Abstract

For the monitoring of good drinking water quality biosensors can be used for the detection of toxicity. A microbial fuel cell (MFC) based biosensor can be used to detect toxicity. In the design external resistors are often used. There does not seem to be a rational basis for the choice of its value and it is even unknown whether this type of control gives the most sensitive sensor. The influence of the external resistor value on the sensitivity and recovery time of the MFC-based biosensor was investigated. A low resistor value resulted in a large signal change and thus a more sensitive sensor, while a high resistor value resulted in a lower recovery time. Furthermore, in stead of using an external resistor, a potentiostat was used to control anode potential or a galvanostat was used to control the electrical current. With both types of control it was possible to detect the presence of a toxic component. Anode potential control resulted in a good signal change, surprisingly also negative currents were observed. Using current control gave an anode potential decrease in the presence of a toxic component as opposed to control with an external resistor where anode potential increased. The recovery time of the sensor both under anode potential control and under current control was longer than when using an external resistor.

Keywords: biosensor; microbial fuel cell; anode potential; recovery time; sensitivity

3.1 Introduction

For good public health, high quality drinking water must be available to the population. To assure high quality drinking water standards, water companies and authorities monitor the quality frequently. To detect toxic events biosensors are the most used available tool. They can be used in-line and real-time and can detect toxicity generically. Currently used online biosensors have some disadvantages amongst others the need of nonlinear transducers to obtain information from the signal [1, 2]. The transducer translates the signal, e.g. light, into a readable signal for operators, usually an electrical signal. To overcome this need for a transducer electrochemical biosensors are available. A microbial fuel cell based biosensor for the detection of toxic components was proposed as an electrochemical biosensor [3, 4].

A microbial fuel cell (MFC) uses bacteria to produce electrical energy [5]. Bacteria oxidize a carbon source and donate their electrons to a first electrode, the anode. The electrons run through an external circuit and reduce an electron acceptor at the second electrode, the cathode. A chemical signal – the oxidation of a chemical substrate – is translated directly into an electrical signal by billions of bacteria. The electrical signal produced by the bacteria depends on several environmental factors that bacteria are exposed to such as pH, substrate availability and toxins concentration [6, 7]. Therefore the signal produced by bacteria can be used as a measure for water quality. A change in water quality can be measured as a change in electrical current or potential. By passing the water to be sampled through the anodic compartment toxins potentially present will affect the bacteria. In this way a continuous online, on site monitor can be developed.

Microorganisms gain energy from the potential difference between electron acceptor and electron donor [5, 8]. In an MFC-based biosensor the anode is the electron acceptor and substrate is the electron donor. This potential difference between substrate and anode is called anodic overpotential. When the anode potential and thus the overpotential are higher, energy released per electron is higher and this leads to a higher reaction rate. Release of electrons is measured as current. A higher reaction rate thus leads to a higher

electrical current. When a toxic component is present in the anodic compartment of the MFC-based biosensor, the bacteria are affected and the reaction rate will change; most likely decrease. This can be measured as a change in current or potential or both. To detect toxic components and to get useful information from changes in the signal, a stable background signal needs to be present. This can be achieved by choosing environmental conditions that lead to a stable signal under non-toxic condition, like controlling pH and working under substrate saturation [4].

When the MFC-based biosensor is operated at a different anode potential, bacteria experience a different energy level. This may influence the sensitivity for toxic components. The change in reaction rate may differ when the MFC-based biosensor is operated under different anode potentials or currents. It was not yet investigated what the settings of the electrodes needs to be to get the signal that is most sensitive to the presence of toxic components.

3.1.1 Control methods

Most MFC-based biosensors, both biological oxygen demand (BOD) and toxicity sensors use, an external resistor in the external electrical circuit [3, 9-15]. The effect of the value of the external resistance on the sensitivity of the sensor has not yet been investigated. There exists no rational basis for a choice of this value. Current is calculated by measuring cell voltage over the external resistor using Ohm's law ($I=V_{cell}/ R_{ext}$; I current (A); V_{cell} cell voltage (V); R_{ext} external resistance (Ohm)). Cell voltage depends amongst others on anode potential. The value of the external resistor influences the level of the anode potential. A lower external resistance gives a higher anode potential. A change in the measured cell voltage is linked to a change in the metabolic activity of microorganisms on the anode and interpreted as a toxic event. Changing water quality is measured as a changed cell voltage. Bacteria adjust the anode potential to such an extent that they need to produce less current.

There is no indication yet that a chosen external resistor value gives a good change in cell voltage when a toxic component passes. It is therefore important to investigate if the choice of resistor value has any influence on the change of cell voltage. A larger change with the same concentration of toxic component is preferable because this will lower the

detection limit of the sensor and this means a more sensitive sensor. The influence of different external resistor values on sensor sensitivity was therefore investigated.

Another important factor for every non-disposable sensor is the robustness of the sensor. The time it takes to recover from the presence of the toxic chemical and whether it recovers to the original value or a different steady state value are important factors to address. Especially in biosensors the disturbance of the biological material and even death may require re-growth of the cells and this takes time. The value of the external resistance may also influence the time the bacteria need to recover.

There are alternatives for using an external resistance to obtain an electrical signal and measure changes due to the presence of toxic components.
An alternative is using a potentiostat to control the anode potential. The potentiostat keeps the anode potential at a pre-specified set value using a reference electrode and current can be measured. Changes at the anode, where the bacteria grow, are measured as a change in current.
Another method is galvanostatic control. Controlling current means controlling the substrate consumption rate. A change in anode potential is measured under changing conditions. It is not yet investigated, however, if the level of potential control or current control has any influence on the signal change when a toxic component passes the sensor.
The bacteria produce an electrical signal that changes when they are affected by a toxic chemical. The cell is more sensitive if a higher change results from the same dose. Of all three methods of control it was investigated if the level of control has an influence on the sensitivity of the sensor and on the recovery time.
The mode of control of the system will influence the way the bacteria can recover and the time this recovery takes. Sensitivity and robustness of a biosensor are difficult to combine. Sensitive means that the cells are easily affected by low concentrations of toxic component. On the other hand, a robust system also demands quick and complete recovery of the cell. The three different control methods each will influence the metabolism of the bacteria and especially the setting of the level of control, will affect the energy level of bacteria and with that how easily bacteria recover from a toxic event.

Currently available electrochemical biosensors also use either current control (potentiometry) or control the working electrode potential (in case of the MFC-based biosensor is this the anode potential). The type of control depends on the type of component that should be detected and on whether reaction rate or concentration should be measured [16, 17].

This paper describes the investigation of three different methods of control of a microbial fuel cell-based biosensor: external resistor; anode potential control and galvanostatic control. It was investigated if the choice for the setting has any influence on the extent of signal change, the sensitivity and on the recovery of the system. The sensitivity and recovery of the MFC-based biosensor for the chemical component sodium dodecyl sulfate (SDS) is tested for the three control methods. SDS is a detergent that can dissolve hydrophobic molecules and has a negative charge attached to it. Therefore the cell membranes can be dissolved in the presence of SDS [18-20]. SDS is used here as a model component for toxic components that can be present in water.

3.2 Material and methods

3.2.1 MFC construction and inoculation

Microbial fuel cells were constructed as in Heijne et al. [21]. The cells were hydraulically and electrically operated individually. Microorganisms were grown in a single MFC with 20 ml of anolyte taken from an active microbial fuel cell [22] at a set anode potential of -0.4 V vs Ag/AgCl. The anode chamber of the fuel cell was continuously fed with medium at a rate of 0.7 ml/min. The medium was the same as used in Rozendal et al. [23] except a buffer concentration of 20 mM phosphate buffer was used. The medium was kept anaerobic by continuously flushing with nitrogen gas. The catholyte contained 30 mM Fe(III)[CN]6/ Fe(II)[CN]6 in a 10 mM phosphate buffer solution flushed with nitrogen gas and replaced regularly with a fresh solution to ensure a high concentration of electron acceptor. The redox potential of the catholyte was measured continuously. Temperature was controlled at 30^{o}C.

3.2.2 MFC operation

The three MFC-based biosensors were electrically controlled individually. One MFC-based biosensor was connected to an external resistor with a set value. Anode potential and cell potential were measured. The second cell was controlled in a three electrode mode by a potentiostat (Bank Electronik GMBH, Germany) and anode potential was controlled. The third cell was controlled by a potentiostat-galvanostat (Iviumstat, Netherlands) in galvanostatic mode. Anode potential and cell voltage were measured. Measurements of potentials and current were taken every minute.

3.2.3 Simulated toxic event

The controlled value (resistance, anode potential or current) was kept constant for at least 16 hours to reach steady state before dosage of sodium dodecyl sulfate (SDS) and several hours after the cell was recovered of dosage. SDS was dosed during two hours with a concentration of 50 mg/l within the medium. Current or anode potential or both changed upon dosage of 50 mg/l SDS and this change is used as a measure of sensitivity. Recovery time is the time it takes for the current to reach a (new) steady state value after it was disturbed due to the presence of a toxic component. Only several hours after recovery, the value at which the cell was controlled was changed to a new value.

3.2.4 Analysis

Acetate and SDS concentrations in the effluent were measured before, 4 times during dosage and after dosage of SDS. Acetate concentrations of the influent and effluent were measured by ion chromatography (Metrohm 761 Compact IC) equipped with a conductivity detector and an ion exclusion column (Metrosep Organic Acids 6.1005.200). SDS concentrations were determined using cuvette tests based on standard methods for anionic surfactants (Dr Lange test kit 332) [24].

When an external resistance is used, current was calculated using Ohm's law: $I=V_{cell}/ R_{ext.}$ Anode potential was measured using a reference electrode (Ag/AgCl).

3.3 Results and discussion

3.3.1 Effect of value of external resistance

When a toxic event is simulated, SDS is added to the sensor that operated in a steady state defined by a constant electrical signal. When an external resistance is used and a toxic component enters the sensor, anode potential changes. As a result cell voltage changes and current changes. Cell voltage decreases and current calculated with Ohms law also decreases. The addition of 50 mg/l SDS led to a decreased cell voltage and current while anode potential increased (Figure 3.1). When SDS washes out of the cell, a new steady state current and anode potential is realized. The presence of a toxic component in the sensor leads to a disturbance of the bacteria and they will adjust to a new situation. The bacterial metabolism is determined both by the anode potential and the current. By increasing the anode potential bacteria can increase the substrate consumption rate and the amount of energy that they gain during this conversion [5, 25]. In this way they can possibly protect themselves from the damage caused by the toxic component.

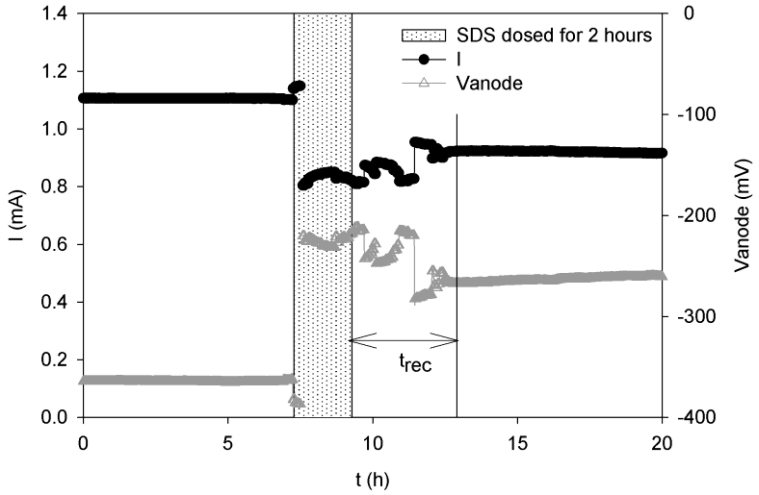

Figure 3.1: Response of an MFC based biosensor with an external resistor of 470 Ohm on addition of 50 mg/l SDS for two hours (grey area). Current (black dot) decreases while the anode potential (grey triangle) increases. Anode potential is measured using a reference electrode (Ag/AgCl). Recovery time (t_{rec}) is indicated with an arrow.

The effect of the value of the external resistor on the change of cell voltage and current was tested. Under nontoxic conditions a lower resistor leads to a higher current. Upon addition of SDS, current changes and as Figure 3.2 shows, when the external resistance was low, e.g. 100 Ohm, the change of current was larger than with a high resistance of 1000 Ohm. This shows that the choice of external resistor has an effect on the size of current change and is thus important in realizing a sensitive sensor.

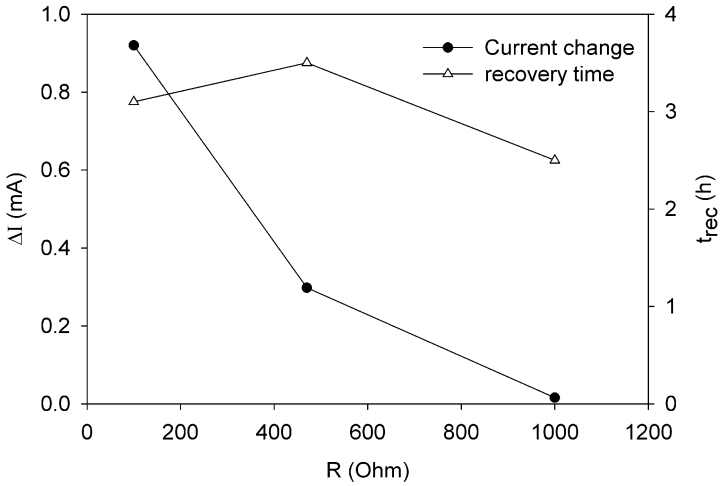

Figure 3.2: change in current (closed symbols) and recovery time (open symbols) as function of external resistance after dosage of 50 mg/l SDS

The recovery time of the current after a toxic event of 50 mg/l SDS was also investigated. Recovery time is the time it takes for the current to reach a (new) steady state value after it was disturbed due to the presence of a toxic component. Although a high change in signal is preferred, a short recovery time is also preferred in a non-disposable sensor. An increased change in signal however also gives an increased recovery time (Figure 3.2, secondary axis). When the resistor was 1000 Ohm, the current change was 0.02 mA and the recovery time was 2.5 hours. At 100 Ohm current changed 0.92 mA and recovery time was 3.1 hours. The hydraulic retention time in the cells is 45 minutes. SDS washes out of the sensor within 3 retention times (2.25h). Recovery is thus not only due to wash out of SDS but also because bacteria are damaged and need to recover.

These results show that the choice of resistor value is important both for the sensitivity and robustness of the sensor.

Cell voltage depends on anode potential, cathode potential and internal resistance that varies with current. The extent that bacteria can adjust therefore also depends on internal resistance and for each system this has to be balanced with the external resistance. The absolute value of the 'best' resistance has to be investigated for every system. It can be concluded that the value of the external resistance is important for the magnitude of signal change and for how fast the bacteria can recover.

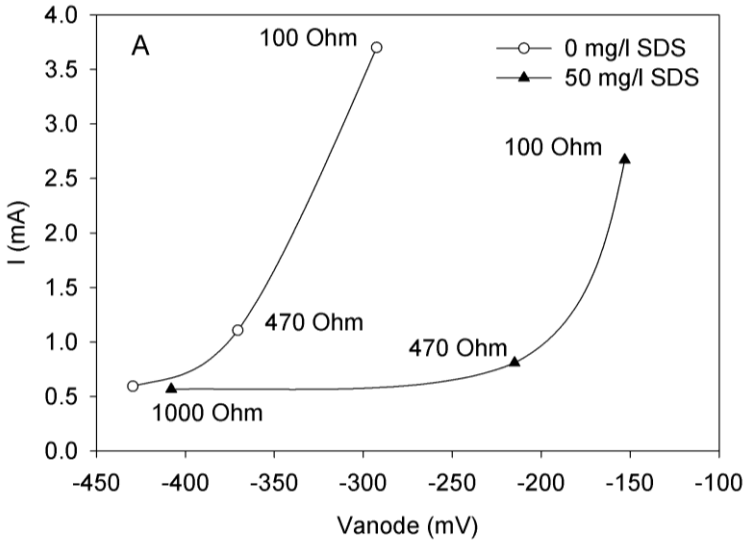

Figure 3.3A: Three measurement methods controlling: A: external resistance

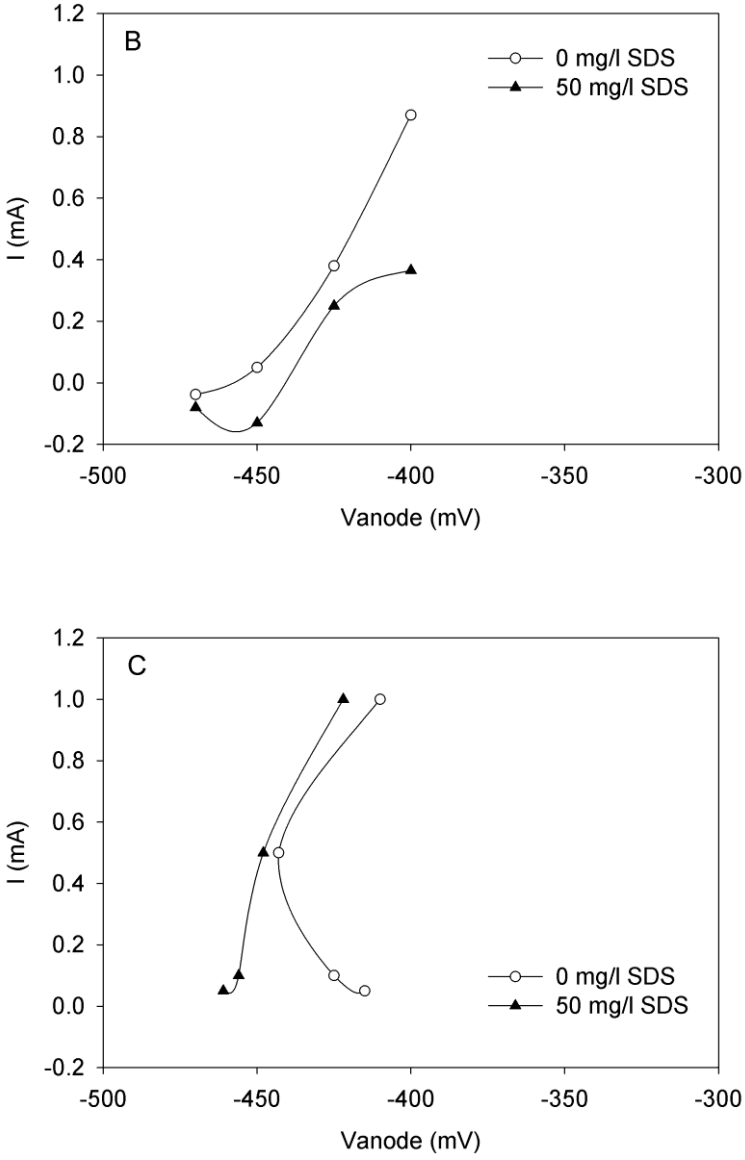

Figure 3.3: Three measurement methods controlling: A: external resistance, B: anode potential, C: current. A shift electrical signal is observed after addition of 50 mg/l SDS

3.3.2 Comparison of control methods

Using an external resistance under non-toxic conditions a low anode potential gives a low current. The addition of a toxic component however leads to an increased anode potential at a lower potential (Figure 3.3A, curve moves right and down). A high resistor value gives a low but measurable change in both anode potential and current and the recovery time is short while at low resistance, change in current and anode potential is much larger. It is interesting to investigate the change when only current or only anode potential can change. It was investigated if a better control of current or anode potential leads to a better response compared to using an external resistor.

When anode potential is controlled, current is measured and a change in current indicates a change in water quality. When anode potential is controlled a change in current can be linked to a change in metabolism of the microorganisms.

A higher anode potential gives a higher current. The current-drop upon addition of SDS is also higher at higher currents. Current drops e.g. from 0.87 mA to 0.27 mA at -0.4V anode potential whereas it drops from 0.05 mA to -0.13 mA at -0.45V (Figure 3.3B). The change of current depends on the anode potential. A selection of anode potential is thus necessary to get the most sensitive sensor. When anode potential is controlled just above OCV_anode, current is low but positive. However on addition of SDS, current dropped from 0.05 mA to negative values, -0.13mA at -0.45V. This is not observed before as far as the authors are aware. Blanks, cells without bacteria, give no current when the anode potential is decreased thus this effect of negative current must be an effect of the presence of the bacteria. Possibly membrane enzymes of bacteria are reduced. After SDS washes out of the sensor, the bacteria recover and current becomes positive again.

In stead of measuring changes in current, current can be controlled and cell voltage or anode potential is then measured. At 0.05 mA, anode potential decreases from -415 mV to -461 mV on addition of 50 mg/l SDS. A higher current of 0.5 mA however gave a decrease from -443 mV to -448 mV on addition of 50 mg/l SDS. (Figure 3.3C). The value of current thus affects the amount of potential change. When current is controlled the change is in the opposite direction than when anode potential and external resistance are

controlled (current moves leftwards). Bacteria can not slow down their metabolism but can only change the anode potential while being forced to have a high reaction rate. Most probably different metabolic mechanisms play a role using current control than when external resistance or when anode potential are controlled. It also shows that the value of control has an effect on the magnitude of signal change.

These experiments show that it is possible to measure a simulated toxic event while controlling either anode potential or current. It also shows that the response of bacteria differs when using an external resistance than when using anode potential control, where we see negative currents, or than when using current control, where we see a shift in polarization curve in the opposite direction.

The recovery time while controlling anode potential and current was also measured. Controlling anode potential resulted in similar recovery times as when using an external resistance: 0.042mA change needed 6.3 hours to recover, 0.42 mA change needed 12.6 hours to recover. (Figure 3.4A) Recovery time of experiments with a set current can be compared on the basis of anode potential change. The change in anode potential was much smaller using a set current than using a set resistor. The recovery times however were much larger. At a set current of 0.5 mA a change in anode potential of 5 mV needed 16 hours to recover while at 0.05 mA 46 hours were needed to recover from a change in anode potential of 44 mV (Figure 3.4B).

From Figure 3.4 one can see that using an external resistance gives a lower recovery time at the same sensitivity than when controlling anode potential or current. These findings suggest that when bacteria can adjust to the toxic situation by a change in both current and anode potential, less damage occurs than in the situation that only one factor can be adjusted.

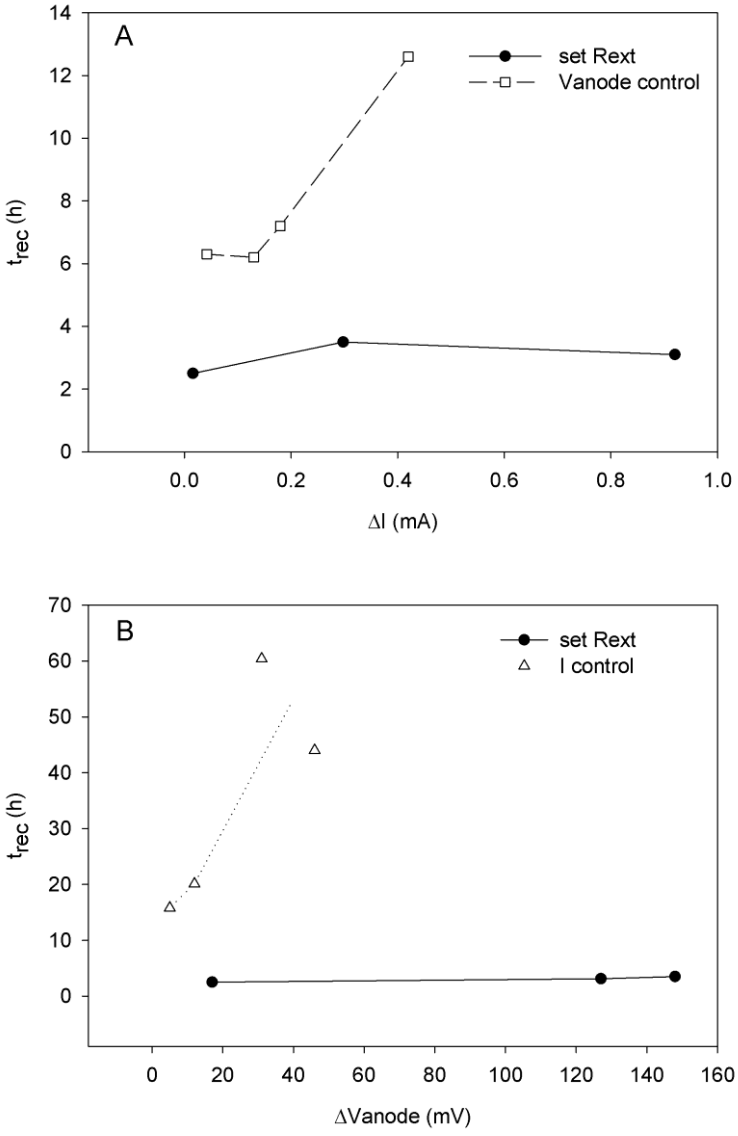

Figure 3.4: recovery time vs. sensitivity on the basis of (A) current change or (B) on the basis of anode potential change. The signal change is induced by dosage of 50 mg/l SDS for two hours.

These results show that using an external resistor gives the shortest recovery time while the response is comparable to the other two tested methods. However no detection limits were tested. It is clear that the direction of the response depends highly on the chosen method. Only a small current-potential range was tested. For further selection of the best sensor system, the most sensitive setting for each method has to be tested. Only then a choice for the best method can be made. However each of the methods seems applicable.
The concentration of SDS used is far above the concentrations allowed in drinking water or surface water [26]. This high concentration gives large signal changes and allows observing trends more clearly. It is not expected that the trends are affected by the concentration. Signal change is expected to be linear to the concentration present.

