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A two-population bio-electrochemical model of a microbial fuel cell

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ABSTRACT

This work presents a two-population model describing the competition of anodophilic and methanogenic microbial populations for a common substrate in a microbial fuel cell (MFC). Fast numerical solution of the model is provided by using ordinary differential equations to describe biomass growth and retention in the anodic compartment. The model parameters are estimated and validated using experimental results obtained in four continuous-flow air–cathode MFCs operated at various external resistances and organic loads. Model analysis demonstrates the influence of operating conditions on MFC performance and suggests ways to maximize MFC power output. The model is suitable both for process optimization and on-line control applications.

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1. Introduction

Organic waste represents a significant source of renewable energy. However, this energy cannot be recovered by traditional chemical processes because organic matter in waste has a complex composition and is often highly diluted. One promising option is to use anodophilic bacteria that oxidize organic matter and transfer electrons to an anode of a microbial fuel cell (MFC) (Logan et al., 2006; Logan and Regan, 2006). This biological system has the potential to convert a large variety of organic compounds into electricity (Debabov, 2008). One of the most attractive energy sources for MFCs is wastewater, where electricity production is combined with wastewater treatment.

MFC application in wastewater treatment implies the presence of a mixed microbial population, including methanogenic, anodophilic, anaerobic and facultative microorganisms (Arcand et al., 1994; Moletta et al., 1986; Quarmby and Forster, 1995). While not all microorganisms take part in electricity generation, the existence of mixed microbial populations might affect the performance of the MFC (Harmand et al., 2008; Sheintuch et al., 1995; Wanner and Gujer, 1985). The operational conditions of MFC may also affect the equilibrium between methanogenic and anodophilic microbial populations (Aelterman et al., 2008).

The major bottleneck of MFC application is its relatively low power density. Although recent research efforts have led to a four order of magnitude increase in power density such that a power

output of 50–200 W/L_a (per anodic compartment volume) was obtained in air–cathode MFCs (Logan, 2008; Rabaey and Verstraete, 2005), this power output density is not high enough for industrial applications. MFC power density can be increased by improving its design but also by optimizing operating conditions such as carbon source load (Aelterman et al., 2008; Behera and Ghangrekar, 2009; Jadhav and Ghangrekar, 2009), pH (He et al., 2008; Rozendal et al., 2006), and electrical load (Aelterman et al., 2008; Woodward et al., 2009). The task of process and design optimization can be facilitated by process modeling, which may allow for a deeper understanding of the operation and help to identify its main bottlenecks.

Despite its importance few MFC models have been reported in the literature. One of the first models was proposed by Zhang and Halme (1995). This single-population model considered the use of an external mediator to transfer electrons and established a dependence between power output and the external mediator concentration. However, modern MFCs don't use external mediators (Bond et al., 2002; Kim et al., 2002). A one dimensional model of a mediatorless MFC with a detailed description of bio-electrochemical reactions was developed by Marcus et al. (2007a). This model was used to evaluate biofilm-related limitations of MFCs. It distinguished between active and non-active anodophilic biomass but the existence of several microbial populations competing for the same substrate was not considered.

Picioreanu et al. (2007, 2008) developed a detailed 3-dimensional model of anodic compartment biofilm. The principal aim of this model was to analyze biofilm formation and species distribution within the biofilm. While Zhang and Halme (1995) oversimplified MFC dynamics, the model of Picioreanu et al. (2007, 2008) provided detailed descriptions of the anodophilic and non-anodophilic populations in the biofilm by using partial differential equations, which led to long computational times.

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The study presented below argues that a simpler model consisting of ordinary differential equations can adequately describe MFC microbial populations at various operating conditions while being suitable for process optimization and control. The model focuses on anodophilic and methanogenic microbial populations and their dependence on operational parameters such as the substrate feeding rate (organic load) and external resistance (electrical load).

2. Methods

2.1. MFC design and operation

Four identical single-chamber membraneless air–cathode MFCs were constructed using polycarbonate and stainless steel plates. Each MFC had an anodic chamber volume of 50 mL. The anodes were made of 5 mm thick carbon felt measuring 10 cm × 5 cm (SGL Canada, Kitchener, ON, Canada). The cathodes were made of a gas diffusion electrode (GDE LT 120EW, E-TEK Division, PEMEAS Fuel Cell Technologies, Somerset, NJ, USA). The electrodes were separated by a J-cloth (Fan et al., 2007) with a thickness of 0.7 mm. An external recirculation loop was installed for improved mixing of the anodic liquid. Anodic chamber temperature was controlled by a PID temperature controller (Model JCR-33A, Shinko Technos Co., Ltd., Osaka, Japan) and a heating plate (120 V–10 W, Volton Manufacturing Ltd, Montreal, QC, Canada). Each MFC had one independent external resistance, which could be set manually. A simplified MFC diagram is shown in Fig. 1.

Each MFC was inoculated with 5 mL of anaerobic sludge with a VSS of approximately 50 g L^{−1} (Lassonde Inc., Rougemont, QC, Canada). Prior to MFC-1 inoculation the sludge was heat-treated for 20 min at 100 °C. MFC-2, MFC-3, and MFC-4 were inoculated with non-heat-treated anaerobic sludge.

Solutions of dilution water, nutrients, and carbon source (acetate) were continuously fed to all MFCs. The MFCs were operated at several influent concentrations of acetate as described in Table 1 while maintaining an HRT of 7.5 h.

Polarization tests (PTs) were performed for each MFC weekly. These tests were performed first by disconnecting the MFC's external resistance for 30 min, and then measuring its open circuit voltage (OCV). Subsequently the external resistance values were decreased with measurements acquired after a delay of 10 min

Fig. 1. A diagram of the MFC set-up showing anode and cathode separated by a J-cloth, heating plate, and sampling ports.

Table 1

Operating conditions. External resistances were adjusted after each polarization test.

MFC	Inoculum heat treatment	Experiment length (days)	Influent acetate concentration (mg-S L ^{−1})	External resistance setting
1	Yes	52	310; 620; 1250; 2500	Set ~10–25 Ω above R_{int}
2	No	60	325; 650; 1275; 2550	Set ~10–25 Ω above R_{int}
3	No	55	275; 550; 1100; 2200	Set ~40–60 Ω above R_{int}
4	No	35	500; 1000; 2000	Set to 5 Ω (below R_{int})

after each resistance change. The polarization tests were used to build polarization curves (PC), i.e. voltage vs. current plots from where the MFC's total internal resistance was estimated (Logan, 2008). Operating conditions for all MFCs are summarized in Table 1.