3.4 Conclusions

It is possible to detect toxic components in the MFC-based biosensor using all control methods, i.e. resistance control, anode potential control and current control. The response of each method depends on the control level of each method, i.e. resistance value in case of resistance control, potential value in case of anode potential control and controlled current level in case of current control. Only when using a high current (>0.5 mA) or high anode potential (>-0.4V), a large change in signal is observed. This large change however, needed a long recovery time when using anode potential control and current control. In practice this may also be an important motivation for choosing the specific settings of control level of the sensor. When bacteria can adjust both anode potential and current the recovery is faster than when they can only adjust one of these. The use of an external resistor is therefore interesting with regards to both signal change and recovery time.
The experiments gave an indication about the sensitivity of the sensor under the studied settings. It was not yet investigated what the optimal setting is to obtain the highest sensitivity.

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Intermezzo

This chapter is based on:

Hamelers HVM, Heijne AT, Stein NE, Rozendal RA, Buisman CJN. 2011. Butler-Volmer-Monod model for describing bio-anode polarization curves. *Bioresour Technol* 102:381-387.

I. Intermezzo

Butler Volmer Monod model for describing polarization curves

I.1 Introduction

The sensor investigated in the thesis uses microorganisms at an anode as a sensing element and the electrical current produced by the microorganisms as the signal. A change in electrical current indicates a change in water quality. Microorganisms grow on the anode of the microbial fuel cell where they convert chemical energy stored in organic material to electrical energy by converting organic material into carbon dioxide, protons and electrons [1, 2]. The anode serves as the electron acceptor in the oxidation reaction. To understand the relationship between the electrical signal in the sensor and the metabolisms of the microorganisms it is important to understand the relationship between the anodic overpotential and the current.

The anode, where the oxidation reaction takes place, is electrically connected to a cathode where oxygen or another electron acceptor is reduced. The oxidation of organic material takes place in the microorganisms and the oxidation rate is determined by enzyme kinetics. The transfer of electrons from the microorganisms to the electrode, however, occurs at the interface between microorganisms and the electrode, and is described by electron transfer kinetics like the Butler-Volmer relationship [3].

Anode polarization curves show the current as a function of anode potential or anodic overpotential η. The overpotential is defined as the difference between the substrate oxidation potential ($_{Esubstrate/\ product}$) and the anode potential (E_{an}),

$$\eta = E_{an} - E_{substrate/\ product} \qquad [1]$$

Polarization curves thus give an indication of the conversion rate of organic material to electrons, measured as electrical current, at a certain potential. The conversion of

chemical energy to electrical energy is generally less than 100% due to losses at the bio-anode. Polarization curves can also be used to quantify these losses [1, 4-6].

A model was developed for the description and analysis of polarization curves of microbial fuel cells under non-toxic conditions [7]. The model is based on a simple representation of the underlying biochemical conversions as described by enzyme kinetics and electron transfer reactions as described by the Butler-Volmer electron transfer kinetics. The combination of biochemical kinetics with electrochemical kinetics gives an analytical solution that can be used for the description and analysis of polarization curves. This newly developed model was referred to as the Butler Volmer Monod-model.

I.2 Model description

The Butler Volmer Monod (BVM) model developed assumes two distinct processes: (i) biochemical oxidation of a substrate and (ii) heterogeneous electron transfer to the electrode. The biochemical conversion of substrate occurs inside the microorganism and yields the products and the released electrons. It is generally assumed that the released electrons that are transferred to a certain redox component, such as NAD^+. The heterogeneous electron transfer occurs via the redox component from the microorganism to the electrode. (Figure I.1). The model makes no assumption on the nature of the electron transfer from the reduced electron component to the final redox component transferring the electron to the anode, whether this is through direct contact, a soluble mediator or a conductive matrix [8]. From Figure I.2, however, one can see that microorganisms grow both directly on the electrode and at some distance. These intermediate transfers are assumed to be very fast. It is assumed that the biochemistry or the electron transfer, or a combination of both processes, is rate limiting.

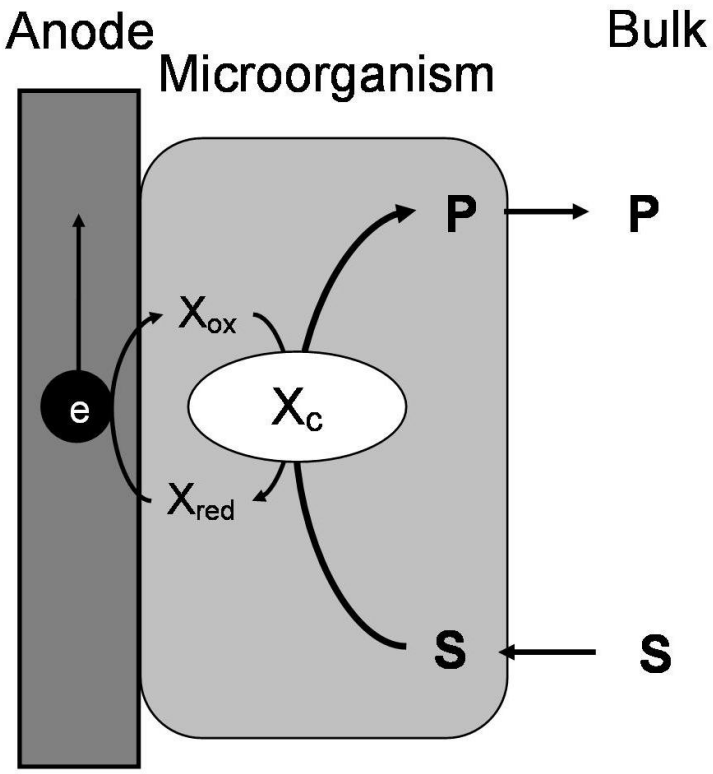

Figure I.1: Kinetic scheme of substrate degradation and electron transfer to the electrode with the use of a redox component.

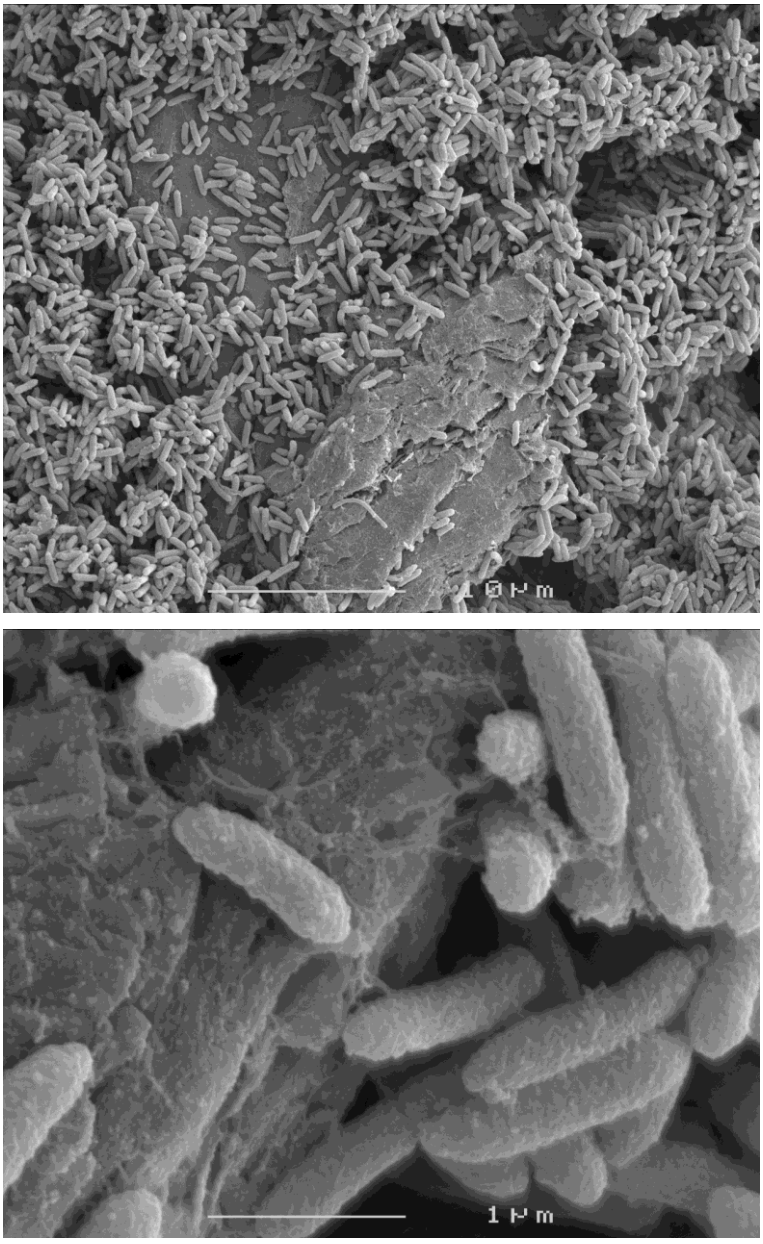

Figure I.2: scanning electrode microscope (SEM) pictures of microorganisms growing on a solid electrode

In the BVM model, the redox component that acts as the electron acceptor in the biochemical oxidation reaction is assumed to be the same redox component that acts as

the electron donor in the heterogeneous electron transfer to the electrode. Accordingly, the bio-anode is described as a three reaction system. The first reaction is the reaction of the substrate (S) and the oxidized redox component (X_{ox}) to form a redox component complex (X_C) via an enzymatic reaction.

$$S + X_{ox} \underset{k2}{\overset{k1}{\rightleftharpoons}} X_c \qquad [2]$$

This reaction is described by the normal mass action rate law and has two constants describing the rate, the forward rate constant k1 and the backward rate constant k2. The redox component complex undergoes a second reaction upon which the products (P) are formed together with the reduced redox component (Xred):

$$X_c \underset{k4}{\overset{k3}{\rightleftharpoons}} P + X_{red} \qquad [3]$$

This reaction is characterized by the forward rate constant k3 and the backward rate constant k4. In the third reaction, the reduced redox component is oxidized at the electrode while the electron is transferred:

$$X_{red} \underset{k6}{\overset{k5}{\rightleftharpoons}} X_{ox} + e^- \qquad [4]$$

This latter reaction is the actual heterogeneous electron transfer. This type of reaction has the specific characteristic that the reaction rate is influenced by the electrode potential, both the forward rate constant k5 and the backward rate constant k6 are thus a function of the electrode potential.

The redox component is thus the intermediate in the reactions. The total redox component concentration in the microorganism, X_T, is assumed to be constant and in given by

$$X_T = X_C + X_{ox} + X_{red} \qquad [5]$$

The concentrations of the different forms may change over time.

I.2.1 Biochemical rates

At low potential, X_{red} is produced relatively fast compared to rate k5 and k6. At higher potential, however, the rate limiting rates are not k5 and k6 but rate k1 to k4. This is due to the biochemical limitations in the microorganisms. The enzymatic reaction reaches a maximum reaction rate $r_{max} = X_T * k3$ and this leads to maximum current density that can be reached according to

$$I_{max} = n \cdot F \cdot r_{max} = n \cdot F \cdot X_T \cdot k3 \quad [6]$$

In which n is the moles of electrons released in the reaction and F is Faraday's constant (C/mol). The maximum current density is only limited by the biochemical reaction as the electrochemical reaction rate can be increased by increasing overpotential.

Substrate consumption rate in microorganisms can be described using the substrate affinity constant, also known as the Monod-constant. This substrate affinity constant can be described by rates k1-k3 following

$$K_M = \frac{k2 + k3}{k1} \quad [7]$$

I.2.2 Electrochemical rates

The transfer of electrons to the electrode is measured as the electrical current and defined as

$$I = n \cdot F \cdot r_{transfer} \quad [8]$$

in which $r_{transfer}$ is the electron transfer rate (mol e^-/sec). Transfer rate r is determined by the forward oxidation of X_{red} and the backwards reduction of X_{ox}.

$$r_{transfer} = k_5 \cdot X_{red} - k_6 \cdot X_{ox} \quad [9]$$

Reaction rates k5 and k6 depend on the electrode potential. This dependency on the electrode potential can be described using the Butler Volmer equation, which is the standard model to describe electrochemical reactions and has shown to be applicable to large class of redox proteins [9].

The electrochemical rate constants k5 and k6 are given by

$$k_5 = k^0 \exp\left[(1-\alpha)\cdot f\cdot(E_{an} - E_X^{0'})\right] \quad [10]$$

$$k_6 = k^0 \exp\left[-\alpha\cdot f\cdot(E_{an} - E_X^{0'})\right] \quad [11]$$

In which, k^0, standard heterogeneous rate constant (cm/s); α, transfer coefficient (–); f = F/ RT (1/V); E_{an}, anode potential (V); $E_X^{0'}$, formal potential of redox component (V). The transfer coefficient has a value between 0 and 1, and is typically around 0.5 [3].
As anode potential increases, reaction rates k5 and k6 also increase.

At equilibrium condition, i.e. at zero-current, the anodic and cathodic current are equal but opposite. The maximum anodic current that can be reached at equilibrium is called the exchange current density (I_{ex}) . It is a measure for the reversibility of the reaction, and thus how fast the electrochemical reaction of the redox couple runs.

I.3 Butler Volmer Monod model

Combining Eq. (5-11) yields the Butler Volmer Monod model, being a steady-state solution for the current density as a function of overpotential:

$$I = Imax \cdot \frac{1 - e^{-\eta\cdot f}}{K1\cdot e^{-(1-\alpha)\cdot\eta\cdot f} + K2\cdot e^{-\eta\cdot f} + \frac{Km}{S} + 1} \quad [12]$$

This equation contains the overpotential η, defined in Eq. (1), and two lumped parameters: K1 and K2. The exact deduction of these parameters can be found in Hamelers et al. [7]. These two parameters are, under steady state conditions, approximated by:

$$K1 \approx \frac{I_{max}}{I_{ex}} \quad [13]$$

Parameter K1 describes the ratio of the biochemical over electrochemical reaction rate constants.

$$K2 \approx \frac{k3}{k2} \quad [14]$$

The parameter K2 describes the ratio between the forward and backward reaction rate constants of substrate oxidation.

These definitions hold when the forward rate constant k3 is much higher than the backward rate constant k4, and when there is no product limitation. At steady state conditions parameters K1 and K2 are constants.

I.4 Polarization curves

The relation between current and overpotential can be described by the Butler Volmer Monod model (eq. 12) and shown in a polarization curve (Figure I.3). The following parameters are based on Hamelers et al. [7] and used for the simulation.

Imax=2 mA; K1=1; K2=15; α=0.5; Km=0.5 mM; T=30^{o}C; S=2.5 and 5 mM

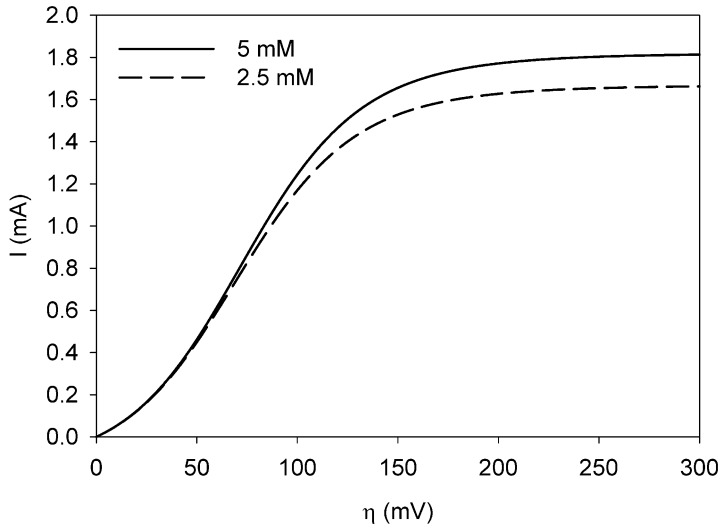

Figure I.3: Polarization curve with a substrate concentration of 5 mM (solid line) and 2.5 mM (dashed line)

At low potentials, the curve shows an increase in current at increasing potential. In this range, the electrochemical reaction is limiting. At higher overpotential, the current starts to level off. When the curve is horizontal the biochemical reaction is rate limiting.

This model, the Butler Volmer Monod model, does not describe the effect of toxic chemicals on microorganisms in the sensor. However, it can predict the current under steady state conditions and a change of the current may thus indicate a change in water quality.

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Chapter 4

Kinetic models for detection of toxicity in a microbial fuel cell based biosensor

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4. Kinetic models for detection of toxicity in a microbial fuel cell based biosensor

Abstract

Currently available models describing microbial fuel cell (MFC) polarization curves, do not describe the effect of the presence of toxic components. A bioelectrochemical model combined with enzyme inhibition kinetics, that describes the polarization curve of a MFC-based biosensor, was modified to describe four types of toxicity. To get a stable and sensitive sensor, the overpotential has to be controlled. Simulations with the four modified models were performed to predict the overpotential that gives the most sensitive sensor. These simulations were based on data and parameter values from experimental results under non-toxic conditions. Given the parameter values from experimental results, controlling the overpotential at 250 mV leads to a sensor that is most sensitive to components that influence the whole bacterial metabolism or that influence the substrate affinity constant (Km). Controlling the overpotential at 105 mV is the most sensitive setting for components influencing the ratio of biochemical over electrochemical reaction rate constants (K1), while an overpotential of 76 mV gives the most sensitive setting for components that influence the ratio of the forward over backward biochemical rate constants (K2).

The sensitivity of the biosensor was also analyzed for robustness against changes in the model parameters other than toxicity. As an example, the tradeoff between sensitivity and robustness for the model describing changes on K1 (IK1) is presented. The biosensor is sensitive for toxic components and robust for changes in model parameter K2 when overpotential is controlled between 118 and 140 mV under the simulated conditions.

4.1 Introduction

Water quality is an important factor that water companies need to monitor. Many methods are used to monitor the chemical components that determine water quality. To measure chemical components, both on-line and offline sensors are available. A drawback of most off-line sensors is, amongst others, that detection takes several hours or even days. Hence this type of sensor is, in general, unsuitable for alarming and process control. Furthermore, these sensors are very accurate for some components but not at all for others. Therefore online, generic sensors are needed as well, especially for real-time applications. In addition to physical-chemical sensors, biosensors like fish, daphnia and bacterial sensors are available. These sensors are sensitive to many components and placed online in systems. They need, however, nonlinear transducers to convert the signal of organisms into a signal that can be read by operators, are costly and need a lot of maintenance [1-6]. A microbial fuel cell (MFC)-based biosensor was therefore proposed as a direct biosensor for toxicity [2, 3].

A microbial fuel cell is a device in which a chemical signal is converted into an electrical signal. Microorganisms convert organic substrate into carbon dioxide, protons and electrons. Microorganisms use an anode as their electron acceptor when no soluble electron acceptor is present. Electrons flow from the anode to the cathode where e.g. oxygen is reduced. The flow of electrons is measured as current. This current is a linear measure of the activity of microorganisms. When a toxic component passes the microorganisms, their activity will change, most likely decrease. Hence, fewer electrons are transported and current then decreases as well. In this way, a microbial fuel cell-based biosensor provides a direct measure for water quality [2, 7].

Activity of microorganisms depends on temperature and pH. Hence to get an accurate signal from the MFC-based biosensor, pH and temperature need to be controlled or they should be measured and corrected for in a signal processing step. The activity of microorganisms also depends on the energy obtained from consumption of substrate. The energy related to the release of electrons at the anode depends on the difference between prevailing anode potential and equilibrium anode potential at zero-current. This potential difference is called overpotential. The overpotential needs to be controlled to

get a stable baseline under nontoxic conditions. However, it was not yet investigated at which overpotential the sensor is most sensitive for detection of toxic components.

The objective of this paper is to offer a methodology how to evaluate at which overpotential the sensor is most sensitive to toxic components.

One has to realize that current is not only determined by the overpotential, but also by other environmental factors such as the substrate concentration. Ideally, changes in e.g. substrate concentration during measurement should not influence the detection of toxic components. The sensitivity of the sensor for toxic components should not decrease when environmental parameters like substrate concentration change. In addition to a high sensitivity with respect to toxic components, the sensor thus also needs to be robust against changes in environmental parameters.

The approach to find an overpotential, at which the sensor is sensitive to toxic components and robust against environmental disturbances, was to use a model that describes current production in microbial fuel cells. There are several models that describe polarization curves [8-13]. The starting point for the analysis here is the Butler–Volmer-Monod (BVM) model as presented by Hamelers et al. (2011). It is given by

$$I = Imax \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot e^{-\eta \cdot f} + \frac{Km}{S} + 1} \qquad [1]$$

Where Imax (mA) is the maximum current determined by maximum enzymatic rates of microorganisms, η is the overpotential (V), K1 is a lumped parameter describing the ratio between biochemical and electrochemical rate constants, K2 is a lumped parameter describing the forward over backward biochemical rate constants, Km (mol/l) is substrate affinity constant and S (mol/l) substrate concentration. Furthermore, f= F/RT with F Faraday's constant, R the gas constant and T temperature. This model is based on a representation of the underlying biochemical conversions and electron transfer reactions that take place on the anode. Hence, it contains a combination of enzyme kinetics and electrochemical kinetics. The kinetic scheme is presented in Figure 4.1.

As yet, this model does not contain any term that accounts for presence of inhibiting components. Hence, in this paper, for the purpose of toxicity detection, the model is extended with an inhibition term. Next, the sensitivity of the sensor for toxic components

as a function of overpotential will be examined. Through simulations it will be investigated how the mode of action of the toxic component influences the change in current when a toxic component is present. Then, it will be determined at which overpotential this change is largest and thus the sensor is most sensitive. This gives the optimal overpotential at which the sensor needs to be controlled under non-toxic conditions. In addition to this, a parameter sensitivity analysis is conducted to investigate the robustness of the sensor and to investigate how the choice of overpotential is influenced by the parameter values in (1).

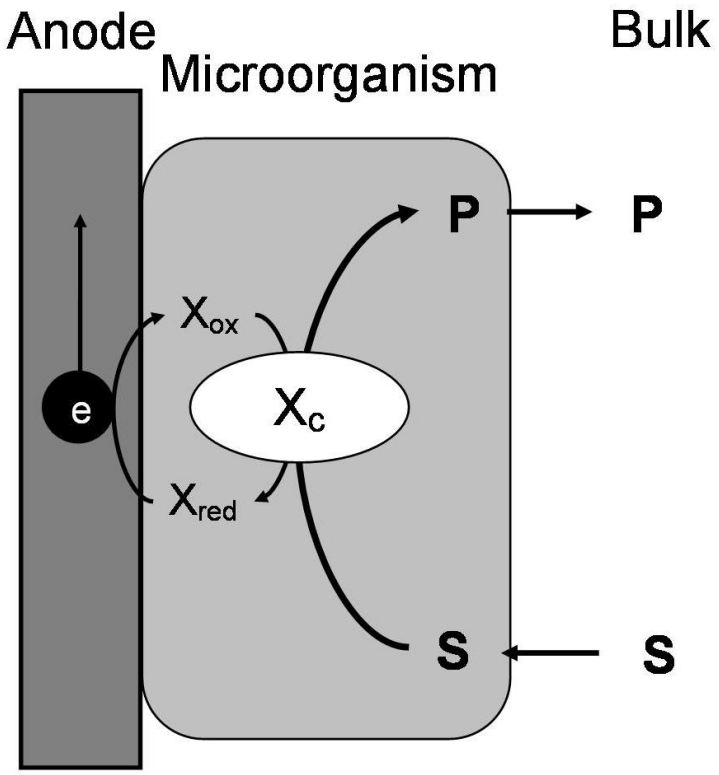

Figure 4.1: Kinetic scheme of the bio-anode. Substrate (S) from the bulk phase diffuses in to the cell and is converted by an enzymatic reaction to form the redox component complex X_C in the micro-organism. This biochemical conversion leads to products (P) that diffuse to the bulk phase again. Electrons that are released in the conversion are transported to the electrode via a redox component (X_{ox}/X_{red}). (Adapted from [9]

4.2 Methods

As a starting point the model (1), as introduced by Hamelers et al. [9], is used. The assumption is that the effect of toxicity can be described and modeled via an effect on the kinetic reaction rates involved in electron transfer. Depending on the mechanisms how the cell is influenced by the component there are several reaction rates that can be influenced by a toxic component. Four models are proposed, containing an inhibition term in which χi is the concentration of toxic component (mmol/l) and Ki the affinity constant (mmol/l). A smaller Ki implies that the component is more toxic to the microorganism.

1) In the first model it is assumed that the toxic component acts as a noncompetitive inhibitor or as an irreversible inhibitor [14]. Consequently, the model is given by:

$$I = I_{\max} \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot e^{-\eta \cdot f} + \frac{Km}{S} + 1} \cdot \frac{Ki}{Ki + \chi i} \qquad [2]$$

This model is referred to as “Itox”.

2) Parameter K1 describes the ratio between biochemical and electrochemical reaction rate constants. This ratio may change when toxic components can act as electron acceptors. In this case the redox complex is not fully oxidized at the anode but partly at the toxic component and hence current decreases. The model for this case is given by:

$$I = I_{\max} \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot \frac{Ki + \chi i}{Ki} \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot e^{-\eta \cdot f} + \frac{Km}{S} + 1} \qquad [3]$$

This model is referred to as “IK1”.

3) Parameter K2 describes the ratio between the forward and backward reaction rate constants of substrate oxidation. This ratio can change when no product is formed anymore or much more product (protons and CO_2) is formed. The ratio also changes when the toxic component is an acid. The increased proton concentration leads to a reduction of the forward reaction rate.

$$I = I_{\max} \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot \frac{Ki + \chi i}{Ki} \cdot e^{-\eta \cdot f} + \frac{Km}{S} + 1} \quad [4]$$

This model is referred to as “IK2”.

4) The substrate affinity constant Km may change when there is a competition between substrate and toxic component to bind to the redox complex [14].

$$I = I_{\max} \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot e^{-\eta \cdot f} + \frac{Km \cdot \frac{Ki + \chi i}{Ki}}{S} + 1} \quad [5]$$

This model is referred to as “IKm”.

As noticed before, the Butler-Volmer-Monod model is based on enzyme and electrochemical kinetics. The proposed models can, therefore, only describe kinetic inhibition of microorganisms and do not describe the various mechanisms of toxic effects on the cell [15, 16]. Consequently, from the results it is most likely not possible to tell if the toxic component is e.g. a DNA damaging agent or a protein damaging agent or is damaging otherwise.

In previous studies several polarization curves were made under non-toxic conditions. From these curves values for model parameters K1, K2 and α were calculated by Hamelers et al. [9]. Similar values as those presented by Hamelers et al. [9] were used for the simulations in this paper. In addition to this, values of parameters Imax, Km and S were estimated from earlier experiments (unpublished). Table 4.1 summarizes parameter values used in the simulations. The overpotential was taken between -50 mV and 250 mV. For the simulations, no explicit component was chosen. Therefore there is no concentration known, nor a value of Ki. As the inhibition term Ki/(Ki+χi) determines the value of dI/dχi and thus the value of the overpotential at which dI/dχi is maximal, it is important to cover a wide range between the possible extreme values 0 and 1. The values

used in the simulations are: Ki=1 mmol/l, χi = 0.1 mmol/l or 2 mmol/l, thus achieving that the inhibition term is 0.9 and 0.33, respectively.

Table 4.1: parameter values used in the simulations.

parameter	*value*	*Unit*
K1	1	-
K2	15	-
α	0.5	-
Imax	2	mA
Km	0.1	mmol/l
S	5	mmol/l
f	0.0383	
Ki	1	mmol/l
χi	0.1 or 2.0	mmol/l

4.3 Results

4.3.1 Current

For each of the four proposed models the electrical current is simulated as a function of the anodic overpotential. As a reference, the results of the BVM-model (eq 1) indicated as I, are also presented. From Figure 4.2 it can be observed that sigmoidal curves result. Compared to the response under non-toxic conditions (fat line) the slope of the curve at the inflection point and final current at high overpotential can be different. From the response of model Itox (dotted line) it can be seen that both the slope and maximum are smaller. This is in line with what is expected, as in this model the inhibition term influences the complete current at all overpotentials. Model IK1 (solid line) also shows a decreased slope but the final current seems to approach the same maximum current as under non-toxic conditions. The slope in model IK2 (large dashed line) is similar to the slope of the non-toxic condition and also the maximum current reached is equal. The graph is only shifted to higher overpotentials (to the right). Model IKm (small dash) shows a slightly lower slope but mainly the maximum is lowered when a toxic component is present.

From these simulated curves it can be observed that, depending on the mode of action of the toxic component, the difference between toxic and non-toxic is significantly influenced by the chosen overpotential set point.

The results in Figure 4.2 suggest that by measuring the polarization curve under non-toxic conditions and for any specific toxic substance under toxic conditions it would be possible to discriminate between the four alternative inhibition mechanisms.

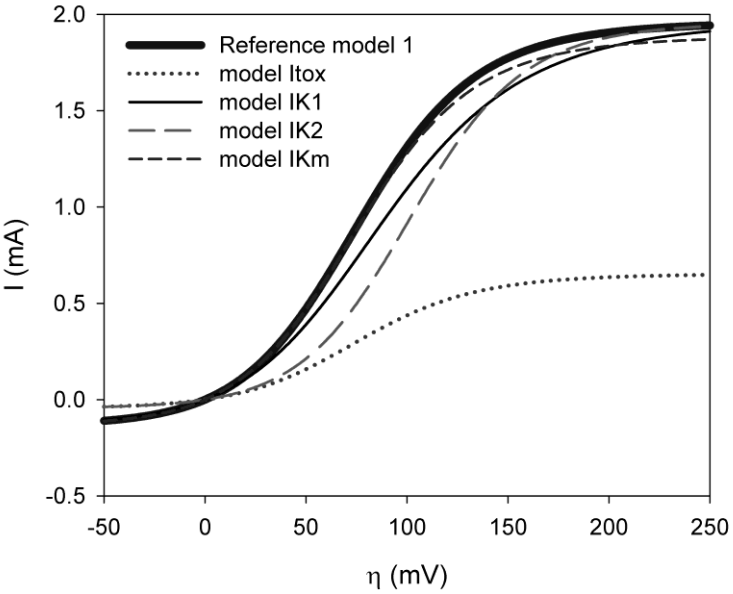

Figure 4.2: polarization curves for all four models (eq 2-5) (with χi=2, Ki=1) and the reference model (eq 1)

4.3.2 Sensitivity for toxic components

The biosensor will be most sensitive for toxic components when the change in current is highest for the same change in concentration of toxic component. This implies that the derivative of the current with respect to the concentration of toxic component, which is a function of η, should be maximal. Consequently, the goal is to find

$$\eta^* = \arg \max_{\eta \in [-50, 250]} \frac{dI(\eta)}{d\chi i} \qquad [6]$$

where $dI(\eta)/d\chi i$ is the sensitivity of the current for toxic components and η^* is the overpotential that gives maximum sensitivity on the interval [-50, 250] mV.

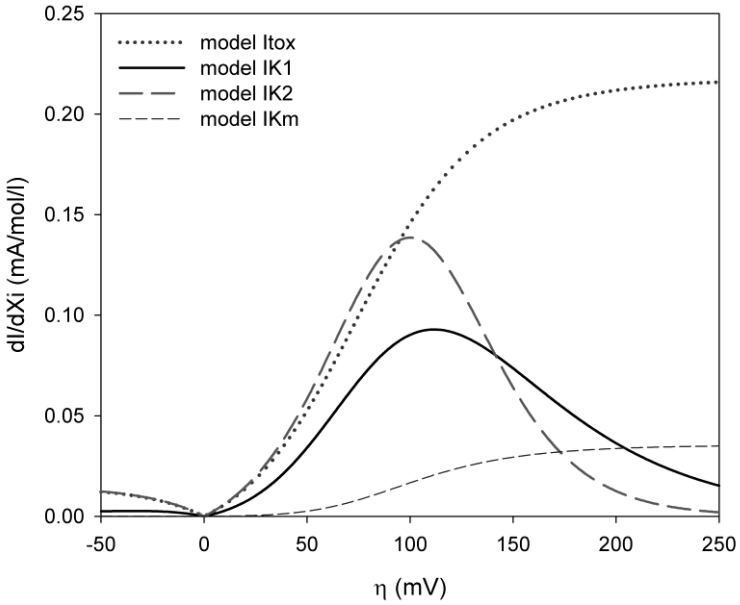

Figure 4.3: Sensitivity to a change of toxic component concentration (χi=2 mmol/l), as a function of overpotential. When $dI/d\chi i$ is maximal, the sensor is most sensitive to toxic components.

In Figure 4.3 the derivative of current to toxicity, $dI/d\chi i$, is plotted as a function of overpotential, η. When $dI/d\chi i$ is at its maximum value, the sensor is most sensitive at this overpotential. From this figure it is observed that model Itox (dotted) and IKm (small dash) are most sensitive when overpotential is as high as possible, in this case 250 mV. Model IK1 and IK2 have their maximum at lower overpotentials. Values of the optimal overpotential, η^*, are shown in Table 4.2 for a toxicity concentration of 2 mmol/l and 0.1 mmol/l calculated with the given parameter values from Table 4.1. Hence, depending on the mechanisms of toxicity for the microorganisms, the optimum overpotential for control of the sensor is different.

Table 4.2: Overpotentials at which the change of current due to toxicity is maximal.

model	*Overpotential η (mV)*	*dI/dχi (mA·l/mmol)*	*Overpotential η (mV)*	*dI/dχi (mA·l/mmol)*
	χi=2 mM		χi=0.1 mM	
Itox	250	0.20	250	1.60
IK1	112	0.09	105	0.13
IK2	100	0.14	76	0.33
IKm	250	0.03	250	0.04

When the toxic component influences the complete cell or just Km, the most sensitive setting is at the highest possible overpotential (250 mV) for every concentration χi. When the toxic component only influences K1 or K2, however, the most sensitive overpotential is lower than 250 mV, that is at 112 and 100 mV. Lower inhibition, thus higher values of Ki/(Ki+χi) shift the goal point somewhat downwards. When the toxic component concentration is, for instance, 0.1 mmol/l, the maximum of dI/dχi from model IK1 lies at an overpotential of 105 mV. This means that, for Ki = 1 mmol/l, the maximum shifts 7 mV to the left as compared to a concentration of toxicity of 2 mmol/l. The optimum of model IK2 shifts from 100 mV at χi = 2 mmol/l to 76 mV at χi = 0.1 mmol/l. We would like to detect very low concentrations of toxic components. Even of components that are not very harmful to the bacteria. This implies that choosing a lower overpotential set point ensures a higher sensitivity at low concentrations.

Thus, for model IK1 and IK2, the overpotential related to a maximum sensitivity is a function of the toxicity itself. This phenomenon increases the difficulty to make a good choice for overpotential. The setting of the most sensitive sensor is not only determined by the mode of action of the toxic component, it also depends on the ratio between Ki and χi in the case of models K1 and IK2.

4.3.3 Robustness

In a real application the sensor will be used in an environment that undergoes changes that are not toxic. For example pH, conductivity or transfer rates may change. These changes in the environment should not influence the detection of toxic components too much. In other words, the detection of toxicity should be robust against changes in parameters other than toxicity. Hence the setting of the biosensor should be such that the sensor is sensitive to change in toxic component concentration and at the same time insensitive to changes over time of other components and model parameters. In what follows, it will be investigated how changes in model parameters affect $dI(\eta)/d\chi i$ and what the consequences are for an optimal choice of η. Recall that the model parameters were estimated under non-toxic conditions. When the sensor is applied, these parameters may change over time. Furthermore, the initial parameter estimates may not be very accurate and may influence the choice for an optimal overpotential. It was investigated how the optimal overpotential setting is influenced by the initial estimates of those parameters that were estimated less accurately.

In the models (eq 2 to 5) the following parameters are present: Imax, K1, Km, α, K2, Ki and the process variable S, the substrate concentration. Imax is determined by the capability of the bacteria under non-toxic conditions. Hence, its initial value can be estimated quite accurately. It is also not likely to change over time. The model parameters α and Km can be determined under non-toxic conditions and are not expected to change over time.

K1 represents the ratio of the biochemical over the electrochemical reaction rate constants that is K1~ Imax/Iex, where Iex (mA) is the exchange current density under zero-current conditions at equilibrium. This exchange current density will not change unless there are redox active components present that can be regarded toxic. Therefore, under non-toxic conditions, K1 will not change. Recall that K1=1 is chosen on the basis of existing datasets [9]. However, if K1 would have a different value, it will possibly affect the sensitivity. Model simulations reveal that in the range $0.1<K1<50$ the optimal overpotential for the model Itox and IKm remains at the highest investigated point, 250

mV. For model IK1 a higher value of K1 results in a higher sensitivity at all overpotentials. The optimal value of the overpotential increases. However, a good sensitivity is still found at an overpotential of 112 mV, as found to be optimal for the parameter values of Table 4.1. Model IK2 on the other hand, gives a lower sensitivity, if K1 increases. The optimal overpotential increases just as in model IK1. Choosing a value to control the overpotential is then dependent on the accuracy of estimation of K1, the accuracy of the current measurements and the value of current itself.

Model parameter K2 represents the forward over the backward biochemical rate constants. This value can change over time especially when water conditions like pH change. Protons are produced as product and pH greatly influences the speed of the forward reaction rate. Therefore, it is important to see if the choice of overpotential for maximum sensitivity of the sensor changes when the value of K2 changes. Also, the substrate concentration can change over time. Hence, the robustness of dI/dχi for changes in K2 and S was determined. The biosensor is more robust to changes in the parameter, if the absolute value of the derivative of dI/dχi with respect to a specific parameter is as low as possible. Hence, with respect to robustness, the goal is to select η such that

$$\eta^{*} = \arg \max_{\eta \in [-50,250]} \frac{\partial^2 I(\eta)}{\partial \chi i \partial \Theta} \text{ with } \Theta \in \{Imax, K1, K2, Ki, S\} \qquad [7]$$

In other words, the change of sensitivity, dI/dχi, is minimal at a change of parameter Θ at overpotential η^{*}. The derivative of dI/dχi with respect to K2 and S is investigated as a measure for robustness. When the toxic component acts on microorganisms according to model Itox and model IKm, the current is most robust for changes in K2 and S when the overpotential is equal to 0mV. However, from Figure 4.2, it can be seen that this is also the overpotential at which the current is least sensitive for changes in concentration χi. The current is most sensitive at a high overpotential, but here it is also least robust.

When the toxic component influences the microorganisms according to model IK1 the sensitivity for toxic components is influenced when model parameter K2 changes. This

influence is largest for an overpotential of 73 mV and smallest when the overpotential is very low (around 0 mV) or very high (250 mV). Changes in S will influence the detection of toxicity the most at overpotential of 120 mV. However, the concentration of substrate is of great influence on this. For a substrate concentration of 5 mmol/l a change will hardly affect the detection of toxic components. However, when the substrate concentration is 0.5 mmol/l, the biosensor will be less robust and a lower substrate concentration will lower the sensitivity and thus the limit of detection of toxic components.

When the toxic component influences the microorganisms according to model IK2, a similar effect is seen for changes in S as compared to model IK1. The sensor would be least robust at an overpotential of 94 mV and most robust at low (0mV) and high overpotentials. For changes in K2, however, the biosensor is most robust at low and high overpotentials but also at 66 mV. An overview of the presented values is given in the Table 4.3. All data were calculated using the parameter values from Table 4.1. When a different system is used the parameter values may be different, but these values can easily be obtained under non-toxic conditions. Also, the values for the optimal control of overpotential in view of sensitivity and robustness have to be adjusted, but the four models still give a good indication for the different type of responses that can be expected from toxic contamination.

Table 4.3: Overview of robustness of sensitivity for changes in the model parameters K1, K2 and S of the four models. Least robust means that the sensitivity is changed most at the given overpotential η when the parameter changes. The sensitivity changes least upon change of the parameter at the overpotential that is indicated as most robust.

Model		*Model parameter that changes*		
		K1	*K2*	*K3*
Itox	Least robust	η= 250 mV	η= 250 mV	η= 250 mV
	Most robust	η= 0 mV	η= 0 mV	η= 0 mV
IK1	Least robust	η= 111 mV	η= 73 mV	S=5 mM η= 120 mV S=0.5 mM η= 115 mV
	Most robust	η= 0 and 250 mV	η= 0 and 250 mV	η= 0 mV
IK2	Least robust	η= 75 mV	η= 39 and 106 mV	η= 94 mV
	Most robust	η= 0 and 250 mV	η=0, 66 and 250 mV	η= 0 mV
IKm	Least robust	η= 250 mV	η= 250 mV	η= 250 mV
	Most robust	η= 0 mV	η= 0 mV	η= 0 mV

4.3.4 Tradeoff between sensitivity and robustness

To control the sensor at an overpotential that gives a sensitive sensor for toxic components, but is also robust for changes in the other parameters, a trade off has to be made. The noise of the current measurement and the absolute value of current should be taken into account when this trade-off is made. In Figure 4.3 the sensitivity for toxicity ($dI/d\chi i$) is plotted, as well as the effect of changes in K2 on this sensitivity ($\frac{\partial^2 I}{\partial \chi i \partial K2}$) for model IK1 and the concentration χi=0.1 mmol/l. The solid line shows the sensitivity and has a maximal value at 104 mV. At this overpotential the sensor is most sensitive. The black dotted line shows the effect a change of K2 has on the sensitivity. The sensor is most robust when this value is very low, thus at an overpotential of 0 or 250 mV and least robust at an overpotential of 72 mV. When the sensor is controlled at 104 mV, a change in K2 will affect the sensitivity. Controlling the sensor at a value between 72 and 104 mV will

make the sensor less sensitive and less robust. However, controlling the sensor at a value just above 104 mV e.g. 120 mV, the sensor will be a little less sensitive for toxicity but more robust for K2. Hence, a feasible interval for η, such that the sensor has the required minimum sensitivity and maximum robustness $\frac{\partial^2 I}{\partial \chi i \partial K2}$, can be chosen graphically. In the area where these two areas overlap, the overpotential should preferably be controlled. In Figure 4.4 the sensitivity is set at a minimum of 0.1 mA/mmol/l and thus the overpotential should lie between 75 mV and 140 mV. The maximum of the influence of K2 is set at 0.002 mA /mmol/l. Hence, the overpotential should then be controlled at values below 31 mV or above 118 mV. The overlapping dashed area shows that the overpotential should be controlled between 83 and 140.3 mV to obey both requirements.

The effect of the substrate concentration has also been investigated. Keeping substrate concentration high, results in a much more robust sensor for changes in substrate concentration than for low substrate concentration (not shown).

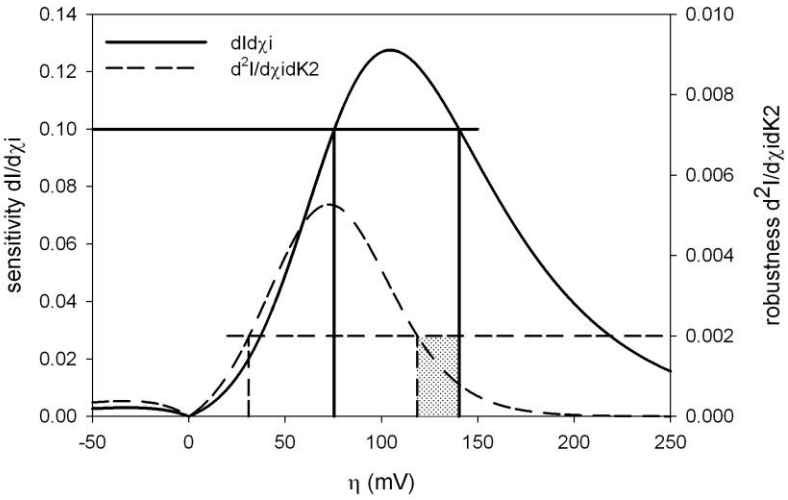

Figure 4.4: Model IK1: sensitivity for toxicity (dI/dχi), χi=0.1 mmol/l, compared with robustness against changes in parameter K2. When the sensitivity is chosen to be at least 0.1 and the influence of K2 on the sensitivity to be at the most 0.002, the overpotential has to lie between 118 and 140 mV. (—) primary y- axis dI/dχi, (- - -) secondary y-axis $d^2I/d\chi i\, dK2$

To determine the best setting for the overpotential, this tradeoff can be made for each model, using parameter values and required levels for sensitivity and robustness applicable for the system used.

4.4 Discussion

When the type of component that may pass the sensor is unknown, the sensor has to be controlled at an overpotential at which the sensitivity is high for all four models. This means that a trade-off has to be made for the choice of the overpotential. Choosing an overpotential between 76 mV and 104 mV gives a very sensitive sensor for components that influence K1 or K2. When a component influences Km, however, and the sensor is controlled at 76 mV, the change in current is very low. Controlling the overpotential at 250 mV gives a sensitive sensor for components that influence the complete metabolism or Km, but a very poor sensitivity for components influencing K1 and K2. The choice of an optimal overpotential is thus very much affected by the kind of contaminations that are expected. If this is not known, an overpotential of e.g. 150 mV gives a relatively good sensitivity for any kind of component, but is not optimal. Another option would be to make the sensor in duplex and control the two sensors at different overpotential, preferably one between 75 and 100mV and the other one at 250 mV. By comparing the change of the current in the set-ups, something may already be concluded about the mode of action of the component and maybe even about the type of component.

For example, a database with different toxic components and related value for Ki and type of kinetic model can be set-up. Values for the model parameters can also be determined from polarization curves made under non-toxic condition. By making a polarization curve at the moment a change in current is observed, it can be determined what the appropriate model is and what the value of the inhibition term $Ki/(Ki+\chi i)$ is. By combining the database of Ki's and the model, with the value of the inhibition term, the concentration for each possible component can be estimated.

The models presented in this paper are derived under steady state conditions. They do not take the specific design of the sensor into account, only the biofilm. To enable online signal processing of the data from the sensor, the modified Butler-Volmer-Monod model

(eq 2-5) must be embedded in a reactor model, in order to account for sensor cell processes such as wash out. When the toxic component enters the sensor, the dynamics of the current change before reaching the new pseudo-steady state can also give information about the type and concentration of component present.

4.5 Conclusions

The sensitivity of current change in a microbial fuel cell based biosensor depends on several factors, as (i) the mode of action of the component, (ii) the affinity for the toxic component, (iii) the concentration of component and (iv) the overpotential at which the sensor is controlled. This paper shows that the choice of the overpotential at which the sensor is most sensitive depends on the mode of action of the toxic component. The choice of overpotential also depends on the robustness of the sensor against changes in the model parameters. To get a sensitive and robust sensor in the same time, a choice on the basis of the tradeoff between sensitivity and robustness has to be made. This theoretical study provides a basis for the design and performance of experiments that can validate the four models for detection of toxicity using a MFC-based biosensor.

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Chapter 5

Online detection of toxic components using a microbial fuel cell-based biosensor

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5. Online detection of toxic components using a microbial fuel cell-based biosensor

Abstract

Safe drinking water without toxic chemicals is crucial for people's health. A recently developed sensor for the detection of toxic components in drinking water is the microbial fuel cell (MFC)-based biosensor. In this biosensor, substrate consumption rate and metabolic activity of bacteria are directly related to the electric current. A reduction in current under otherwise similar conditions is an indication of toxic inhibition. Under steady state conditions, current can be described by the Butler-Volmer-Monod (BVM) model. Knowing which parameters of this model change under toxic contamination can give an indication on the type of toxicity. The model requires that the substrate concentration is known. It is shown in this paper that the ideal to estimate both the substrate concentration as well as the BVM parameters on-line from current data at constant overpotential is not possible. However, it appears that substrate and substrate rate can be estimated on line, and that the BVM parameters can be estimated by linear regression from a polarization curve that is generated as soon as a suspect change in current occurs. An analysis shows that weighted least squares is necessary to secure a good fit at the overpotentials where current is most sensitive to changes in kinetic parameters. A protocol is provided for operation of the sensor and online for detection of toxicity and of the type of kinetic inhibition.

Keywords: online estimation; toxicity detection; microbial fuel cell; biosensor; least square estimation; linear regression

5.1 Introduction

Safe and clean drinking water is important for every person. The monitoring of water quality is therefore important. Sensors have been developed to monitor water quality both online and offline in surface water, water intake basins and in water distribution networks (see e.g. [1, 2]. A recently developed sensor is the microbial fuel cell (MFC)-based biosensor in which bacteria act as the sensor for toxic components in water [3, 4]. Bacteria in an MFC catalyze the degradation of organic material and electrons released in this degradation process are transferred to the anode in the sensor. The anode is electrically connected to a cathode where an electron acceptor is reduced. Current is a measure for the degradation rate of organic material and is directly proportional to the substrate consumption rate of bacteria [5]. Bacteria affected by toxic components in water have a lowered metabolism and hence a lower substrate consumption rate which shows as a decrease in current [3, 4].

Consuming substrate, bacteria release electrons to the anode and gain energy from the potential difference between the equilibrium redoxpotential of substrate and anode potential. This potential difference is called overpotential. The equilibrium redoxpotential of substrate at zero-current (also called open circuit potential, OCV) can be determined by the Nernst equation and the anode potential can be controlled using, for instance, a potentiostat and reference electrode. The overpotential is calculated according to:

$$\eta = E_{an} - E^{0} + \frac{RT}{nF}\ln\frac{[ox]}{[red]} \qquad [1]$$

Where η (V) is overpotential, E_{an} (V) anode potential, E^0 (V) standard potential of reaction, R (J/mol/K) gas constant, T (K) temperature, n the number of electrons released in the reaction, F (C/mol) is Faraday's constant, and [ox] and [red] (mol/l) are the concentration of the oxidized and reduced species of the redox couple, respectively.

Current depends on the overpotential and, under steady state conditions, is described by a polarization curve. A polarization curve can be described by the Butler-Volmer-Monod (BVM) model (Hamelers et al. 2011) given by:

$$I = Imax \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot e^{-\eta \cdot f} + \frac{Km}{S} + 1} \quad [2]$$

Where Imax (mA) is the maximum current determined by maximum enzymatic rates of microorganisms, K1 (-) is a lumped parameter describing the ratio between biochemical and electrochemical rate constants, K2 (-) is a lumped parameter describing the forward over backward biochemical rate constants, Km (mol/l) is substrate affinity constant and S (mol/l) substrate concentration. Furthermore, f= F/RT. This model is based on both electrochemical kinetics and biochemical kinetics of bacteria. The model can be extended to describe the effect of four types of enzyme inhibition kinetics distinguishing four types of toxicity each affecting one of the four kinetic parameters K1, K2, Km and Imax (Stein et al. 2011). In the extended model a toxicity term (Ki/(Ki+χi)) is introduced, with Ki the kinetic inhibition constant (mol/l) and χi the concentration of toxic component (mol/l). Depending on the mechanisms of kinetic inhibition the toxicity term can be introduced giving four models. The toxicity term, or its inverse, in short denoted by β_1 – β_4, is introduced at one of four places in the model which is then given by,

$$I = Imax \cdot \frac{1 - e^{-\eta \cdot f}}{\beta_1 \cdot K1 \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + \beta_2 \cdot K2 \cdot e^{-\eta \cdot f} + \frac{\beta_3 \cdot Km}{S} + 1} \cdot \beta_4 \quad [3]$$

The extended models are thus based on (2) combined with enzyme inhibition kinetic [6, 7].

Table 5.1 lists the four models and shows the associated values of β_i for each of the models.

Table 5.1: Overview of the different values of term $\beta_1 - \beta_4$ for the four extended model versions describing four types of toxicity.

constant / *Model*	β_1	β_2	β_3	β_4	*Affecting parameter*
Itox	1	1	1	Ki/(Ki+χi)	Imax
IK1	(Ki+χi)/Ki	1	1	1	K1
IK2	1	(Ki+χi)/Ki	1	1	K2
IKm	1	1	(Ki+χi)/Ki	1	Km

To be able to detect changes in current and thus in water quality, overpotential has to be controlled at a set value. Evaluation of the extended model gives the overpotential at which the sensor is most sensitive for toxic components depending on the type of toxicity and the value of the kinetic parameters Imax, K1, K2 and Km. The selection of the overpotential at which the cell is operated thus provides, in principle, a means to tune the sensor to to a specific type of toxic inhibition [7]. Changes in current indicate a change in water quality. If a change in current is detected, it is not known *a priori* which type of toxic effect occurs. To know more about the type of toxicity, it is necessary to determine which of the kinetic parameters has changed.

In the (extended) Butler-Volmer-Monod (BVM) models (eq 2 and 3), biochemical and electrochemical rates in the biofilm of the sensor are taken into account. Consequently, the substrate concentration in the biofilm (S in eq 2 and 3) is needed to determine the values of the kinetic parameters (Imax, K1, K2, and Km) using the BVM model and the measured current. The substrate concentration S in the biofilm is hard to determine. However, if diffusion is relatively fast compared to the substrate consumption rate then S in the biofilm is approximately equal to S in the bulk. Substrate concentration in the bulk is much easier to determine. To monitor changes online it is necessary to embed the BVM

model in a mass balanced based reactor model of the anode compartment of the MFC sensor.

If it is possible to determine which of the kinetic parameters change under toxic load, a statement can be made about the type of kinetic inhibition. It is conceivable to set up a database of toxic components that may be present in water and the type of kinetic inhibition they cause. When current changes while monitoring water and the type of kinetic inhibition is then determined it is possible to exclude many components based on their type of inhibition. This will narrow down the possible contaminants and may make the identification of the component quicker.

The aim of the paper is to investigate if it is possible to estimate the states and kinetic parameters of the sensor online in order to detect the type of toxicity when current changes. Based on state estimation theory and linear regression techniques, a protocol is provided for operation of the sensor and for online estimation of the sensor parameters.

5.2 Methods

MFC-based biosensors were constructed as in Heijne et al. [8]. Bacteria were grown and medium was supplied continuously with 0.7 ml/min giving a residence time of 47 min. Medium was as in [9] except that a buffer concentration of 20 mmol/l phosphate buffer was used. During start-up anode potential was controlled at -0.3V using a potentiostat/ galvanostat (Ivium, Netherlands). Polarization curves, when needed, were made by controlling anode potential at a set value for 10 minutes, measuring current every 10 sec. Current stabilized after approximately 7 minutes at the new anode potential. Average current was calculated from 11 datapoints at the end of the ten minute interval. Every 10 minutes anode potential was increased by 0.025V covering a range from -0.4 to -0.15 V. With nickel as a model toxic component, a step input of 20 mg/l or 30 mg/l was supplied to the medium and the polarization curve measurement started two hours after the start of dosing nickel. Dosing of nickel stopped about one hour after the polarization curve was finished. Open circuit potential was measured two hours after dosing had stopped to determine the zero-current equilibrium potential.

5.3 Theory

In this section some theory is explained that will be applied for the estimation of the substrate parameters and the kinetic parameters K1, K2 and Km in the MFC-based sensor. The parameter Imax is estimated separately from polarization curves. To estimate S online the theory of state estimation in linear-time invariant systems is employed, whereas the kinetic parameters are estimated by linear regression. Some relevant details of these methods are described briefly below.

5.3.1 State estimation: theory

A linear time invariant (LTI) dynamical system can be represented in the state space form

$$\frac{dx(t)}{dt} = Ax(t) + Bu(t)$$
$$y(t) = Cx(t) + Du(t) \qquad [4]$$

The variables x, called the states, are related to the outputs y (usually containing measurements) and the inputs u of the system by a set of first order differential and algebraic equations. In general, x, y, and u are vectors of appropriate dimensions. Given input- output data, the states in (4) can be estimated online and this is known as state estimation. However, the states can only be estimated when the system is observable or at least detectable. The formal definitions of observability and detectability are given in the following definitions:

Definition 1: [Observability]: The system is observable if there exists $t_1 < \infty$ such that $y(t; t_0, x_0, u) = y(t; t_0, x_0', u)$, $t_0 < t < t_1$ for all u(t), $t_0 \le t \le t_1$, implies that $x_0 = x_0'$.

It has been proven that the system is observable if and only if the n columns of observability matrix $O = \begin{bmatrix} C \\ CA \\ CA^2 \\ \dots \\ CA^{n-1} \end{bmatrix}$ are independent, with n the number of states, and thus matrix O has full rank [10].

Thus when a system as (4) is observable in theory it is possible to reconstruct the initial state and all present and past states from present measurements.

Definition 2: [Detectability]: A linear time invariant system is detectable if its unobservable subspace is contained in its stable subspace [10].
In other words, when a system is detectable, after some time the unobservable states become independent of the initial states and are only driven by the input of the system according to the model.

Some of the states may, in fact, represent model parameters. The expected model for these states is dx(t)/dt = 0, as parameters are assumed constant. However, to allow tracking of time variation in the model parameters, a more suitable model in practice is to assume that the rate of change is driven by a zero-mean unknown disturbance input:

$dx(t)/dt=\omega$ [5]

with ω being an unknown zero-mean disturbance vector. The value of ω is unknown but it is frequently assumed that the covariance matrix of ω is given by a diagonal pxp matrix Q (p being the number of parameters). Equation 5 is called a random walk model. The value of Q has to be chosen. It is common to assume that Q is a diagonal matrix. If the elements of Q are chosen large the parameter estimates are allowed to vary more and thus changes in the parameters can be detected easier, but it makes the estimate also more sensitive to measurement noise. Hence, given the parameter model (5), online parameter estimation can be put into a state estimation framework.

5.3.2 Linear regression: theory

If the detectability test described in the previous section fails, online estimation is not possible, but it may still be an option to estimate the states and parameters off-line. Offline estimation can be done by obtaining an additional dataset and afterwards fitting the data to a model. For the BVM this can be done by measuring a polarization curve, i.e. by varying η and measuring I. If the model can be cast in the form of a linear relationship between independent and dependent variables, linear regression can be applied. A linear regression model in vector-matrix form is given by,

$$y = \Phi \cdot \vartheta + e \quad [6]$$

y is the measured data and Φ the regressor matrix, an Nxp matrix with N the number of measurements and p the number of parameters. ϑ is the parameter vector and e the prediction error-vector. The value of the parameters can be uniquely identified if and only if matrix Φ has at least p rows and if all the columns are independent. This can be checked by using singular value decomposition of Φ , that is Φ =U*S*V^T with matrix S full rank [11]. Although matrix S may have full rank, in practice ϑ is only identifiable if the singular values in S are of similar order of magnitude.

Given (6), the least square estimate of ϑ is given by,

$$\hat{\vartheta} = (\Phi^T \cdot \Phi)^{-1} \cdot \Phi^T \cdot y \qquad [7]$$

with covariance matrix

$$Cov(\hat{\vartheta}) = \sigma_\varepsilon^2 (\Phi^T \cdot \Phi)^{-1} \qquad [8]$$

Furthermore, an estimate of the variance of the residuals (ε) is given by:

$$\sigma_\varepsilon^2 = \frac{1}{N-p} \sum_{t=1}^{N} \varepsilon^2(t) \qquad [9]$$

with N the number of measurements and p the number of parameters [11, 12].

5.4 Results and discussion

5.4.1 State estimation: analysis

The anodic compartment of the sensor contains the biofilm and the bulk of the sensor. When substrate enters the bulk of the sensor, it diffuses into the biofilm where it is consumed by the bacteria. The substrate concentration in the biofilm will quickly reach the concentration in the bulk of the anodic compartment if diffusion is fast. The diffusion time will be $t=L^2/D$ in which L is the thickness of the biofilm and is estimated to be maximum 100 micrometer. D is the diffusion coefficient and is approximately $10^{-8} – 10^{-9}$ as reported for acetate in biofilms [13-15]. The diffusion time will then be t= 1 to 10sec. This is considered much faster than changes that occur due to the effect of toxic events. Thence, the biofilm substrate concentration that is needed in the BVM- model (2) can be set equal to the bulk concentration.

A mass balance over the anode compartment in conjunction with a relation between substrate consumption rate and current yields the following dynamical model.

$$\frac{dS}{dt} = \frac{Q}{V} * (S_{in} - S) - \frac{r}{V} \quad [10]$$

$$I = n * F * CE * r \quad [11]$$

In which:

S= substrate concentration in the bulk/ biofilm (mol/l)

S_{in}= substrate concentration of influent (mol/l)

Q= flow (l/sec)

V= volume (l)

r = substrate consumption rate (mol/sec)

I= current (A)

n = electrons released per mol substrate (-)

F= Faradays constant (C/mol electrons)

CE= columbic efficiency (-) (measured current/ theoretical current)

Provided that the substrate concentration S is constant under non-toxic conditions, the substrate consumption rate r is constant as well, hence the appropriate model under non-toxic conditions is

$$\frac{dr}{dt} = 0 \quad [12]$$

When the bacteria get affected by a toxic component in the sensor, the biomass or biomass yield on substrate or the maximum substrate consumption rate, measured as Imax, changes. And thus the substrate consumption rate changes. To allow for fast changes in state r, dr(t)/dt=ω is assumed for online estimation.

Model equations (10)-(12) can be cast in formal state space form (4) by defining

$$x = \begin{bmatrix} r \\ S \end{bmatrix}$$

$$u = S_{in}$$

$$A = \begin{bmatrix} 0 & 0 \\ -1/V & -Q/V \end{bmatrix}$$

$$B = \begin{bmatrix} 0 \\ Q/V \end{bmatrix}$$

$$C = \begin{bmatrix} n * F * CE & 0 \end{bmatrix}$$

$$D = 0$$

To check if the states S and r can be estimated, the observability of the system has to be checked.

The observability matrix $O = \begin{bmatrix} n * F * CE & 0 \\ 0 & 0 \end{bmatrix}$ does not have full rank since there are two states and rank(O)=1 and thus the system is not observable. The unobservable state is the substrate concentration S. However, the eigenvalues of A are given by λ(A)=[0 ;–Q/V] and $\mathrm{Re}\,\lambda_i \leq 0$ which implies that the system with states r and S is stable. Consequently the system is detectable because the effect of the initial value of S becomes negligible when time increases and the value of S becomes dependent on the past input trajectory [10].

Thus although the states S and r are not observable directly they can be detected, provided CE is known. Under non-toxic conditions the estimates of S and r converge after a number of measurements. It is thus possible to estimate r and S from measurements of I, provided CE is known.

Parameter Imax will be estimated separately from polarization curves. To estimate the values of the kinetic parameters K1, K2 and Km online, however, a theoretical option would be to expand the model (10)-(12) with the BVM model. This would lead to a non-linear estimation problem with five states (S,r,K1,K2,Km). This is hard to solve, if at all possible. Therefore, it is an easier option to decompose the system into two sub- one that contains states related to the bulk conditions and allows estimating state r and S (10 to 12) and the second sub-system that describes the biofilm and thus contains the possibly time

varying kinetic parameters K1, K2 and Km (13). In linear, time-invariant state space form this second sub-system can be represented by

$$\frac{dK1}{dt} = \omega_1$$
$$\frac{dK2}{dt} = \omega_2$$
$$\frac{dKm}{dt} = \omega_3 \qquad [13]$$
$$\frac{Imax}{I} * (1 - e^{-\eta \cdot f}) - 1 = [e^{-(1-\alpha)\cdot\eta\cdot f} \quad e^{-\eta\cdot f} \quad \frac{1}{S}] * \begin{bmatrix} K1 \\ K2 \\ Km \end{bmatrix}$$

The output equation in (13) is the result of re-writing the BVM in linear form. Independent and dependent variables are now functions of the truly measured variables. This subsystem uses the inverse of the estimated substrate concentration S as a regression variable. From the previous observability/ detectability analysis, substrate concentration can in principle be estimated online using the first subsystem. Overpotential can be controlled. This gives a system with only the kinetic parameters K (K=[K1 K2 Km]^T) as unknown states. Consequently, given an on-line estimate of S, a next step is to evaluate the observability of the system presented in (13). The observability matrix is given by:

$$O = \begin{bmatrix} C \\ CA \\ CA^2 \end{bmatrix} = \begin{bmatrix} e^{-(1-\alpha)\cdot\eta\cdot f} & e^{-\eta\cdot f} & \frac{1}{S} \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}.$$

The rank of the observability matrix equals 1, while three states are unknown and marginally stable. Hence, it is not possible to estimate the kinetics parameters online under constant overpotential. To estimate, after a detected change in current, the time-varying parameter vector K=[K1 K2 Km]^T, a different measurement is required. When a polarization curve is made by varying the overpotential, it appears that K and the changes in K, can be estimated by linear regression, as shown in the next section.

5.4.2 Estimation of K using polarization curves: analysis

Direct estimation using equation 2 leads to a nonlinear estimation problem in terms of K1, K2 and Km. Fitting the data with nonlinear least-squares techniques can easily lead to local minima [16]. A more suitable option in this case is to re-parameterize the Butler Volmer Monod model to make it linear in its kinetic parameters K1, K2 and Km, as shown in (13). This becomes than a linear regression.

The linear regression and extended with an error term (e) can be represented in a general vector matrix form, as in (6), with

$$y = \left[\frac{Imax}{I}(1 - e^{-\eta_1 \cdot f}) - 1 \quad \ldots \quad \frac{Imax}{I}(1 - e^{-\eta_N \cdot f}) - 1 \right]^T$$ using the measured values of current I.

$$\Phi = \begin{bmatrix} e^{-(1-\alpha)\cdot\eta_1 \cdot f} & e^{-\eta_1 \cdot f} & \frac{1}{\hat{S}_1} \\ \ldots & \ldots & \ldots \\ e^{-(1-\alpha)\cdot\eta_N \cdot f} & e^{-\eta_N \cdot f} & \frac{1}{\hat{S}_N} \end{bmatrix}$$

With $\hat{S}$ the substrate concentration estimated from (10 to 12) and Φ an Nxp matrix with N the number of measurements and p the number of parameters (p=3).

$$\vartheta = \begin{bmatrix} K1 \\ K2 \\ Km \end{bmatrix}$$ the parameter vector.

The parameters are identifiable for $\alpha \neq 0$ and when measurements are available for at least three overpotentials η. Column one and two in Φ contain only a small error because the value of η is controlled with large precision using advanced equipment but column three contains the substrate concentration estimated from (10 to 12). So, strictly speaking, the problem is a so-called errors-in-variables regression problem. The errors in the variables will give a bias in the estimate. However, the goal is to detect changes in the kinetic parameters, not to know the exact values. Hence it was decided to disregard the errors in variables problem in the current application. One can also chose to keep the substrate

concentration much higher than the half-saturation concentration (S>>Km). In this way Km/S→ 0 and thus Km doesn't need to be estimated and current will not be very sensitive for changes in Km. For completeness, in this paper, the estimation of Km is maintained.

5.4.3 Experimental results

To test the linear regression, first a polarization curve was performed by measuring current at several overpotentials in a sensor without toxic chemicals (Figure 5.1). Parameter Imax is estimated directly from the data. Given the data of the polarization curve, values for K were estimated. Ordinary least square estimation (OLS) of the linear regression results in

$$\hat{K} = [\text{estimate} \quad \pm \text{standard deviation}]$$

$$\hat{K} = \begin{bmatrix} 3.1 & \pm 0.27 \\ 0.7 & \pm 0.32 \\ -0.45 & \pm 0.16 \end{bmatrix}$$. This shows that a reasonable fit is obtained but that the estimate of Km is negative. Hence, this cannot be interpreted physically as Km represents the substrate concentration at half maximum substrate consumption rate. Km is hard to estimate accurately because of the high substrate concentration.

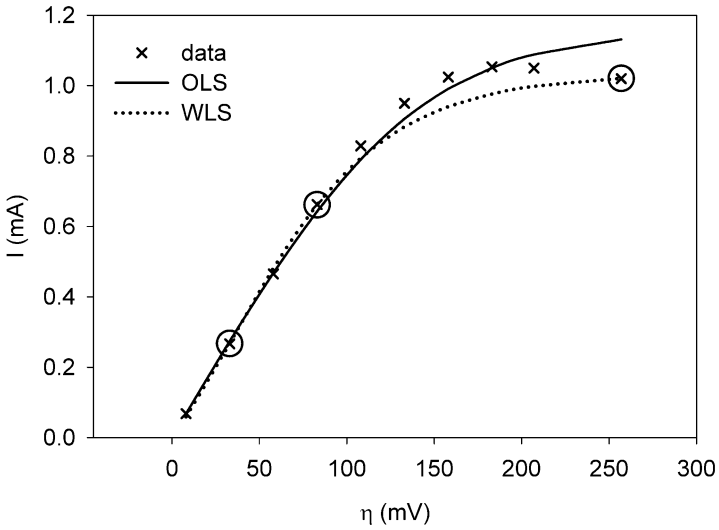


Figure 5.1: Data (x) from a polarization curve were used to estimate parameter values K by ordinary least square (OLS, solid line) and by weighted least squares (WLS, dotted line) obtained by giving enhanced weight to overpotentials where dI/dK is maximum (circles)

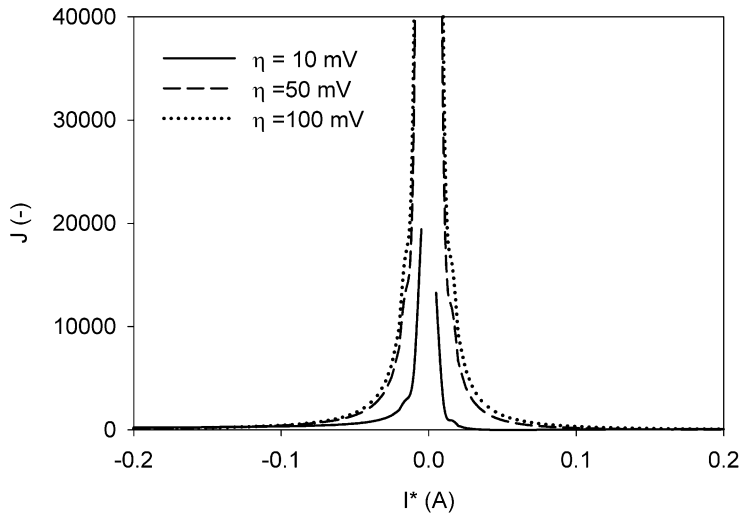
Estimating the values of K of the re-parameterized model (13) by an ordinary least square method leads to an output that is inversely proportional with current. The squared error of each measurement is given by

$$J_i = e_i^2 = \left(\frac{Imax}{I_i^*} \cdot (1 - e^{-\eta_i f}) - 1 - (K1 \cdot e^{-(1-\alpha)\eta_i f} + K2 \cdot e^{-\eta_i f} + \frac{Km}{S}) \right)^2 \qquad [15]$$

with I_i* the measured current, which is not necessarily equal to I (eq 2). It can be seen that it is not possible to estimate K accurately using small values of current because the squared error values at low overpotential will dominate the sum of squared errors. The squared error J_i as a function of I is shown in Figure 5.2A and the sensitivity of J for a change in K as a function of I is shown in Figure 5.2B. Notice from figure 5.2B that a change in K results in a major change of the squared error J_i near I*=0 mA, The fit of the curve will be very good near zero-overpotential because the sensitivity of J is very high

while the fit at higher overpotentials is poor. Furthermore, there is no feasible solution for η=0 V and I*=0 mA (Figure 5.2A-B).

A



B

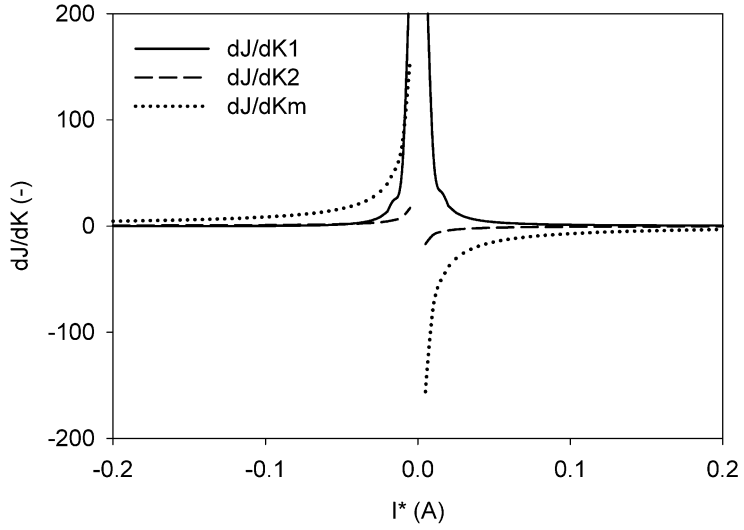


Figure 5.2: A: squared error of estimation J as a function of current. B: derivative of J to K1, K2 and Km.

To compensate for the large effects of small values of I in the squared error, in the next step a weighted least square (WLS) method is applied with weighing matrix W. This weighing matrix should favor measurement point away from overpotentials where I=0 and should put less weight on measurement points near I close to zero. The overpotentials where current is most sensitive for changes of K1, K2 ad Km should be weighted more heavily. The sensitivity of current for changes in kinetic parameter K at overpotential η, is given by $\frac{\partial I(\eta)}{\partial Kn}$ with index n= 1or 2 or m.

Using the data from the polarization curve (Figure 5.1) and the estimated values from the ordinary least squares, a selection of data points was made that should be given extra weight. The overpotentials where dI/dKn is maximal are given by:

$abs\left(\frac{\partial I(\eta)}{\partial K1}\right) \rightarrow \max$ η=81mV, $abs\left(\frac{\partial I(\eta)}{\partial K2}\right) \rightarrow \max$ η=39 mV, $abs\left(\frac{\partial I(\eta)}{\partial Km}\right) \rightarrow \max$ η=250 mV.

The overpotentials chosen in the experiments that are closest to the theoretical values above are represented by a circle in Figure 5.1 and are 34, 84 and 258 mV.

For the polarization curve with η =[9 34 59 84 109 134 159 184 208 258] the weighing matrix was chosen as follows:

$$W = diag\ (\eta_1 \quad \eta_2\left(\frac{\partial I}{\partial K2} \rightarrow \max\right)^2 \quad \eta_3 \quad \eta_4\left(\frac{\partial I}{\partial K1} \rightarrow \max\right)^2 \quad \eta_5 \ldots$$

$$\ldots \eta_6 \quad \eta_7 \quad \eta_8 \quad \eta_9 \quad \eta_{10}\left(\frac{\partial I}{\partial Km} \rightarrow \max\right)^2 \quad)$$

where operation diag(.) forms a diagonal matrix from the given vector and the index represents the measurement-number.

The weighted least square estimates are given by $\hat{K}_W = \begin{bmatrix} 1.93 & \pm 1.06 \\ 2.76 & \pm 1.26 \\ 0.086 & \pm 0.63 \end{bmatrix}$, with all estimated values positive and very good fit for η equals 34, 84 and 258 mV.

Using the data from the polarization curve (Figure 5.1) and the estimated values from the weight least squares, the overpotentials at which $abs\left(\frac{\partial I(\eta)}{\partial Kn}\right) \rightarrow \max$ was determined again and found to be $abs\left(\frac{\partial I(\eta)}{\partial K_w 1}\right) \rightarrow \max$ η=78 mV, $abs\left(\frac{\partial I(\eta)}{\partial K_w 2}\right) \rightarrow \max$ η=45 mV, $abs\left(\frac{\partial I(\eta)}{\partial K_w m}\right) \rightarrow \max$ η=250 mV. This still leads to the same weighing matrix and thus the same values for $\hat{K}_w$.

5.4.4 Optimizing the polarization curve set-up

When a polarization curve is made to estimate the values of K after a change in current has been detected, for an efficient experimental design, the time needed for a polarization curve should be as short as possible while predicting accurate estimates of K. It may not be necessary to obtain polarization data for a large number of overpotentials just to estimate values of K. There are three unknown parameters that should be estimated and the minimum amount of overpotentials that need to be considered is therefore three. K can be estimated most accurately when $abs\left(\frac{\partial I(\eta)}{\partial Kn}\right) \rightarrow \max$ with index n= 1or 2 or m. The sensitivity dI(η)/dKn as a function of η normalized by multiplication with the nominal values for $\bar{K}$ and $\bar{I}$ =1 mA, is shown in Figure 5.3 using the data from the polarization curve and the estimated values for K from the weight least squares. From this figure it can be seen that at low overpotentials, current does hardly change when K changes. It is thus not possible to estimate the value of K at low overpotentials showing again the need for weighted least squares-estimation. Current changes most due to changes in K1 at 78 mV overpotential and current changes most due to changes in K2 at 45 mV overpotential. Therefore K1 and K2 can be estimated most accurately when a polarization curve is made covering at least these two overpotentials. Km can be estimated best at high overpotentials (250 mV) but at these overpotentials K1

and K2 cannot be estimated accurately. The sensitivities of current for a change of K, however, are very small at low current (See Figure 5.3).

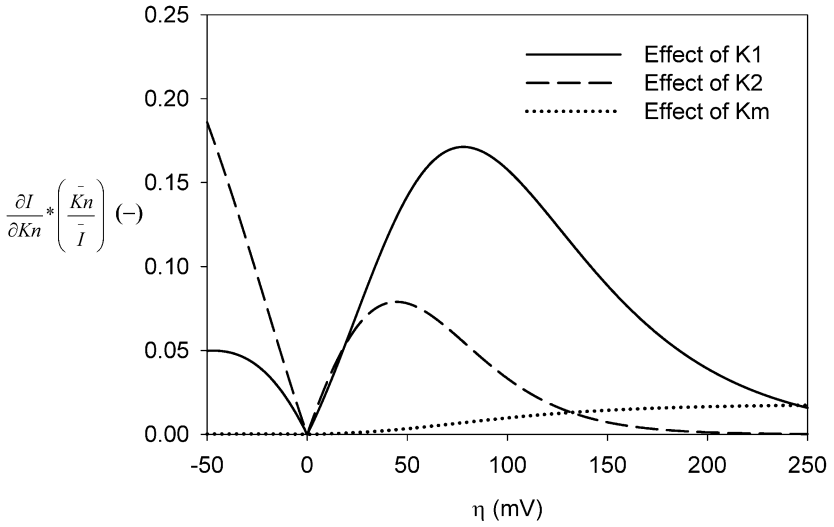


Figure 5.3: dI/dKn*(Kn/I) with index n= 1or 2 or m as a function of overpotential η

5.4.5 Effect of nickel on the sensor

A polarization curve was made without toxic components in the sensor. (Figure 5.1). Nickel was then dosed and a new polarization curve was made (see Figure 5.4). The values of K are estimated during the toxic situation (20 mg/l Ni) using WLS and this gives the following estimated values: $\hat{K}_1$ = -0.09, $\hat{K}_2$ = 4.02, $\hat{K}_m$ = 0.035 mmol/l.

In stead of fitting the data to the BVM-model, it can be fit to the extended BVM models (equation 3 and Table 5.1). In that case, the values of K as estimated from the clean conditions, still apply and the value for the toxicity term β can be estimated for each of the four extended models. As the fit to model Itox (Table 5.1) gives the smallest error compared to the other models available ($\Sigma J = 0.09$ for Ki=31.6 mg/l) it may be assumed that nickel has an effect on the whole organism.

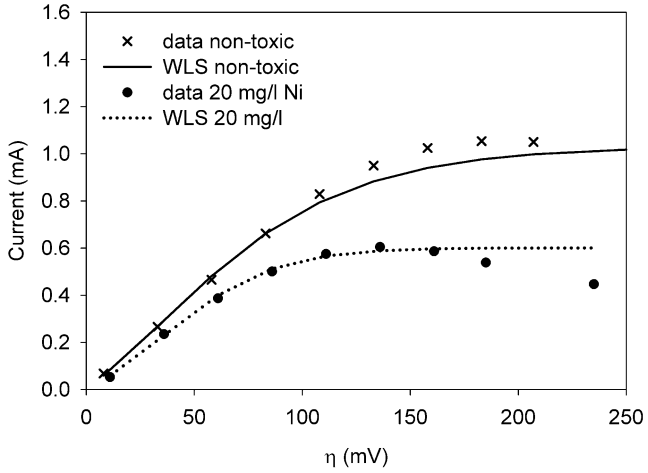


Figure 5.4: polarization curve of a clean MFC-based biosensor (crosses) and when nickel is present in the system (dots). Parameter values were estimated to be K1=1.93, K2= 2.76 and Km= 0.086 mmol/l under clean conditions and K1=-0.09, K2= 4.02 and Km=0.035 mmol/l when 20 mg/l nickel is present.

5.4.6 Effect of Columbic efficiency

Notice from the previous section that substrate concentration and substrate consumption rate can be estimated as a function of time if columbic efficiency is known and assumed constant (eq 9). This assumption may, however, not be correct. At the moment a toxic component enters the system and affects for instance the cell membrane, it is possible that the efficiency of the transfer of electrons drops. Although it is useful to have an online estimate of the substrate concentration, in this paper it is mostly used to estimate the kinetic parameters of the Butler-Volmer-Monod model and with that to estimate the type of toxicity present in the system. Hence, accurate on-line estimates of the substrate concentration are required.

In our experiments (Figure 5.1 and 5.4) an MFC-based biosensor was used under constant anode potential and non-toxic conditions. If CE is assumed to be 0.95, the estimated substrate concentration in this experiment was 4.89 mmol/l. Recall that from the polarization curve (figure 5.1 and 5.4) the following parameter values for K were

estimated using weighted least square estimation: $\hat{K}_1$ = 1.93, $\hat{K}_2$ =2.76, $\hat{K}_m$ = 0.086 mmol/l.

After dosing nickel, a new polarization curve was made (see Figure 5.4). If it is assumed that the substrate concentration and columbic efficiency did not change with respect to the non-toxic conditions and were constant over the time of the polarization curve, the weighted estimated values for K, during the toxic situation (20 mg/l Ni) are given by

$\hat{K}_1$ = -0.09, $\hat{K}_2$ = 4.02, $\hat{K}_m$ = 0.035 mmol/l.

However, when it is assumed that in the time before the polarization curve was made, the current already dropped down to 0.575 mA and columbic efficiency had dropped to 0.05, substrate concentration is then estimated to be 3.72mmol/l. Using this CE and substrate concentration, new values for $\hat{K}$ are found and given by: $\hat{K}_1$ =-0.09, $\hat{K}_2$ = 4.02, $\hat{K}_m$ =0.026. Notice from equation 10 that the substrate concentration only has a direct effect on the estimate of Km. The ratio of Km/S gives the same value in both cases: 0.035/4.89=0.026/3.72=0.0071. The kinetic parameters K1 and K2, however, are also changed compared to the non-toxic conditions. Thus although the estimate of Km may not be very accurate, it can still be concluded that nickel has an effect on the whole organism. This implies that, although the assumption of a constant CE may not be correct, the conclusion about the type of toxicity is still the same. It is therefore valid to assume CE constant.

5.5 Protocol

Let us summarize what has been obtained so far. To detect changes in water quality, an MFC-based biosensor can be used. Current is measured under constant potential and substrate concentration and substrate consumption rate can be estimated. Changes in current indicate a change in the substrate consumption rate, changed columbic efficiency or a combination of these. To get a better idea about the type of toxic component present in the system, as introduced by Stein et al. (2011), the kinetic values K1, K2 and Km have to be determined which can be done by making a polarization curve.

A protocol for the online use of the sensor might be as follows (Figure 5.5):

- Start measuring current under clean condition (I) and make a polarization curve (II).
- Determine the values of Imax and the kinetic parameters K1, K2 and Km and determine the overpotential where current is most sensitive to changes in K1, K2 or Km.
- Measure current as a function of time and estimate substrate consumption rate and substrate concentration online (III).
- When a change in current is observed, this is an indication that a toxic component is present in the system. A polarization curve should be made again (IV) and the values of the kinetic constants can be determined again. This polarization curve should be short but include the overpotentials where current is most sensitive to changes in K, as determined after the full polarization curve under clean conditions.
- Fitting the data to the four models will give according to what type of toxicity the component is acting.

The type of toxic component can be determined by fitting the data to the four modified models of the BVM-model (3).

If a database is present with information on how components act according to which type of toxicity and with corresponding parameter Ki, immediately a selection of components can be made based on the type of toxicity that is found during the measurements with corresponding estimate of the concentration χi. The work in the paper gives a starting point for making the database.

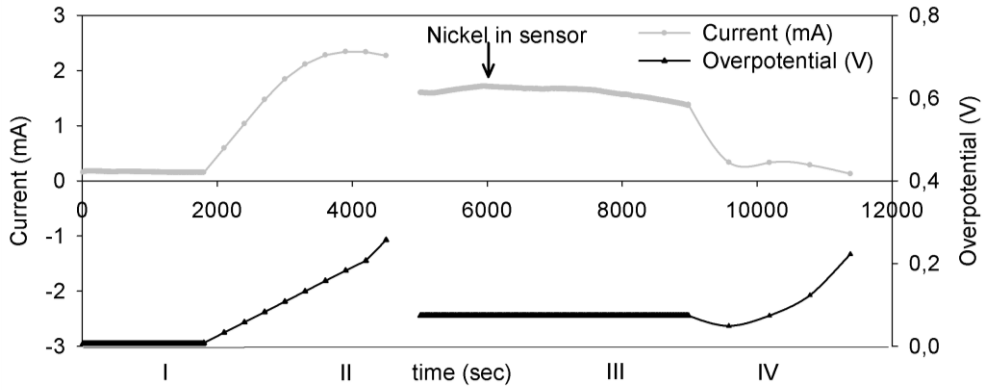


Figure 5.5: online use of an MFC-based biosensor. At t=7500 sec, 30 mg/l nickel starts to enter the sensor.

5.6 Conclusions

It is possible to measure current and to detect online changes in water quality as a change in the current. However, it is not possible to estimate the kinetic parameters K1, K2 and Km in the BVM-model online at a fixed overpotential. To determine the type of toxicity, a polarization curve should be made under clean conditions and as soon as a change in the current is observed. Finally a protocol for measuring current and estimating parameters (K1, K2 and Km) and states (substrate concentration and substrate consumption rate) has been presented for a prototype MFC-based biosensor for toxicity detection with intelligence. It is conceivable to create a database containing many toxic components and their kinetic inhibition type and the value of Ki. The type of toxicity detected can then be coupled to the database, narrowing down the amount of possibly contaminating components.

Acknowledgements:

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Chapter 6

Effect of toxic components on microbial fuel cell-polarization curves

6. Effect of toxic components on microbial fuel cell-polarization curves

6.1 Introduction

In a microbial fuel cell bacteria oxidize organic material to carbon dioxide, protons and electrons at an anode. An electron acceptor at the cathode is reduced so that the electrons flow through the electrical circuit and thus produce an electrical current.

The microbial fuel cell (MFC) has many applications such as a source for electricity production or to use it as a sensor for toxic components in water [1-3]. The MFC-based biosensor produces a constant current under constant conditions. However, when a toxic component enters the sensor, the bacteria are affected by the toxic component, resulting in a change of current. This change is often a decrease [2, 4].

As an important tool in research on MFCs the cells are characterized using polarization curves. Anode potential is varied and the resulting current gives information related to the energetic losses in the system and the metabolic state of the bacteria under the applied conditions.

Models were developed to describe polarization curves of the MFC under nontoxic conditions, called the Butler Volmer Monod (BVM) model and under toxic conditions [5, 6]. These models are based on biochemical and electrochemical kinetics. The models adapted to describe the toxic conditions are also based on enzyme inhibition kinetics. Theoretically it is possible to distinguish between four types of toxic inhibitions.

1. Overall inhibition of the bacteria. The toxic component acts as an irreversible inhibitor. This gives the following model

$$I = I_{max} \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot e^{-\eta \cdot f} + \frac{Km}{S} + 1} \cdot \frac{Ki}{Ki + \chi i} \qquad [1]$$

This model is referred to as “Itox”.

2. Inhibitions that affect the ratio between biochemical and electrochemical rate constant, e.g. when the component acts as an electron acceptor. This gives the following model:

$$I = I_{max} \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot \frac{Ki + \chi i}{Ki} \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot e^{-\eta \cdot f} + \frac{Km}{S} + 1} \quad [2]$$

This model is referred to as “IK1”.

3. Inhibition effect on the ratio between the forward and backward reaction rate constant of substrate oxidation. This gives the following model:

$$I = I_{max} \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot \frac{Ki + \chi i}{Ki} \cdot e^{-\eta \cdot f} + \frac{Km}{S} + 1} \quad [3]$$

and is referred to as ‘IK2’

4. Competition between substrate and toxic component to bind to the redox complex.

$$I = I_{max} \cdot \frac{1 - e^{-\eta \cdot f}}{K1 \cdot e^{-(1-\alpha)\cdot\eta \cdot f} + K2 \cdot e^{-\eta \cdot f} + \frac{Km \cdot \frac{Ki + \chi i}{Ki}}{S} + 1} \quad [4]$$

and is referred to as ‘IKm’.

In these models I (mA) is the current, Imax (mA) is the maximum current determined by maximum enzymatic rates of microorganisms, η (V) is overpotential, K1 (-) is a lumped parameter describing the ratio between biochemical and electrochemical rate constants, K2 (-) is a lumped parameter describing the forward over backward biochemical rate constants, Km (mol/l) is substrate affinity constant, and S (mol/l) substrate concentration. Furthermore, f= F/RT with F (C/mol) Faraday’s constant, R (J/mol/K) the gas constant and T (K) temperature. Ki is the inhibition constant of component I and χi is the concentration of toxic component i.

Ki is the inhibition constant and the value shows how toxic the component is for the bacteria and thus how sensitive the sensor is for the toxic component. A low value for Ki gives a very sensitive sensor.

For each of these inhibition mechanisms the polarization curve looks different. For each of the mechanisms it is possible to determine at which overpotential current changes most when a toxic component enters the cell [6]. In practice, however, it may not be one type of inhibition or the other. Combinations of inhibitions may be possible and the concentration of toxic component may also play a role in determining the type of inhibition. No data on polarization curves in the presence of toxic components are available in literature yet. We investigate therefore, in this chapter, whether it is possible to fit one of the models to the polarization curves when toxic components are present in the system. Furthermore we study if it is possible to distinguish between different types of toxic components based on the different enzyme inhibition kinetics.

First, polarization curves under non-toxic conditions and under toxic conditions with four components at three different concentrations were experimentally determined. These polarization curves were then compared with the model responses (1-4) describing the four types of toxic inhibitions.

6.2 Material and methods

6.2.1 Experimental

Microbial fuel cells were constructed as in Heijne et al. [7] and medium was used as in Stein et al. [4]. The medium was purged with nitrogen to keep it anaerobic and the continuous flow was 0.7 ml/min. Microbial fuel cells were operated at a constant anode potential of 0.3V. For polarization curves, the anode potential was increased stepwise with 0.025V every ten minutes from -0.4V to -0.15V. Current was measured every ten seconds. Average current of the last 10 data points per potential was calculated and used in the simulations. Open circuit potential was measured approximately two hours after the polarization curve was made.

To measure the influence of toxic components, the component was added to the medium in the wanted concentration and medium was continuously supplied at least two hours (>3 HRT) before the polarization curve was made. The following components were added: nickelchloride (10, 20, 30 mg/l nickel), sodiumdodecylsulfate (10, 25, 50 mg/l), bentazon (1 and 3 mg/l) and potassium ferricyanide (0.5, 1, 2 mM).

6.2.2 Estimating the type of kinetic inhibition

Given the experimental data of the polarization curves, the BVM models were subsequently fitted to the data to determine the values of kinetic parameters K1, K2 and Km and the type of toxicity. The curve under clean conditions was used to determine the values of K1, K2 and Km. This fitting can be done by using linear regression techniques [8, 9]. Notice that the BVM-model can be written linear in its kinetic parameters [10, 11], as

$$\frac{Imax}{I} * (1 - e^{-\eta \cdot f}) - 1 = [e^{-(1-\alpha)\cdot\eta\cdot f} \quad e^{-\eta\cdot f} \quad \frac{1}{S}] * \begin{bmatrix} K1 \\ K2 \\ Km \end{bmatrix} \qquad [5]$$

After writing (5) in the general linear regression form

$$y = \Phi\, \vartheta + e \qquad [6]$$

in which the error term e is added represent measurement and model errors, the ordinary least square estimate ϑ is given by

$$\hat{\vartheta} = (\Phi^T \Phi)^{-1} \Phi^T y \qquad [7]$$

with $\hat{\vartheta}$ the estimate of [K1 K2 Km]^T. The Nx3-dimensional regressor matrix

$$\Phi = \begin{bmatrix} e^{-(1-\alpha)\cdot\eta_1\cdot f} & e^{-\eta_1\cdot f} & \frac{1}{\hat{S}_1} \\ \dots & \dots & \dots \\ e^{-(1-\alpha)\cdot\eta_N\cdot f} & e^{-\eta_N\cdot f} & \frac{1}{\hat{S}_N} \end{bmatrix}$$ containing the N values of the overpotential at which the current is measured and the substrate concentration S in the biofilm.

$y = \left[\frac{Imax}{I^*}(1 - e^{-\eta_1\cdot f}) - 1 \quad \dots \quad \frac{Imax}{I^*}(1 - e^{-\eta_N\cdot f}) - 1 \right]^T$ containing the measured current (I*) at each overpotential.

In what follows, S is assumed to be constant. Notice from chapter 5 that, if the columbic efficiency is known, S can be estimated online from measurements of the electrical current. Alternatively, the concentration can also be measured in the bulk of the sensor under the assumption that the substrate concentration in the bulk and in the biofilm are equal.

A weighted least squares, as in chapter 5, is used to estimate K1, K2 and Km, where the estimate is given by:

$$\hat{\vartheta}_W = (\Phi^T W \Phi)^{-1} \Phi^T W y \quad [8]$$

With W the weighing matrix.

Subsequently, the four extended BVM models (2-5) were fitted to the data by estimating one at the time each of the Ki, using the estimates of Imax, K1, K2 and Km of the clean curve, and the known concentration of toxic component χi. However, this best fit is not equally good for each of the four types of toxicity, resulting into different values for the sum of squared errors (J_N). The sum of squared errors for each of the fitted models is used as a criteria to judge the quality of the fit.

$$J_{N\,J} = \sum_{k=1}^{N} \varepsilon_k^{\,2} = \sum_{k=1}^{N} (I_{meas\,k} - \hat{I}_k)^2 \quad [9]$$

With N the number of measurements, $\hat{I}_{meas\,k}$ the k-th measurement of the current and $\hat{I}_k$ the estimated current at measurement index k using the estimated parameter values of K1, K2 and Km. Index j denotes the model type (Itox, IK1, IK2, or IKm) with the corresponding equation to calculate the estimated current.

For example, for model Itox this equation looks as follows:

$$J_{N\,Itox} = \sum_{k=1}^{N} \varepsilon_k^{\,2} = \sum_{k=1}^{N} \left(I_{meas\,k} - I_{\max} \cdot \frac{1 - e^{-\eta_k \cdot f}}{K1 \cdot e^{-(1-\alpha)\cdot\eta_k \cdot f} + K2 \cdot e^{-\eta_k \cdot f} + \frac{Km}{S_k} + 1} \cdot \frac{Ki}{Ki + \chi i} \right)^2 \quad [10]$$

A smaller J_N means a better fit to the data.

6.3 Results and discussion

6.3.1 Polarization curves

Polarization curves were made under clean conditions and were also made when a toxic component is present in the sensor. Through experiments we investigated in a systematic way what the effect of toxic components, nickel chloride (nickel), sodium dodecyl sulfate (SDS), bentazon and potassium ferricyanide, is on the current at different overpotentials.

The effect of the concentration is also investigated. These four components were chosen because they are very different types of toxic components. Nickel is a heavy metal, SDS is used in soaps as a surfactant, bentazon is an herbicide acting on photosynthetic activity and ferricyanide is very fast electron acceptor.

6.3.2 Effect of Nickel

Nickel was dosed at three concentrations: 10, 20 and 30 mg/l. Data are shown in Figure 6.1. Comparing the polarization curve when nickel is present with the curve under non-toxic conditions, the curve seems to shift down when nickel is present in the sensor. The higher the concentration the larger the shift. Hence, there seems to be a dose-response relationship of current change to nickel concentration. This was already found for experiments in the MFC-based biosensor at constant anode potential [4] but it is thus also found from the polarization curves.

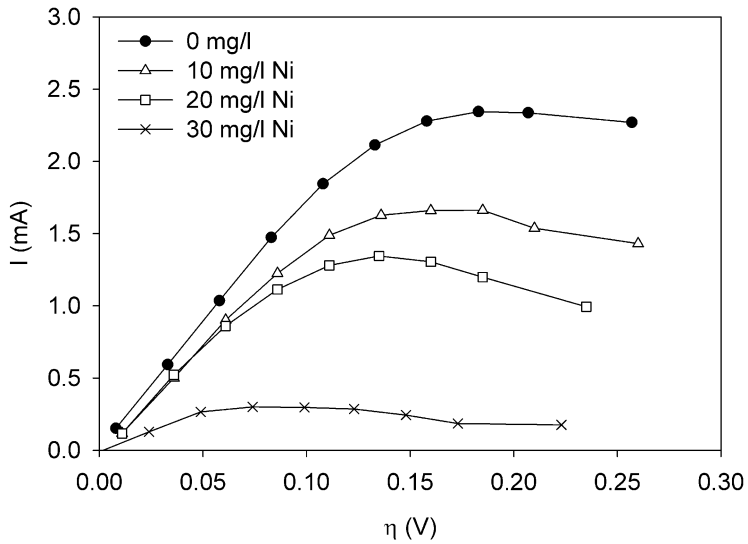

Figure 6.1: polarization curves in the presence of nickel.

6.3.3 Effect of sodium dodecyl sulfate

Notice from Figure 6.2 that a concentration of 10 or 25 mg/l SDS did not show a strong effect on the bacteria and the polarization curve is more or less the same as under non-toxic conditions. When the concentration was increased to 50 mg/l, however, the curve shifts down and a lower current is observed at all overpotentials.

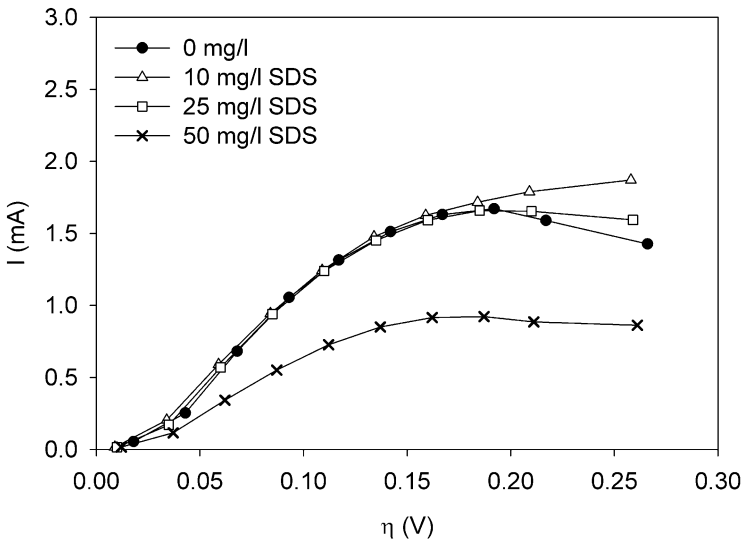

Figure 6.2: Polarization curve in the presence of sodium dodecyl sulfate (SDS)

6.3.4 Effect of bentazon

Bentazon was added to the sensor at a concentration of 1 and 3 mg/l. In Figure 6.3 it can be seen that at all overpotentials the polarization curve with 1 mg/l bentazon is lower than the polarization curve without any toxic components. The curve related to 3 mg/l bentazon has shifted to the left compared to the curve without any bentazon present and reaches a lower maximum than the clean curve. This gives a higher current at low overpotentials and a lower current at overpotentials above 0.1V.

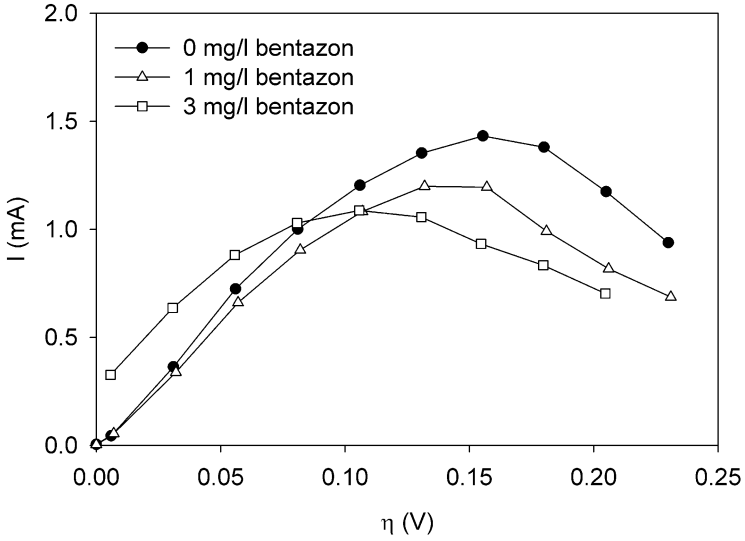

Figure 6.3: Effect of bentazon on the current

6.3.5 Effect of Ferricyanide

Ferricyanide is a negative ioncomplex ($FeCN_6^{3-}$) that can take up one electron to form ferrocyanide ($FeCN_6^{4-}$). Ferri/ferrocyanide is known to be a very fast electron couple. It is frequently used at the cathode in research on MFC's, as to prevent the electron uptake being the rate limiting step [1, 12]. When ferricyanide is present in the anode compartment, it is expected that it will take up electrons from the bacteria. Hence, it could act as an electron shuttle, or act as the final electron acceptor. If it acts an an electron shuttle it takes up electrons from the bacteria, forming ferrocyanide, and then donate the electron to the anode forming ferricyanide again. The standard electron potential of the couple $FeCN_6^{3-}$/ $FeCN_6^{4-}$ is E_0=+0.14V vs. a silver/silver chloride reference

electrode. The equilibrium potential of the bacteria is around -0.46V vs. Ag/AgCl. The bacteria can thus donate the electron to ferricyanide which is then oxidized. When making the polarization curve, the anode potential is varied between -0.45V and -0.150V, corresponding to 0V to 0.25V overpotential, and thus ferrocyanide can be reduced at the anode for the whole range of the polarization curve. Ferricyanide is used in several applications as electron shuttle/ mediator (e.g. in a BOD sensor [13, 14]) and is considered nearly non-toxic [13, 15]. It also happens that some of the oxidized form is washed out of the compartment before it is reduced at the anode. Then it acts as a final electron acceptor and, in the MFC, is thus lowering the measured current.

Ferricyanide was dosed to the sensor at concentrations of 0.5 mM, 1 mM and 2mM.

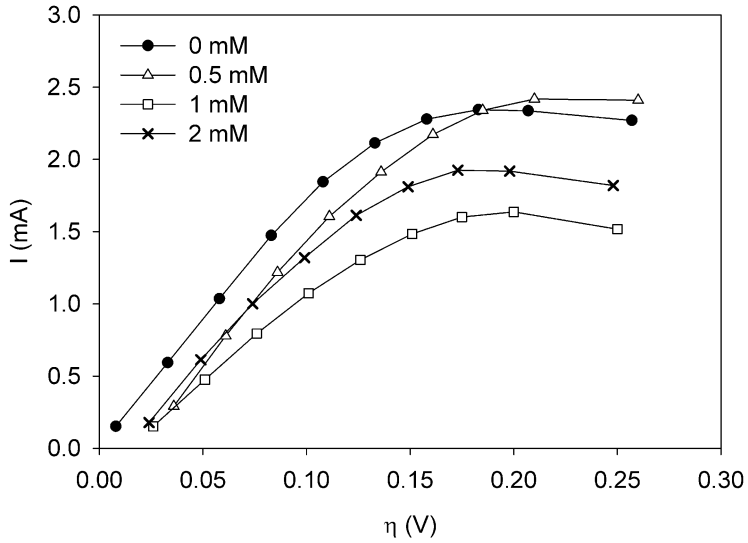

Figure 6.4: polarization curve in the presence of ferricyanide ($FeCN_6^{3-}$)

As can be seen from Figure 6.4, the current decreases due to the presence of ferricyanide. The decrease of current was larger when 1 mM was dosed then when 2 mM was dosed. The polarization curve when 0.5 mM was present is lower for overpotentials below 0.175V overpotential than the polarization curve when no toxic component was present.

6.3.6 Fitting the model to nickel-data

First, the BVM model ($\chi i=0$) was fit to the polarization curve without toxic component. The following estimates for K1, K2 and Km were found using weighted least squares techniques:

$$\begin{bmatrix} K1 \\ K2 \\ Km \end{bmatrix} = \begin{bmatrix} 1.93 & \pm 1.06 \\ 2.76 & \pm 1.26 \\ 0.086 & \pm 0.63 \end{bmatrix}$$

The models were then fitted to the polarization curve made with 20 mg/l nickel in the sensor. The value of Km cannot be estimated accurately because S>>Km and this results in Km/S→0. Fitting the data to model IKm will therefore result in inaccurate estimations of Ki. For the three remaining types of toxicity, the following values were found (Table 6.1):

Table 6.1: fitting of data of polarization curve with 20 mg/l Ni to three extended BVM model

toxtype	*Ki (mg/l)*	*J_N (mA^2)*	*Factor J_N*
Itox	**31.68**	**0.30**	**1**
IK1	14.72	3.53	11.87
IK2	14.87	3.70	12.44

The sum of squared errors (J_N) shows that model Itox gives the best result. The fits are shown in Figure 6.5. The fits for model IK1 and IK2 are not very good, while the fit for model Itox is much better. The factor J_N (last column table 6.1) shows the normalized value of the sum of squared errors to the sum of squared errors of Itox (J_{N_model}/ J_{N_Itox}).

When the model is fit to the data for 10 mg/l nickel, a similar result was found. The sum of squared errors is lowest for model Itox and the estimated value for Ki was similar: 30.1 mg/l. With 30 mg/l nickel in the sensor model Itox also gives the best fit. The value for Ki was estimated to be 4.57 mg/l. The value of Ki is expected to be constant because it should be intrinsic to the bacteria. A changing Ki with changing concentration of toxic component may imply that the toxicity term Ki/(Ki+χi) is not yet the right term. All results can be found in Table 6.3A-3C in the supplemental data.

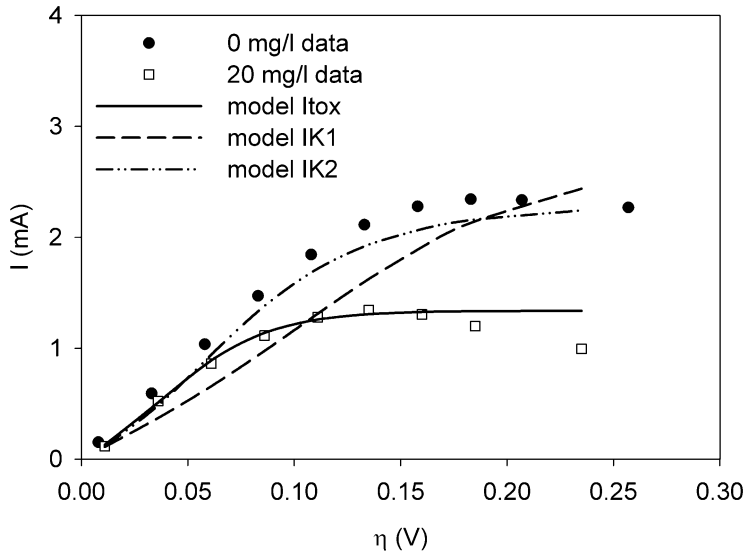

Figure 6.5: Polarization curves for a clean sensor (dots) and with 20 mg/l Ni present (squares). The data for 20 mg/l Ni were used for fitting according to model Itox (solid line), IK1 (dash), and IK2 (dot-dash-dot).

6.3.7 Fitting the model to SDS-data

The four models were fit to the polarization curve made with 50 mg/l SDS in the sensor (data in Figure 6.2). The best fit was obtained for model IK1 but this gives a negative value for Ki which cannot be interpreted physically. The fit for model IK2 is not significantly worse, only a factor 1.5 and the fit for model Itox is only 2 times worse than for model IK1. In this case there is no significant difference between the fit of the three models using weighted least squares estimation, and it is therefore hard to say according to which inhibition type SDS acts upon the bacteria. Furthermore, using ordinary least squares estimation, model Itox gave a significantly better fit than model IK1 and IK2. For model Itox, the calculated value for Ki was found to be 85.37mg/l. This explains the very small change at lower concentrations of SDS in the sensor. Hence, the bacteria are not very sensitive to SDS. The calculations for all models can be found in Table 6.4 in the supplemental data.

6.3.8 Fitting the model to Bentazon-data

Fitting the models to the data in Figure 6.3, model Itox gave the best fit. When 1 mg/l is dosed the difference between the sums of squared errors between the three models is not very large. Model Itox gives a J_N of 0.32 mA^2. The estimated Ki is 3.0 mg/l when model Itox is fitted. When 3 mg/l is dosed it is clear that model Itox fits best with a sum of squared errors of 0.63 mA^2. The calculations for all models can be found in Table 6.5A-5B in the supplemental data.

6.3.8 Fitting the model to ferricyanide-data-

Polarization curves with ferricyanide present were compared to the curve when no toxic chemical was present. The models were fit using weighted least square estimations and the sum of squared errors (J_N) determined. J_N was smallest when the curve was fit to model IK2 when 0.5 mM $FeCN_6^{3-}$ was present while J_N was smallest when fitting to model Itox when 2 mM $FeCN_6^{3-}$ was present (see Table 6.2).

Table 6.2: Fit to polarization curves with potassium ferricyanide in the sensor

toxtype	*Ki (mM)*	*J_N (mA^2)*	*Factor J_N*
Itox (0.5 mM)	48.44	0.46	3.73
IK2 (0.5 mM)	0.11	0.12	1
Itox (2 mM)	10.26	0.11	1
IK2 (2 mM)	0.51	0.39	3.54

At 0.5 mM $FeCN_6^{3-}$ the fit of model IK2 is 3.73 times better than model Itox. When the concentration is increased model Itox fits better. The factor is more or less the same. This means that model IK2 does not fit significantly better at 0.5 mM than model Itox at 2 mM. Thus if the fits are equally good, also seen from the similar value of J_N, there is a change in the mode of inhibition of ferricyanide at increasing concentration.

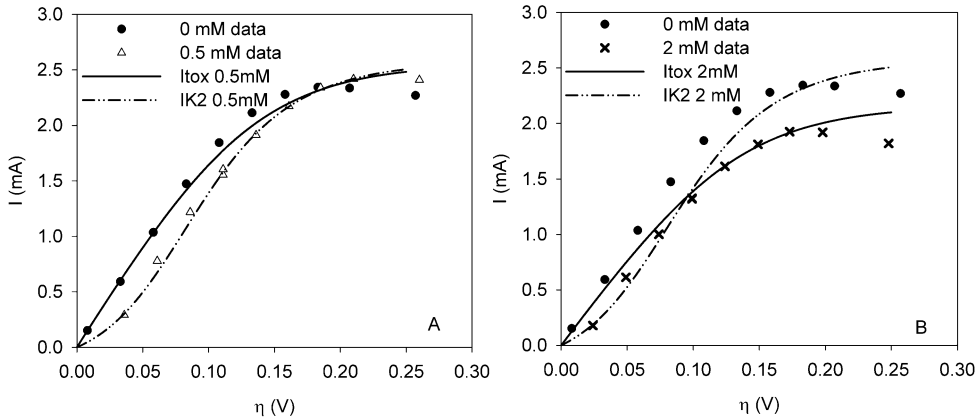

Figure 6.6: polarization curves under clean conditions (dot) and when ferricyanide is present in the sensor A: 0.5 mM (trangle) or B:2 mM (cross). Model Itox (solid line) and model IK2 (dot-dash-dot) were fitted to data from the curves with ferricyanide.

If ferricyanide acts as an electron shuttle, it is expected to inhibit the sensor according to model IK1 because parameter K1 describes the ratio between the electrochemical and biochemical rate constants. An electron shuttle could affect this ratio because part of the redox complex is not fully oxidized at the anode but partly at the toxic component and hence current decreases. However, this was not found from the analysis of the curves. The sum of squared errors is 0.65mA^2 at 0.5 mM and 1.09 mA^2 at 2 mM. The calculations for all models can be found in Table 6.6A-6C in the supplemental data.

6.3.10 Comparing the effects of toxic components

For nickel, SDS and bentazon, it was found that model Itox gives the best fit. Ferricyanide fits best to model IK2 or Itox, depending on the concentration. It thus seems that we can distinguish according to which type of toxicity a component acts on bacteria. Measuring more components will result in a better overview for different types of toxicity. In total nine polarization curves of sensors containing toxic components were fitted to the extended BVM models. Seven of these polarization curves fit best to model Itox while one ($FeCN_6^{3-}$ 0.5 mM) fits best to model IK2 and one cannot be clearly distinguished (SDS, 50 mg/l). It is interesting to see whether model Itox gives the best fit with many other

components as well. The models are based on enzyme inhibition kinetics and combined effects may also play a role. This has not been taken into account yet with the models used. However, the models used already give good fits in most experiments.

6.4 Conclusions

From experimentally determined polarization curves, it is clear that toxic components have an effect on the bacteria in the cell, seen as a shift in the polarization curve. The way the polarization curve changes, depends on the type of components and the concentration. There seems to be a dose-response relationship for all the tested components, meaning that current decreases more when the concentration of toxic component is higher. As seen for nickel and SDS, a higher concentration leads to a lower current at all overpotentials.

It was possible to fit the extended BVM models (1-4) to the polarization curves related to all the components used. Comparing the sum of squared errors for each fit, it is clear that model Itox fits best for nickel and SDS and bentazon. For potassiumferricyanide it seems that combined effects play a role. At low concentrations, only one type of kinetic inhibition plays a role (model IK2). When the concentration increases, however, model Itox, representing the overall inhibition of the cell, gives a better fit.

The results show that the extended BVM model can be used to distinguish between different types of kinetic inhibitions for a single component, although in most cases the same type of inhibition was observed, being the inhibition of the whole organisms (model Itox). The extended BVM-model has the potential to determine the type of kinetic inhibition of a component. The model may need to be improved for combined effects or for a concentration effect.

The value of Ki can be determined. It is expected that Ki is constant for each concentration of toxic component because it is a property of the bacteria. When it is found that the value of Ki changes with concentration, this may mean that the toxicity term, $Ki/Ki+\chi i$, may not be the right term for toxicity. The value of Ki tells something about the sensitivity of the sensor for the measured component. This is important to know when determining if the sensor is suitable for the application that one wants.

6.4 Supplemental Data

The results in the table below give the calculations for the fit of all data to the three models Itox, IK1 and IK2. Each polarization curve within the presence of a toxic component in a known concentration was compared to the polarization curve without toxic component and fit to the four models. This could be used to calculate the value of the inhibition constant Ki using least square methods. Both ordinary least square (OLS) methods and weighted least square (WLS) methods were used as described in Chapter 5. The components and concentrations used are: Nickelcloride (10, 20 30 mg/l-Ni), SDS (50 mg/l), Bentazon (1,3 mg/l), potassiumferricyanide (0.5, 1, 2 mM).

Nickel

Table 6.3A: Nickel, 10 mg/l

toxtype	*Ki (mg/l)*	*J_N (mA^2)*	*Factor J_N*
OLS			
Itox	**24.39**	**0.30**	**1**
IK1	10.63	2.08	6.98
IK2	1.63	2.93	9.82
WLS			
Itox	**30.31**	**0.15**	**1**
IK1	7.62	1.97	13.03
IK2	7.43	1.81	11.95

Table 6.3B: Nickel 20 mg/l

toxtype	*Ki (mgl)*	*J_N (mA^2)*	*Factor J_N*
OLS			
Itox	**27.74**	**0.45**	**1**
IK1	20.20	3.90	8.70
IK2	3.22	5.61	12.49
WLS			
Itox	**31.68**	**0.30**	**1**
IK1	14.72	3.53	11.87
IK2	14.87	3.70	12.44

Table 6.3C: Nickel 30 mg/l

toxtype	*Ki (mg/l)*	J_N (mA^2)	*Factor* J_N
OLS			
Itox	**4.12**	**0.09**	**1**
IK1	5.22	5.99	67.69
IK2	4.94	19.13	216.17
WLS			
Itox	**4.57**	**0.07**	**1**
IK1	9.02	8.70	118.25
IK2	24.42	16.26	220.95

Sodium dodecyl sulfate (SDS)

Table 6.4: SDS 50 mg/l

toxtype	*Ki (mg/l)*	J_N (mA^2)	*Factor* J_N
OLS			
Itox	**76.29**	**0.05**	**1**
IK1	-18.24	2.80	53.61
IK2	71.36	1.22	23.46
WLS			
Itox	85.37	1.49	2.10
IK1	**-7.82**	**0.71**	**1**
IK2	49.60	1.07	1.50

Bentazon

Table 6.5A: Bentazon, 1 mg/l

toxtype	*Ki (mg/l)*	*J_N (mA^2)*	*Factor J_N*
OLS			
Itox	**4.96**	**0.18**	**1**
IK1	-71.13	0.47	2.68
IK2	-43.55	0.48	2.76
WLS			
Itox	**3.00**	**0.32**	**1**
IK1	0.51	1.03	3.24
IK2	13.29	0.35	1.10

Table 6.5B: Bentazon3 mg/l

toxtype	*Ki (mg/l)*	*J_N (mA^2)*	*Factor J_N*
OLS			
Itox	**14.43**	**0.39**	**1**
IK1	1.30	280.87	719.08
IK2	-3.98	1.45	3.72
WLS			
Itox	**9.11**	**0.63**	**1**
IK1	-0.15	15.11	24.00
IK2	-2.95	1.44	2.28

Ferricyanide

Table 6.6A: Ferricyanide, 0.5 mM

toxtype	Ki (mM)	J_N (mA^2)	Factor J_N
OLS			
Itox	8.00	0.25	7.36
IK1	0.31	0.65	19.00
IK2	**0.03**	**0.03**	**1**
WLS			
Itox	48.44	0.46	3.73
IK1	0.19	0.65	5.21
IK2	**0.11**	**0.12**	**1**

Table 6.6B: Ferricyanide, 1 mM

toxtype	*Ki (mM)*	*J_N (mA^2)*	*Factor J_N*
OLS			
Itox	**1.88**	**0.08**	**1**
IK1	0.35	0.93	10.97
IK2	0.04	2.53	29.90
WLS			
Itox	**2.18**	**0.12**	**1**
IK1	0.22	0.45	3.67
IK2	0.16	1.61	13.12

Table 6.6C: Ferricyanide, 2 mM

toxtype	*Ki (mM)*	J_N (mA^2)	*Factor* J_N
OLS			
Itox	**7.81**	**0.10**	**1**
IK1	0.56	1.50	15.14
IK2	0.13	0.97	9.80
WLS			
Itox	**10.26**	**0.11**	**1**
IK1	0.71	1.09	9.97
IK2	0.51	0.39	3.54

Reference:

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Chapter 7

Influence of membrane type, current and potential on the response to chemical toxicants of a microbial fuel cell based biosensor

This chapter is submitted as

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7. Influence of membrane type, current and potential on the response to chemical toxicants of a microbial fuel cell based biosensor

Abstract

Drinking water free of chemical toxicants is important for people's health. A microbial fuel cell based biosensor can be used to detect the presence of toxic chemicals. The sensitivity of this type of biosensor for nickel was investigated. There was no delay in the response of the sensor and the sensitivity was 0.0027 A/m^2/mg Ni/l at an anode potential of -0.4V. The effect of four types of ion exchange membranes (cation exchange, anion exchange, monovalent cation exchange and bipolar membranes) on the sensitivity was not significant.

Current density correlates with the decrease of the nickel concentration in the sensor with 16.5 mg/l per A/m2 by causing a flux of nickel towards the membrane and the catholyte. However, the sensitivity is higher at higher overpotential and thus at higher current density. Thus although nickel concentration is lower, the response is higher at high overpotentials. The sensitivity still has to be increased because even at an overpotential of -0.16V the sensitivity is too low to be able to measure the concentrations that is maximally allowed by European directives on (drinking)
water quality.

Keywords: microbial fuel cell, biosensor, sensitivity, toxicity

7.1 Introduction

Drinking water free of chemical toxicants is important for people's health. An early warning system is needed to indicate the presence of any chemical toxicant. After detection of a chemical toxicant, measures can be taken to stop the intake of the contaminated water [1]. Therefore generic detection methods are needed. After intake, water stays in the distribution system approximately three days in the Netherlands [2]. Methods for the rapid detection of chemical toxicants in drinking water within this time are thus needed. Rapid detection can be done by online detection methods.

Among sensors that are generic are many biosensors. Biosensors currently available use higher organisms, eg fish, or use algae or (bio)luminescent microorganisms [3-13]. These sensors give various types of signals such as change of movement or light intensity. One drawback of these available biosensors is the need for a transducer to translate the signal given by the organisms into an electrical signal that can be interpreted by operators. The use of a transducer can lead to a loss of accuracy due to difficulty to measure the signal accurately or due to difficult, often nonlinear, interpretation of the signal. When a new sensor is to be designed the signal produced should be easy to translate into information [14, 15]. A direct signal in the form of electricity is an easily readable signal and there is no need for a transducer [16]. Therefore, a microbial fuel cell- based biosensor is proposed as online sensor for chemical toxicants in water.

7.1.1 MFC as sensor

A microbial fuel cell (MFC) is a device in which microorganisms convert chemical energy into electrical energy. Electrochemically active microorganisms oxidize organic matter anaerobically and transfer electrons to an anode. Organic matter serves as electron donor for these microorganisms and the electrode serves as electron acceptor. Electrical current in an MFC is thus a direct measure for metabolic activity of electrochemically active microorganisms [17].

Chemical toxicants have an inhibitory effect on the metabolic activity of microorganisms and in case of electrochemically active microorganisms, an inhibitory effect on the transfer rate of electrons to the electrode. Presence of chemical toxicants can thus

decrease the electrical current. This means that the microbial fuel cell can act as sensor for the presence of chemical toxicants in water [18]. The water that has to be investigated is fed as anolyte because the microorganisms grow on the anode. When a toxic substance is present, this is indicated by the sensor as a decrease in electrical current. An MFC can thus be used as an online biosensor for the metabolic activity of the microorganisms and no transducer is needed.

The concentration of chemical toxicant that bacteria are exposed to determines the decrease of metabolic activity and thus the decrease of electrical current. This concentration may not be the same as the concentration of chemical toxicant in the influent. To understand to what concentration bacteria are exposed, a look has to be taken at the design of the MFC-based biosensor and the fluxes of the chemical toxicant (Figure 7.1). The toxicant that enters the anodic compartment of the sensor can move towards the biofilm (flux 1), into the cathode (flux 2) and into the membrane (flux 3). The rest leaves the sensor convectively.

An MFC-based biosensor consists of an anode, at which the bacteria grow, an anodic compartment where the water that may contain the toxicant enters the sensor, a membrane that separates the anodic compartment from the cathodic compartment, the cathodic compartment that contains an electron acceptor, and finally a cathode. Anode and cathode are connected through an external circuit containing a potentiostat or external resistor (Rext). The chemical toxicant enters the anodic compartment via the water and is convectively transported through the compartment. Inside the anodic compartment it can move into several directions. First it can diffuse into the biofilm, where the bacteria are exposed to it. The concentration in the biofilm reaches the same concentration as in the bulk. This is the concentration that determines the decrease in electrical current.

Secondly the chemical toxicant can diffuse and migrate towards the cathode, where it arrives at the membrane that is present. The type of ion exchange membrane mostly used in MFC-based biosensors is a cation exchange membrane [18-21]. The use of a CEM forces cations to pass the membrane to keep the charge balanced [17, 22-24]. Therefore positively charged chemical toxicants that can be present in drinking water such as nickel might also pass the membrane. This means that positively charged chemical toxicants can

move into the cathodic compartment. Thirdly positively charged chemical toxicant can be absorbed in the membrane due to ion exchange that takes place in cation exchange membranes. These latter two fluxes can be sustained over a long period and lower the concentration in the bulk of the anodic compartment and thus in the biofilm. The chemical toxicant that does not enter the cathodic compartment or membrane can flow out of the sensor convectively.

If the two fluxes away from the anode (arrow 2 and 3 in figure 7.1) are kept as low as possible, the concentration in the anodic compartment and thus in the biofilm (1 and outflow in figure 7.1) is nearly as high as the influent concentration. Decreasing the flux away from the anode and thus increasing the concentration in the bulk and in the biofilm increases the reduction of metabolic activity. Thus the electrical current in the sensor changes more at the same influent concentration. This influences the lowest concentration of chemical toxicant that is detected, the lower detection limit. Therefore it is important to consider diffusion and migration factors when designing a new sensor.

The sensitivity is determined by the decrease of electrical current per amount of chemical. The sensor is more sensitive when the current density changes more under the same toxic chemical concentration. When the current density change is divided by the influent concentration, the apparent sensitivity is calculated. However the real concentration may be different from the influent concentration. When the real concentration in the sensor is used, the real sensitivity is calculated. Decreasing flow 2 and 3 in figure 7.1 will keep the concentration in the sensor similar to the influent concentration and will give an apparent sensitivity that is close to the real sensitivity. The flux towards the cathode (flux 2 and 3) may be influenced by electrical current, cell voltage and type of ion exchange membrane. Cation exchange membranes are mostly used; however other types of ion exchange membranes are available and include anion exchange membranes, monovalent selective cation exchange membranes and bipolar membranes. These membranes are tested in microbial fuel cells used for electricity production or hydrogen production but not in microbial fuel cell-based biosensors [23-27].

The response of the sensor in the form of sensitivity and response time, to nickel was investigated with different types of ion exchange membranes. Furthermore, the influence

of the current and potentials on the flux of nickel was investigated. The different parameters are compared on the basis of sensitivity, defined as change in current density per milligram/l of toxic component.

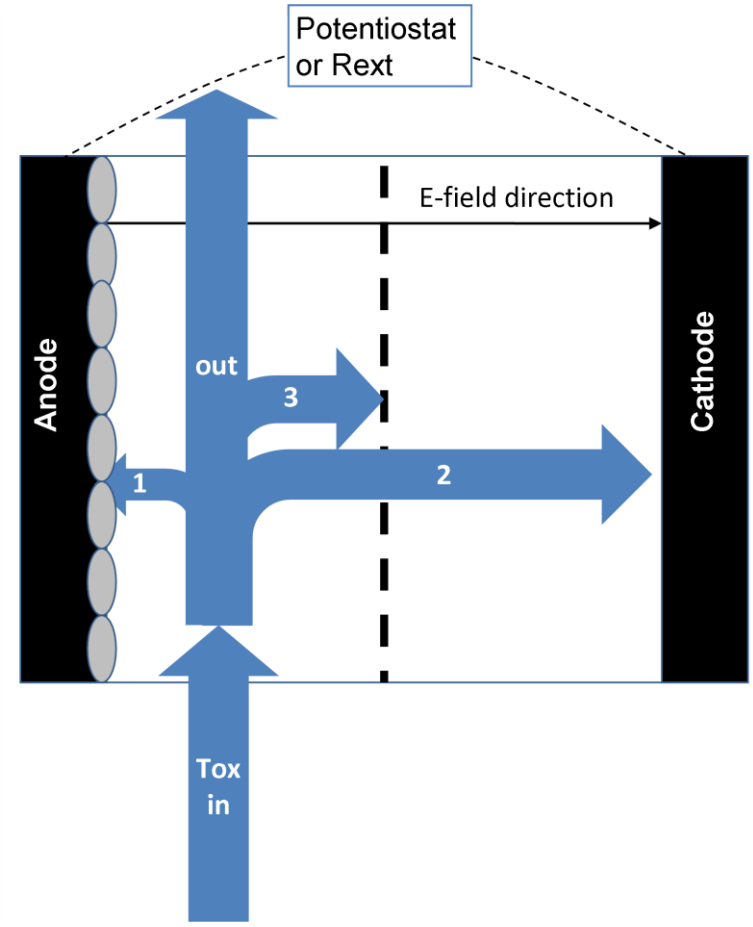

Figure 7.1: Fate of positively charged chemical toxicant in reactor. The concentration bacteria are exposed to, depends on 1) flux of the chemical toxicant towards and inside the biofilm, 2) flux through the membrane into the cathodic compartment, 3) absorption into the membrane. The remainder is in the convective outflow of the mfc-based biosensor.

7.2 Material and methods

7.2.1 MFC construction and start up

Two microbial fuel cells were constructed as in Ter Heijne et al 2008 [28] and were operated individually. The anode chamber was inoculated with 20 ml of anolyte taken from an microbial fuel cell operated under the same conditions [26]. and were operated at an anode potential of -0.4 V vs Ag/AgCl. The anode chamber of the fuel cell was continuously fed with medium at a rate of 0.7 ml/min. The hydraulic retention time in the cell is approximately 45 minutes. The medium was the same as used in Rozendal et al.[29] except a buffer concentration of 20 mM phosphate was used. The medium was kept anaerobic by continuously flushing with nitrogen gas. The catholyte contained a 10 mM phosphate buffer solution continuously flushed with pressurized air to supply oxygen. Temperature was controlled at 30C. During start-up a cation exchange membrane (Fumatech GMBH, Germany) was present in the system.

7.2.2 MFC operation and testing chemical toxicants.

In the toxicity tests nickelcloride was added to the medium and fed to the MFC for two hours in the wanted concentration (13.2 – 187.6 mg/l of nickel ions). After these two hours, clean medium was provided again. The medium flow (clean or contaminated) was continuously 0.7 ml/min. Nickel was washed out of the cell when clean medium was provided again to wash out the nickel. Maximum one experiment of two hours dosing was done per day to let the biofilm recover completely and make sure no nickel was left in the sensor before the start of a new experiment. Catholyte was replaced before each experiment. After each component was tested twice at each concentration, the membrane was replaced. During the experiments testing the effect of the membrane type, anode potential was controlled at -0.4V. The effect of overpotential was tested at anode potentials of -0.3V to -0.45V. Electrical current was measured every minute, 24h/day and open circuit anode potential was measured at least twice per week.

7.2.3 Analysis.

Acetate and nickel concentrations in the effluent were measured before, four times during dosage and four times after dosage with 30 minutes interval. The influent concentration of nickel and acetate were also measured. Acetate concentrations 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). Nickel concentrations were analyzed with inductive-coupled plasma (Optima 3000XL, Perkin Elmer).

7.2.4 Membrane selection:

Cation exchange membranes contain negatively charged groups fixed to the membrane backbone and allow transport of all cations over the membrane. This membrane is generally used because protons produced at the anode are supposed to migrate towards the cathode and reduce oxygen to water. However positively charged components other than protons can also pass the membrane [23]. Positively charged chemical toxicants can therefore also pass the membrane and the concentration in the anodic compartment will decrease.

Anion exchange membranes contain positively charged groups fixed to the membrane backbone. Anions, like hydroxyl can pass the membrane while cations are repelled [30]. It is expected that when an anion exchange membrane is present in the MFC-based biosensor, most charge will be balanced by negative ions migrating from the cathode towards the anode compartment, most likely hydroxyl ions and buffer components like phosphate ions [24]. This means that nickel is not expected to pass the membrane.

Monovalent cation selective membranes only pass ions that have a single positive charge while ions that are multivalent positively charged and anions are repelled [31-33]. Thus multivalent positively charged ions such as nickel-ions are expected to be retained in the anodic compartment while protons can still migrate towards the cathode to balance the charge.

A bipolar membrane (BPM) is a membrane that consists of a layered ion-exchange structure composed of a cation selective layer (with fixed negative charges) and an anion selective layer (with fixed positive charges). On the borderline between anion selective layer and cation selective layer water is split into hydroxyl ions and protons when

exceeding a potential difference between anode and cathode of approximately 0.8 V. The voltage over the MFC however does not exceed 0.8V and therefore water splitting does not make up for 100% of the charge. Previous research with MFCs and microbial electrolysis cells (MEC) estimate that around 70% of the charge is balanced by the transport of protons/ hydroxyl ions while the rest is made up by the transport of other ions, mainly potassium. [23, 26] This suggests that the transport of positively charged (toxic) species such as nickel across the membrane can happen.

The following membranes were used: cation exchange membrane (Fumasep FKS, FuMA-Tech GmbH, Germany), an anion exchange membrane (Neosepta AMX, Tokuyama Co., Japan), a monovalent cation selective membrane (Neosepta CMS, Tokuyama Co., Japan) and a bipolar membrane (Fumasep FBM, FuMA-Tech GmbH, Germany).

7.3 Results and discussion

7.3.1 Response to nickel

The MFC-based biosensor gives electrical current as a signal that is used for water quality measurements. Under non-toxic conditions electrical current is stable while under toxic conditions current is disrupted. To investigate the response of the sensor to nickel, it was dosed to the sensor in a concentration range from 22.7 to 187.6 mg/l at a sensor that was operated at an anode potential of -0.4V. Figure 7.2 shows a typical experiment in time. Current density decreases at the start of dosing of a chemical toxicant e.g. nickel (t=0min) and increases again when the dosage stops (t=120 min) and the concentration of the chemical toxicant in the anodic compartment decreases due to washout. When nickel enters the anodic compartment of the sensor (t=0 min), it takes a while for the concentration at the effluent to reach the maximum concentration. Part of the nickel diffuses towards the cathode or is absorbed on the membrane. The concentration in the sensor is therefore lower than the concentration in the influent, even after three hydraulic retention times.

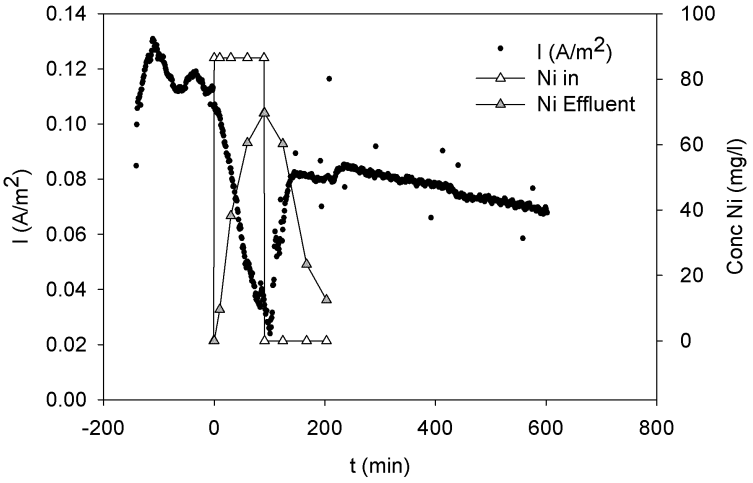

Figure 7.2: Response of current density on the presence of nickel. Nickel is dosed for two hours >3HRT).

In Figure 7.3 the response for all nickel concentrations is shown. The response at the maximum concentration is given because at that concentration the largest effect can be observed. As can be observed from Figure 7.3 an increase in concentration of nickel leads to an increase of the response of the current density. An increased nickel concentration thus leads to a larger decrease in current density. The slope of this graph represents the sensitivity of the sensor. The sensitivity of the sensor to nickel is 0.0027 A/m^2/mg/l.

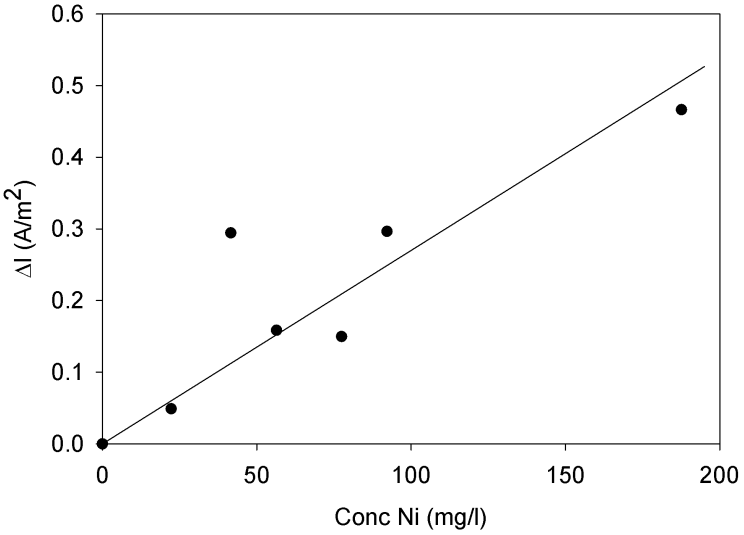

Figure 7.3: influence of nickel concentration on decrease of current density. Anode potential: -0.4V, CEM membrane. Sensitivity, represented by the slope of the graph, is 0.0027 A/m2/mg/l (R^2=0.63)

The response at the maximum concentration in the cell is shown in figure 7.3. However the current starts to decrease as soon as nickel enters the sensor. The decrease at the prevailing concentration follows the same trend as the sensitivity at the maximum concentration in the cell. This shows that the response is fast. The time it takes to reach the same concentration of e.g. 20 mg/l in the sensor is much longer when the influent has a concentration of 25 mg/l, than when the influent has a concentration of 180 mg/l because of dilution effects. In each case however, the same response is observed when a certain concentration is reached (Table 7.1). This shows that the sensitivity is the same for

different concentrations and that the response is not delayed by phenomena like absorption onto the biofilm.

Table 7.1: Sensitivity during experiment. This is the slope of the dose response curve per experiment. R^2 is the ratio of the variability of the modeled values to the variability of the original data values

Influent concentration (mg/l)	*Sensitivity during experiment (A/m^2/mg/l)*	*R^2*
42.6	$9.9*10^{-3}$	0.83
56.6	$3.2*10^{-3}$	0.83
77.5	$2.0*10^{-3}$	0.73
92.2	$2.2*10^{-3}$	0.77
187.6	$3.6*10^{-3}$	0.81
overall	$2.7*10^{-3}$	0.63

The concentration in the biofilm will quickly reach the concentration in the bulk of the anodic compartment. The diffusion time will be $t=L^2/D$ in which L is the thickness of the biofilm and is estimated to be maximum 100 micrometer [34]. D is the diffusion coefficient and is approximately $10^{-8} – 10^{-9}$ as reported for acetate in biofilms [35, 36]. The diffusion will then be t= 1 to 10sec. This flux into the biofilm is therefore only a temporary flux compared to the two hours that the toxic ions are dosed. When the pulse of chemical toxicant is longer then 10 sec the concentration in the biofilm will be the same as in the bulk. No delay was thus expected from diffusion.

7.3.2 Ion exchange membrane type

The flux of positively charged chemical toxicants away from the anode may be influenced by the type of ion exchange membrane in the sensor.

When a CEM is used cations will exchange in the membrane and can partly be absorbed. The used CEM (Fumatech FKS, Germany) has an ion exchange capacity (IEC) of 1.0 meq/g. If a membrane density of 1 kg/m^3 is assumed and the surface area of the membrane is 22

cm^2, the total IEC is 0.242 meq. This means that 0.242 mmol of charge can be exchanged. For a two-valent species this equals 0.121 mmol. As an example, 7.3 mg nickel is dosed in two hours and that equals 0.124 mmol. When 100% of the mobile groups in the membrane is replaced by nickel ions, 97% of the nickel will be present in the membrane and regarded as loss. This means that in the two hours of the experiment no equilibrium is reached and that the flux towards and into the membrane is continuous. Moreover the preference of the membrane is four times higher for divalent ions than for monovalent ions.

The membrane in the sensor can retain ions in the anode compartment if the membrane is not permeable for that type of ions. Using anion exchange membranes, bipolar membranes and monovalent selective membrane may greatly reduce the flux of multivalent toxic cations into the membrane. However the perm selectivity of all used membranes is lower than 100% and therefore a small flux will exist. To test the effect of the flux through the membrane, four different types of ion selective membranes were applied in the mfc-based biosensor.

MFC-based biosensors were operated at an anode potential of -0.4V resulting in similar current densities (average 0.65 A/m^2, in the 2.5 hours before dosing average st dev: 0.016 A/m^2). The sensitivity after dosage of 86 mg/l of nickel was measured for each of the cells containing a different membrane (Figure 7.4).

The decrease in current density is a measure for the concentration chemical toxicant the bacteria are exposed to. The average sensitivity upon dosage of nickel is $2.56*10^{-3}$ (stdev= $0.65*10^{-3}$) $A/m^2/mg/l$. Using statistical analysis visualized with a box-plot, and performing t-tests, shows that there are no significant differences between the sensitivities using different membranes.

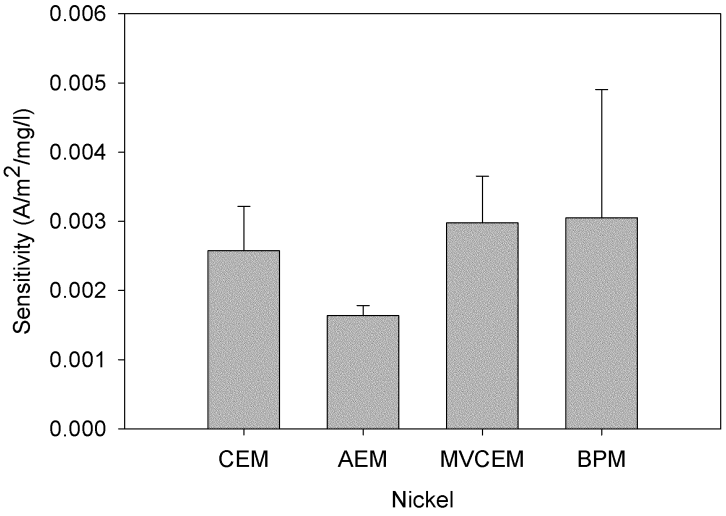

Figure 7.4: sensitivity upon addition 86 mg/l nickel in mfc-based biosensors containing ion selective membranes: CEM= cation exchange membrane, AEM= anion exchange membrane, MVCEM= monovalent cation selective ion exchange membrane, BPM=bipolar membrane. Error bars: deviation from mean.

7.3.3 Influence of current density on concentration.

The total concentration of positive ions in the anolyte is 59.8 mM while the proton concentration at pH 7 is 10^{-4} mM. Therefore, most of the charge that migrates across the membrane is in the form of ions other than protons. This can be non-toxic salts that are present in the electrolyte such as potassium and sodium, but also the chemical toxicants. In this way the concentration of toxic component in the anode compartment can decrease. The bacteria experience a lower concentration than the concentration in the influent as can be seen in Figure 7.5.

After three hydraulic retention times (HRT), the concentration in the anodic compartment will have reached a stable concentration when the influent concentration is stable. It can be seen from Figure 7.2 that the effluent concentration is lower than the influent concentration, even after three HRT. This is due to diffusion towards the cathode and absorption on the membrane. The anode potential as set by the potentiostat determines the electrical current and influencing the current will affect the ion flow. A higher current

leads to a higher migration of ions to balance the charge that is transported in the sensor. The influence of the current on the change in nickel concentration is shown in Figure 7.5

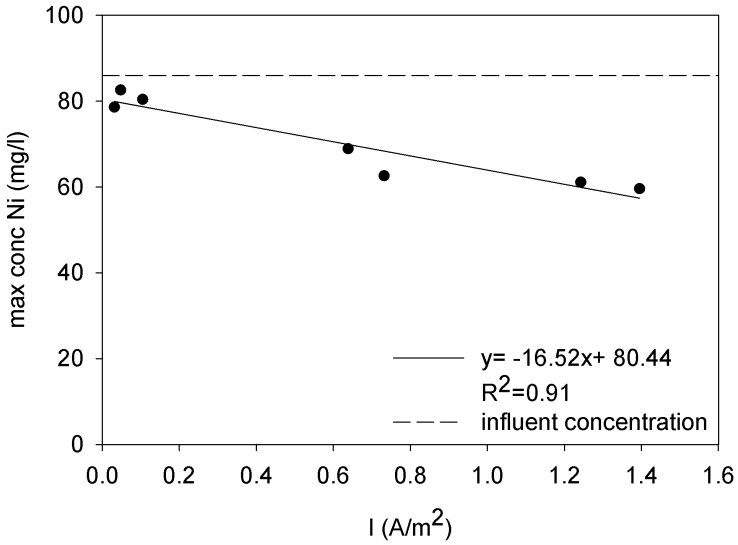

Figure 7.5: Influence of current density on nickel transport. Maximum concentration Ni, using CEM membrane after 86 mg/l Ni is dosed for two hours.

The concentration difference between anodic compartment and cathodic compartment is also a driving force for the continuous movement of toxic ions towards the cathodic compartment and this leads to the initial decrease from 86 mg/l to 80.4 mg/l at zero-current.

The nickel concentration in the effluent of the anodic compartment is higher when the electrical current is lower. This shows that influencing the current density has influence on the concentration in the bulk in the anode compartment. Influencing the current density can be done by choosing the anode potential. Figure 7.5 shows that a higher current density gives a lower maximum concentration of nickel in the sensor.

7.3.4 Anode potential

Apart from influencing the migration of toxic component and with that the concentration in the bulk, the anode potential also influences the energy level of the electrons that are released to the anode. Bacteria gain energy from the release of electrons and thus from the potential difference between the open cell anode potential and the controlled anode potential. This potential difference is called overpotential. For an anode potential of -0.45V this overpotential is approximately 0.015 V and this is lower than for an anode potential of -0.3V where overpotential is approximately 0.165 V. Hence the energy that is gained by the bacteria is lower at lower anode potential. This can be seen in the current because current is positively correlated to the overpotential. The average current density for an anode potential of -0.45V is 0.12 A/m2 in the used set-up, while the average current density in the set-up with anode control at -0.3V was 1.3 A/m2 and thus more than a factor 10 higher. The sensitivity of the bacteria for nickel was investigated was for the energy level set by the overpotential. Figure 7.6 shows that a higher overpotential, and thus a higher baseline current, leads to a higher sensitivity when nickel was dosed. The closed symbols show the apparent sensitivity. This is the decease in current density if the concentration in the sensor would be the same as in the influent, namely 86 mg/l. However as shown in Figure 7.5, the concentration in the sensor is lower than in the influent. This means that the real sensitivity is actually even higher. The open symbols show the sensitivity if corrected for the real concentration. Using the adjusted model of Hamelers et al (2011) (Stein 2011, model 2), the sensitivity can also be predicted, both for the apparent concentration and the real concentration.

The difference between the real and apparent sensitivity increases when overpotential increases.

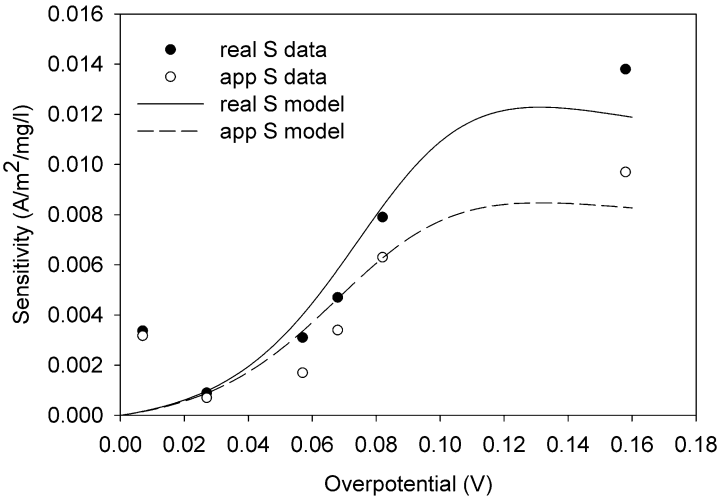

Figure 7.6: Sensitivity when 86 mg/l Ni is added into the sensor for two hours. The different overpotential results in a different baseline current. This affects the sensitivity. Colsed symbols: apparent sensitivity: dI/conc_in, open symbols: sensitivity corrected for the real concentration.

Although the concentration of nickel decreases at increasing current and thus at increasing overpotential (figure 7.5), the sensitivity keeps increasing leading to an even higher real sensitivity. Figure 7.5 shows that a higher current density gives a lower maximum concentration of nickel in the sensor. A higher current density is reached when the overpotential is higher. Figure 7.6 however shows that a higher overpotential gives a higher sensitivity, both when the influent concentration and the real (lower) concentration are taken into account. Thus although at high potentials the concentration is up to 30% lower, the current changes more. Therefore we can conclude that anodic overpotential is a more important factor for current density changes than concentration change of nickel in the used concentration range.

At a nickel concentration of 22 mg/l the current density decreases 0.05 A/m^2 at an anode potential of -0.4V. The concentration that is allowed in drinking water in the Netherlands is 20 microgram/l [37]. At an overpotential of 0.16V the apparent sensitivity is maximal and is around 10^{-2} $A/m^2/mg/l$. To measure 20 microgram/l in the influent, the change in

current will be $2*10^{-4}$ A/m^2. The noise level of the measurements is higher than 10^{-4} A/m^2. To detect changes in current due to concentrations of nickel in the microgram range, it is important that the baseline-current under non-toxic conditions is stable otherwise the change in current can be in the same order of magnitude as the noise. A stable current can be reached by controlling the anodic overpotential, keeping pH stable and under substrate saturation [38]. The detection threshold for nickel was not investigated but the condition to reach a high sensitivity, being a high overpotential with a high current density, leads to high loss of nickel ions in the anodic compartment. This will thus negatively influence the detection threshold. The sensor should be optimized further to get a larger response to low concentrations of toxic component.

7.4 Conclusions

A higher concentration of toxic chemical gives a higher response of the sensor. The concentration in the biofilm can be influenced due to migration and diffusion. The type of membrane in the sensor does not seem to influence the sensitivity of the sensor for nickel. A more important factor is the current density in the reactor. A higher current density leads to a decreased concentration but nevertheless it gives a higher sensitivity of the sensor. From the research described in this paper we can conclude that the overpotential and thus the energy level of the bacteria is a more important factor for sensitivity than the concentration differences caused by migration and diffusion.

Acknowledgements

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Chapter 8

8. General conclusions and outlook

8.1 Introduction

The thesis deals with the development of a new microbial fuel cell (MFC) based biosensor for the detection of toxic components in water. Water of a good quality and without toxic components is crucial for peoples' health. Many sensors for the monitoring of toxic components in water are available but they all have some drawbacks such as measuring specific components, high maintenance costs or inability to work online. To overcome some drawbacks the microbial fuel cell was proposed as a new online sensor for the monitoring of toxic components in water. Bacteria produce a flow of electrons and this electrical current is used as a measure for water quality. A change in current indicates a change in the bacterial metabolism as a result of a change in water quality and thus indicates the possible presence of toxic components. The sensor can thus be used as an early warning system that provides early detection and awareness of toxicity.

8.2 Research conclusions

Experiments showed that the MFC-based biosensor can detect the presence of toxic components when the overpotential, pH and substrate concentrations are kept constant (chapter 2). In general, current did decrease upon dosage of toxic components.

Bacteria grow on the anode and the anode potential is directly related to the overpotential. A higher overpotential results in a higher energy gain for the bacteria. The sensor can work using three different modes of electrical operation that influence the anode potential: amperometry based on controlling anode potential, potentiometry based on controlling current, or measuring cell voltage over a fixed external resistance. The method that gives the most response and is thus most sensitive is using a fixed external resistance. A lower external resistance gives a higher response. The detection method and setting also affects the recovery time of the bacteria. The shortest recovery time occurs when external resistance is controlled. The shortest recovery time was reached while using an external resistance of 1000 Ohm (chapter 3). However, controlling external

resistance gives a lower degree of control of the overpotential than controlling anode potential. This control is needed for accurate detection of current changes and the prevention of false positive alarms as shown in chapter 2. In the research further performed, anode potential was controlled.

The relationship between anode potential and current in microbial fuel cells can be described by the model that was developed by Hamelers et al [1], the Butler-Volmer-Monod (BVM) model. This model is based on biochemical and electrochemical kinetics (Intermezzo). The BVM model does not describe the effect of toxic components on current. The model was therefore extended to describe four types of kinetic inhibitions based on enzyme kinetics theory resulting in four models (chapter 4). Of each of the four described types of kinetic inhibitions the overpotential that gives a sensor that is most sensitive to toxic components was determined. At least two sensors that work parallel at different overpotentials are needed to be able to detect all possible toxic components. One has to control the overpotential at 250 mV to detect changes affecting the substrate affinity constant or affecting the whole cells. The second sensor should control the overpotential at a value between 76mV and 104 mV for the simulated parameter values, to detect changes affecting the other two kinetic rate constants, K1 and K2. The sensor also has to be robust against changes other than toxicity, such as pH, temperature or conductivity. The potential that results in the most robust sensor differs from the potential that gives a sensitive sensor. To get a sensitive and robust sensor at the same time, a choice for an optimal overpotential has to be made on the basis of the tradeoff between sensitivity and robustness. This choice depends e.g. on the noise level in the system and the application regarding the allowed concentration of toxic components.

Recall that the aim is to develop a sensor that detects the presence of toxic components using online measurements. Current changes indicating the possible presence of toxic components can be measured online at a fixed anode potential. Substrate concentration and substrate consumption rates can also be estimated online. However, the kinetic parameters and the type of kinetic inhibition cannot be determined using the online measurements but only by making polarization curves. A protocol is proposed for operating the sensor in an intelligent way that combines online measurements with polarization curves. First, a polarization curve has to be made under non-toxic conditions

to determine the kinetic parameters. Then current can be measured online at a fixed potential. This should be a potential giving a sensitive sensor. When current decreases to below a pre-set threshold value, this indicates the presence of toxicity. A new polarization curve should be made. The type of inhibition can then be determined, using the values of the kinetic parameters determined under non-toxic conditions and estimating the toxicity term containing the concentration of toxic component and the toxicity constant. (chapter 5)

To validate the protocol, polarization curves were made. It is possible to fit the extended BVM model on experimental data from polarization curves made in the presence of toxic components. In most cases, the model representing inhibition of the whole cell fits best. The other models, however, could also be fitted to some polarization curves. The model may need to be extended to include extra effects when concentration of toxic component increases. Also combined effects when a toxic component causes more types of kinetic inhibition may need to be included (chapter 6).

Operation and design of the sensor affect the exposure of bacteria to toxic components and thus the sensitivity of the sensor. Effect of type of membrane, current and overpotential on the sensitivity were tested. The type of ion exchange membrane did not have a significant effect on the sensitivity of the sensor in the used concentration range of nickel. The current and overpotential, however, had an effect on sensitivity. For nickel an increased current gave a decrease in nickel concentration in the anodic compartment where the bacteria are present. However an increased overpotential, resulting in an increased current and thus lower concentration, gave a larger response to nickel. This phenomenon is confirmed by the extended BVM model, Itox. (chapter 7)

In general, it can be concluded that the MFC-based biosensor can be used as an online sensor for the detection of toxic components in water. The thesis describes conditions that are needed to get a robust sensor with a short recovery time and to prevent false positive alarms. Already the settings are determined to get a sensitive sensor. Even the type of kinetic inhibition can be determined after making polarization curves, although this is done off-line. If a database would be present containing the inhibition type and value of the inhibition constant, this would directly result in a pre-selection of possible

contaminations when the sensor detects the presence of toxic components combined with the estimated concentration.

8.3 Future research

Future research on the MFC-based biosensor should focus on the improvement of the sensor regarding sensitivity. The sensitivity for e.g. nickel is not yet high enough to be able to detect concentrations below the limits from the Water Framework Directive (chapter 8) [2]. As yet, the detection limit of the sensor is not determined for other toxic components. It would also be informative to get more insight in the population of bacteria present. The type of bacteria present might have an effect on the components that can be detected. The evolution of the biofilm after a toxic event may also affect the sensitivity.

Improvement of the sensitivity may be possible by influencing the thickness and structure of the biofilm. Bacteria growing in a biofilm form an extracellular matrix that makes it possible for bacteria to adhere to surfaces and this matrix mainly contains extracellular polymeric substances (EPS) [3]. The toxic component cannot easily enter the biofilm and this is mainly due to the EPS structures surrounding the biofilm. EPS can lower the diffusion rate and toxic components may also accumulate and bind to this EPS layer preventing the toxic component to reach the electrochemically active bacteria. Improvement of sensitivity could therefore also focus on the minimization of the EPS production by bacteria. Addition of surfactants may promote detachment of bacteria from the biofilm carrying away EPS and leaving a thinner biofilm. Ortho-phtalaldehyde (OPA), sodium hydroxide (NaOH) and sodium hypochlorite, dodigen, and NaOCl have been seen to catalyze the detachment of upper layers of biofilms [4, 5]. This may also work well for electrochemically active biofilms although the extracellular matrix may also contain molecules that allow for direct interspecies electron transfer [6]. Detachment of these molecules may decrease the electrical current and the sensitivity of the sensor for toxic components.

The bacterial population and with that the detection of toxic components may be influenced by the substrate of the bacteria. The concentration of substrate may affect the sensitivities per component. The type of substrate may be a means to direct the population and with that to direct the type of components that can be detected. A biofilm

that is fed with e.g. hydrogensulfide or ammonium will contain different bacteria than a biofilm fed with glucose or acetate.

Electrochemical measurements methods that are not standard yet in research on microbial fuel cells may give much extra information about the biofilm. Using for instance, electrochemical impedance spectroscopy is a noninvasive method that may give information about the ability of bacteria to donate electrons to the anode and thus about the quality of the biofilm. This can be important after a toxic event. Other dynamic methods to control the anode potential, such as cyclic voltammetry, may help to estimate the type of enzymatic inhibition and concentration of toxic component while measuring current constantly in the online sensor [7].

The full development of the sensor also includes determining the lower detection limit of many different components. Bacteria are sensitive to many components that may be present in water but they cannot detect all components that are in the water framework directive. For example endocrine disruptors have an estrogenic effect on mammals but bacteria cannot experience hormonal effects. However, some of these components may cause cellular toxicity and by this mechanism can still be detected by bacteriological sensors [8-10].
The effect of oxygen in water should also be investigated. According to preliminary experiments the presence of oxygen does not affect the current significantly. Dissolved oxygen concentration in most drinking waters is low and it does not penetrate into the biofilm well. This is advantageous because it means that there is no need to de-aerate water before leading it to the sensor. However the effect of the presence of oxygen on the detection limit of toxic components should be investigated.

The thesis described the development of an online microbial fuel cell-based biosensor. The developed sensor is generic, cheap, low in maintenance, and self regenerating. These are important properties for sensors that can be used as early warning system in e.g. the distribution system of drinking water. Early warning systems for the detection of toxic chemicals are needed in view of terrorism attacks but also when pipes start to leak to

prevent contaminated water from reaching households [11]. As opposed to many other online electrochemical biosensors, the sensor developed does not contain genetically modified bacterial cells [12, 13]. This is another advantage of the sensor. The results of the study give a theoretical, mathematical framework that also can be used for the system analysis and operation of other types of sensors. The research described in the thesis provides results that lead to better early warning systems that can even already make a selection of possible contaminants.

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Summary

The thesis investigates the applicability of a microbial fuel cell as an online biosensor for the detection of toxic components in water.

Safe drinking water without toxic chemicals is crucial for people's health. Many biosensors for the detection of toxic components are currently available, but all have some drawbacks such as the need of a transducer to translate the measured variable to a signal that can be read by operators and the inability to work online. A microbial fuel cell directly produces an electrical signal and thus no transducer is needed. It can also work online. In a microbial fuel cell bacteria degrade organic material as substrate to carbon dioxide, protons and electrons. In the presence of a solid electron acceptor in the compartment where the bacteria are, the bacteria donate the electrons to the solid electrode, the anode. The anode is electrically connected to another electrode, the cathode, where an electron acceptor such as oxygen, is reduced. This flow of electrons is measured as electrical current. The degradation rate of organic material is proportional to the measured current and current is thus a measure for bacterial activity. Consequently, a change in bacterial activity, measured as a change in electrical current, can be caused by the presence of toxic components.

To work as a generic, online sensor the device has to be robust against changes that are not toxic to prevent false positive alarms (ch 2 and 3), the recovery time should be short to allow re-use and not to miss subsequent toxicity (ch 3), the sensor has to be sensitive to toxic components (ch 4, 7) and work online (ch 5). To allow for faster identification of the component, the sensor should also be able to eliminate classes of components. This could be done by combining theoretical knowledge with the measurements (ch 6).

Microorganisms gain energy from the difference between anode potential and the redox potential of the substrate. This potential difference is called anodic overpotential. The current between anode and cathode strongly depends on the anodic overpotential. To detect changes in current and thus to detect the presence of toxic components, but also to prevent false positive alarms, control of the anodic overpotential is necessary. In **chapter 2** the conditions are investigated that are needed to reach a constant

overpotential under non-toxic conditions. The anodic overpotential, and thus current, is influenced by anode potential, pH, substrate and bicarbonate concentrations. The induced change on the overpotential caused a comparable effect on the current for all factors: 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. At high acetate concentration, the control of acetate and bicarbonate concentration can be less strict than control of anode potential and pH. Current density changes due to changes of the anode potential and pH are in the same order of magnitude as changes due to the presence of toxic components eg. copper. To reach a constant baseline of the current under nontoxic conditions, a MFC-based biosensor should be operated at *controlled anode potential*, controlled pH and saturated substrate concentrations.

The *anode potential* can be controlled via different types of electrical control of the sensor. **Chapter 3** investigates the effect of the type of control and the associated response on the detection of toxic components. Three responses are investigated: (i) amperometry based on controlling anode potential, (ii) potentiometry based on controlling current, or (iii) measuring cell voltage over a fixed external resistance. For all three methods it was possible to detect the presence of toxic components. Anode potential control resulted in a good response. Surprisingly also negative currents were observed, meaning that there must be a reduction reaction at the anode. The electrical current and thus the bacteria recovered well and thus negative current did not have a negative effect on the bacteria. Using current control an anode potential decrease in the presence of a toxic component was observed, as opposed to control with an external resistor where anode potential increased. The method that gave the highest sensitivity is using a fixed external resistance. The setting of the electrical control affects the response. A lower external resistance gives a higher response. Recovery time of the bacteria after a toxic response is also affected by the measurement method and setting. The recovery time of the sensor, both under anode potential control and under current control, was longer than when using an external resistor. A high resistor value resulted in a shorter recovery time. The shortest recovery time was reached when using an external resistance of 1000 Ohm. However, the most

stable control of overpotential is reached by controlling the anode potential and this is crucial in preventing false positive alarms.

Current depends on anodic overpotential. The **Intermezzo** describes a mathematical relationship between anodic overpotential and electrical current based on biochemical and electrochemical kinetics. The relationship is called the Butler Volmer Monod (BVM) model. Maximum current and three other, lumped, kinetic parameters are defined to describe the biochemical and electrochemical rate constants. The Butler Volmer Monod model does not describe the effect of toxic components on the current. In **chapter 4** the BVM-model is extended to include the effect of toxic components. Based on general theory for inhibition of enzyme kinetics by toxic substances four types of toxic inhibitions can be distinguished. Each type of intoxication affects predominantly one of the three kinetic parameters or the maximum current parameter, so knowing which parameter has changed is an indicative of the type of toxic component. Knowing this effect on the kinetic parameter values, it can be determined at what overpotential the sensor has to be controlled to get the most sensitive setting with respect to this inhibition. The sensor also has to be robust against changes other than toxicity, such as pH, temperature or conductivity. The potential at which the sensor is most robust differs from the potential that gives the most sensitive sensor. To get a sensitive and robust sensor at the same time, a choice for an optimal overpotential has to be made on the basis of the tradeoff between sensitivity and robustness.

In **chapter 5**, a protocol is provided for the operation of the sensor, based on the mathematical analysis of the sensor. The analysis showed that current changes and thus the presence of toxic components can be detected during online measurements at a fixed potential. However, to determine the values of the kinetic parameters and the type of toxic inhibition, a polarization curve is needed. First, a polarization curve should be made under non-toxic conditions to determine the values of the kinetic parameters. Then current can be measured during online measurements at a fixed current. When current changes, a new polarization curve should be made to determine the type of kinetic inhibition. No data were available on the effect of a toxic component on the polarization curve. **Chapter 6** shows polarization curves of four different toxic components with three

concentrations, making it possible to validate the extended BVM models. These polarization curves show that current decreases when toxic components are present and that a higher concentration in general leads to a higher change in current. The extended BVM model can be fit to the polarization curves. The type of toxic inhibition and the inhibition constant were determined using least squares methods. For the tested components, it was found that model Itox, representing the overall inhibition of the bacteria, gave the best fit for most of the polarization curves.

In **chapter 7** the effect of ion exchange membrane type, current and anode potential on the detection of positively charged toxic components was investigated. Protons are produced at the anode. To keep the charge balanced positively charged ions have to reach the cathode or negatively charged ions have to move from the cathode to the anode. The need for electroneutrality causes thus a flux of charged ions between anode and cathode over the membrane. Ion exchange membranes allow selective transport of ions with either a positive, negative or monovalent postitive charge over the membrane. The effect of the four tested types of ion exchange membranes (cation exchange, anion exchange, monovalent cation exchange and bipolar membranes) on the sensitivity was not significant. Nickel was used as a toxic component to test the effect of the current density and overpotential on the sensitivity of the sensor. To balance the electron flow through the electrical circuit, there is a flux of nickel towards the membrane and the catholyte. This ion flux results in a decrease of the nickel concentration in the sensor and correlates with current density with 16.5 mg/l per A/m^2. At lower concentration of toxic component a lower response is expected. However the sensitivity is higher at high overpotentials and high current densities. Thus, although nickel concentration is lower, the overall response is higher at high overpotentials. The sensitivity still has to be increased because even at an overpotential of -0.16V the sensitivity is too low to be able to measure the concentrations that is maximally allowed by European directives on (drinking) water quality.

Chapter 8 gives an outlook on the status and further development of the MFC-based biosensor. The thesis describes conditions that are needed to get a robust sensor with a short recovery time and to prevent false positive alarms. Already the settings are determined to get a sensitive sensor. The crucial element that has to be improved further is the sensitivity. This can be done by influencing the biofilm thickness, structure or

community. A more dynamic control of the anode potential may lead to a signal that gives more information than just a change of current without having to make polarization curve to detect the type of toxic inhibition. When the sensitivity of the sensor is improved, the MFC-based biosensor can be applied to detect the presence of toxic components and to give an indication of the type of toxic inhibition. Therefore the sensor can be used as an early warning system. This result constitutes an important contribution to safeguarding the quality of water.

Samenvatting

Dit proefschrift beschrijft de toepassing van een microbiële brandstofcel als een online biosensor voor de detectie van giftige stoffen in water.

Veilig drinkwater zonder giftige chemicaliën is van cruciaal belang voor de gezondheid van mensen. Op dit moment zijn er al veel biosensoren beschikbaar voor het detecteren van giftige chemicaliën in water, maar ze hebben allemaal een aantal nadelen. Zo hebben ze bijvoorbeeld vaak een extra stap nodig om de gemeten variabele om te zetten naar een signaal wat begrepen kan worden door operators en veel sensoren kunnen niet online werken. Een microbiële brandstofcel produceert direct een elektrisch signaal en heeft geen extra vertaalstap nodig en kan daarbij ook online gebruikt worden. In een biobrandstofcel breken bacteriën organisch materiaal af tot koolstofdioxide en protonen en elektronen. In de aanwezigheid van een vaste elektronacceptor in het compartiment waar de bacteriën zijn, zullen zij de elektronen hieraan afgeven en is dit de anode. De anode is elektrisch verbonden met een andere elektrode, de kathode, waar een elektron acceptor zoals water, wordt gereduceerd. Deze stroom van elektronen wordt gemeten als elektrische stroom. De afbraaksnelheid van organisch materiaal is proportioneel met de gemeten stroom en de stroom is daarmee een maat voor de bacteriële activiteit. Een verandering in bacteriële activiteit, gemeten als een verandering in elektrische stroom, kan veroorzaakt worden door de aanwezigheid van giftige chemicaliën.

Om als een generieke, online sensor te functioneren, moet de apparatuur robuust zijn tegen veranderingen die niet giftig zijn om vals-positieve alarmen te voorkomen (ho 2 en 3), de hersteltijd moet kort zijn zodat de sensor meermalen te gebruiken is (ho 3), de sensor moet gevoelig zijn voor giftige stoffen (ho 4 en 7), en online werken (ho 5). Om snellere identificatie van de giftige stof mogelijk te maken moet de sensor in staat zijn om verschillende klassen van stoffen te elimineren. Dit kan gedaan worden door het combineren van theoretisch kennis met de metingen (ho 6).

Micro-organismen krijgen energie door het verschil tussen de potentiaal van de anode en de redoxpotentiaal van het organisch materiaal, het substraat. Dit potentiaalverschil wordt anodische overpotentiaal genoemd. De stroom tussen anode en kathode hangt

sterk af van deze anodische overpotentiaal. Om veranderingen in stroom te meten en dus om de aanwezigheid van giftige stoffen te detecteren, maar ook om vals-positieve alarmen te voorkomen, is controle van de anodische overpotentiaal nodig. In **hoofdstuk 2** worden de omstandigheden onderzocht die nodig zijn om een constante overpotentiaal te bereiken onder niet-giftige omstandigheden. De anodische overpotentiaal, en dus stroom, wordt beïnvloed door de anode potentiaal, pH, substraat en bicarbonaat concentratie. De veroorzaakte verandering op de overpotentiaal zorgde voor een vergelijkbaar effect op de stroom voor al deze factoren. Anode potentiaal: 1,2% verandering in stroomdichtheid per mV verandering van overpotentiaal, pH 0,43% per mV, bicarbonaat 0,75% per mV en acetaat 0,8%per mV. Bij substraatverzadiging wordt de maximale substraat-omzettingssnelheid bereikt en dus ook een constante bicarbonaatconcentratie. Bij hoge acetaatconcentraties kan de controle van de acetaatconcentratie en van de bicarbonaatconcentratie minder strikt zijn dan de controle van de anode potentiaal en de pH. De stroomdichtheid verandert door de verandering van anodepotentiaal en pH in dezelfde orde van grootte als door de aanwezigheid van giftige stoffen zoals koper. Om een constante basiswaarde van de stroom te bereiken onder niet-giftige omstandigheden, moet de sensor bedreven worden met een *gecontroleerde anode potentiaal*, gecontroleerde pH and verzadigde substraat concentraties.

De *anode potentiaal* kan gecontroleerd worden via verschillende methodes van elektrische controle van de sensor. **Hoofdstuk 3** onderzoekt het effect van het type controle en de bijbehorende respons op de detectie van giftige stoffen. Drie responsies zijn onderzocht: (i) amperometrie, gebaseerd op het controleren van de anode potentiaal, (ii) potentiometrie, gebaseerd op het controleren van de stroom, of (iii) het meten van de celpotentiaal over een gecontroleerde, vaste externe weerstand. Met alle drie de methodes was het mogelijk om de aanwezigheid van giftige stoffen te meten. Controle van de anode potentiaal gaf een goede respons. Verassend genoeg werden ook negatieve stromen gemeten, wat aangeeft dat er een reductie reactie moet plaatsvinden aan de anode. De elektrische stroom, en dus de bacteriën, herstelden hierna goed en de negatieve stroom had dus geen negatief effect op de bacteriën in de sensor. Bij het controleren van de stroom werd een afname van de anodepotentiaal waargenomen, in

tegenstelling tot het gebruik van een externe weerstand waar de anodepotentiaal juist steeg in aanwezigheid van giftige stoffen. De methode die de hoogste gevoeligheid voor giftige stoffen gaf, is het gebruik van een externe weerstand. De instellingen van de controle beïnvloeden de responsie. Een lagere externe weerstand geeft een hogere responsie. De hersteltijd van de bacteriën na een giftig incident wordt ook beïnvloed door de meetmethode en de instellingen hiervan. De hersteltijd van de sensor bij het controleren van zowel de anodepotentiaal als de stroom, was langer dan de hersteltijd bij het gebruik van een externe weerstand. Een hoge weerstand gaf een korte hersteltijd. De kortste hersteltijd is gemeten bij het gebruik van een weerstand van 1000 Ohm. De meest stabiele controle van de overpotentiaal werd echter bereikt bij het controleren van de anodepotentiaal.

Stroom hangt af van de anodische overpotentiaal. Het **Intermezzo** beschrijft de wiskundige relatie tussen de anodische overpotentiaal en de elektrische stroom gebaseerd op biochemische en elektrochemische kinetiek. De relatie wordt het Butler Volmer Monod (BVM) model genoemd. De maximale stroom en drie andere samengestelde, kinetische parameters zijn gedefinieerd om de biochemische en elektrochemische snelheidsconstanten te beschrijven. Het Butler Volmer Monod model beschrijft het effect van giftige chemicaliën op de stroom niet. In **hoofdstuk 4** wordt het BVM-model uitgebreid om ook het effect van giftige stoffen toe te voegen. Gebaseerd op algemene theorie voor de remming van enzymkinetiek door giftige stoffen, kunnen vier verschillende soorten remming worden onderscheiden. Ieder type remming heeft voornamelijk invloed op één van de drie kinetische parameters of op de maximale stroom. Wanneer bekend is welke parameter het meest beïnvloed is, is dat een indicatie welk type toxische stof aanwezig is.

Wanneer het effect op de kinetische parameters bekend is, kan worden vastgesteld bij welke overpotentiaal de sensor gecontroleerd moet worden om de meest gevoelige instelling te hebben wat betreft deze remming. De sensor moet ook robuust zijn tegen niet-giftige veranderingen, zoals pH, temperatuur of geleidbaarheid. De potentiaal waarbij de sensor het meest robuust is, verschilt van de potentiaal waarbij de sensor het meest gevoelig is. Om een sensor te krijgen die zowel gevoelig als robuust is, moet een keuze

voor de beste overpotentiaal gemaakt worden, gebaseerd op een afweging tussen gevoeligheid en robuustheid.

In **hoofdstuk 5** wordt een protocol gegeven voor het bedrijven van de sensor, gebaseerd op de wiskundige analyse van de sensor. De analyse laat zien dat veranderingen in stroom en dus de aanwezigheid van giftige stoffen, aangetoond kunnen worden tijdens online metingen van de stroom bij een vaste anodepotentiaal. Echter, om de waarde van de kinetische parameters en het type remming te bepalen, is een polarisatiecurve nodig. Eerst moet er een polarisatiecurve gemaakt worden onder niet-giftige, schone, omstandigheden om de waarde van de kinetische parameters te bepalen. Daarna kan stroom gemeten worden tijdens online metingen bij een vaste stroom. Wanneer de stroom verandert, moet er een nieuwe polarisatie curve gemaakt worden om het type remming te bepalen.

Data van het effect van giftige stoffen op een polarisatie curve waren nog niet beschikbaar. **Hoofdstuk 6** laat polarisatie curves zien van vier verschillende giftige stoffen bij drie verschillende concentraties, wat het mogelijk maakt om het uitgebreide BVM model te valideren. De polarisatie curves laten zien dat stroom afneemt wanneer er een giftige stof aanwezig is in de sensor en dat een hogere concentratie over het algemeen leidt tot een grotere verandering in stroom. Het uitgebreide BVM model kan gefit worden aan de polarisatie curves. Het type remming en de remmingsconstante konden bepaald worden met behulp van kleinste kwadraten technieken. Voor de gemeten stoffen werd gevonden dat model Itox, dat algehele remming van de bacteriën aangeeft, het beste overeenstemt met de meeste polarisatie curves.

In **hoofdstuk 7** is het effect onderzocht van membraantype, stroom en anodepotentiaal op de gevoeligheid van de sensor voor positief geladen componenten. Protonen worden geproduceerd aan de anode. Om de lading gebalanceerd te houden, moeten positief geladen ionen de kathode bereiken of moeten negatief geladen ionen van de kathode naar de anode bewegen. De eis van elektronneutraliteit veroorzaakt een flux van geladen ionen tussen de anode en kathode over het membraan. Ion-wissel-membranen staan het

selectieve transport toe van ionen met een positieve, een negatieve of een monovalente positieve lading over het membraan. Het effect van vier geteste types van ion-wissel-membranen (kation wissel, anion wissel, monovalent kation wissel en bipolaire membranen) op de gevoeligheid van de sensor was niet significant. Nikkel werd gebruikt als giftige stof om het effect van stroomdichtheid en overpotentiaal op de gevoeligheid te meten. Om het effect van de elektronenstroom door het elektrische circuit te neutraliseren, is er een stroom van nikkelionen in de richting van het membraan en het katholyt. Deze ionenflux resulteert in een afname van de nikkelconcentratie in de sensor en correleert met stroomdichtheid met 16.5 mg/l per A/m^2. Bij een lagere concentratie van giftige stof wordt ook een lagere responsie verwacht. Echter, de gevoeligheid van de sensor is hoger bij hogere overpotentiaal en hogere stroomdichtheden. Dus hoewel de nikkel concentratie lager is, is de algemene responsie hoger bij hogere overpotentialen. De gevoeligheid van de sensor moet nog wel verhoogd worden want zelfs bij een overpotentiaal van -0.16V is de gevoeligheid te laag om concentraties te kunnen meten die als maximale concentratie zijn toegestaan door de Europese Kaderrichtlijn water voor (drink)waterkwaliteit.

Hoofdstuk 8 geeft een vooruitblik op de status en verdere ontwikkelingen van de op een microbiële brandstofcel gebaseerde sensor. Het proefschrift beschrijft omstandigheden die nodig zijn om een robuuste sensor met een korte hersteltijd te krijgen en om vals-positieve alarmen te voorkomen. De instellingen om een gevoelige sensor te krijgen zijn al bepaald. Het cruciale element wat verder verbeterd dient te worden, is de gevoeligheid van de sensor. Dit kan gedaan worden door het beïnvloeden van de biofilmdikte, -structuur of bacteriële samenstelling. Een meer dynamische controle van de anode potentaal kan leiden tot een signaal wat meer informatie bevat dan alleen de verandering van de stroom en waarmee zonder het hoeven maken van een polarisatiecurve het type remming al bepaald kan worden. Wanneer de gevoeligheid van de sensor is verbeterd, kan de op een biobrandstofcel gebaseerde sensor worden toegepast om de aanwezigheid van giftige stoffen te detecteren en om een indicatie van het type remming te kunnen geven. Daardoor kan de sensor gebruikt worden als een vroeg waarschuwingssysteem. Dit resultaat is een belangrijke bijdrage aan het beveiligen van de waterkwaliteit.

List of symbols

I	current
e	measurement or estimation error
E^0	standard reduction potential of half reaction
$E^{0'}_x$	formal potential of redox complex
Ean	anode potential
Eelectron_donor	electron donor potential under the present conditions
Es/p	ES/P, thermodynamical anode potential (V), which is the theoretical anode potential as calculated using the Nernst equation
F	Faradays constant (C/mol)
f	RT/F
i	current density
Iex	exchange current density
If	forward anodic current density
Imax	maximal current
J	squared error of the estimate
k0	standard heterogeneous rate constant (cm/s)
K1	kinetic rate constant 1
k1-k6	reaction rate constant
K2	kinetic rate constant 2
Ki	kinetic inhibition constant
Km	substrate affinity constant
Kp	product inhibition constant
n	number of electrons
N	number of measurements
P	product
Q	flow
Q	charge
r	substrate consumption rate
$r_{transfer}$	electron transfer rate

r_{max}	maximum reaction rate
Rext	external resistance
S	substrate concentration
T	temperature
t	time
U	energy
V	volt
V	volume
Vcell	cell voltage
X_c	redox component complex
X_{ox}	oxidized redox component
X_{red}	reduced redox component
X_T	total amount of redox component

α	transfer coefficient (-)
ε	error of measurement i
η	overpotential
χ	toxic component concentration

SDS	sodium dodelyl sulfate
MFC	microbial fuel cell
OLS	ordinary least squares
WLS	weighted least squares
ocv	open circuit potential/ zero current potential

List of publications

Stein NE, Hamelers HVM, Buisman CNJ. 2010. Stabilizing the baseline current of a microbial fuel cell-based biosensor through overpotential control under non-toxic conditions. *Bioelectrochemistry* 78:87-91.

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T.H.J.A. Sleutels, N.E. Stein, 'Biobrandstofcel: een nieuw perspectief voor groene elektriciteit', *NPT Procestechnologie* editie 2007-5, pp. 11-13

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Now it is time to wait for the final words: hora est.

Curriculum Vitae

Nienke Stein was born on August 24, 1981 in De Bilt, The Netherlands. After high school at vwo-level in Utrecht and a year of traveling in Australia, she moved to Wageningen. Here she began to study biotechnology. In the second year a case study on bacteria in an anaerobic water treatment plant started her interest in environmental biotechnology. During her specialization in Process Technology she did a thesis at the subdepartment of Environmental Technology on the biological oxidation of hydrogen sulfide. A second thesis was performed at Delft University of Technology on mixed culture fermentations. An internship led to Penn State University in the US where she did research on microbial fuel cells. Finally an internship at Intervet International introduced her to working in a company.

The work on microbial fuel cells was continued when she started in 2006 as a PhD-student for Wageningen University. The project was a collaboration between the subdepartment of Environmental Technology and the Systems and Control group. She performed the research described in this thesis at Wetsus, centre of excellence for water technology.

In 2009 she also started working part-time as a chemistry teacher at a high school in Leeuwarden.

In 2011, after finishing her PhD-research, Nienke started working at TNO as physical-chemical technologist in the water treatment-group.

Sense certificate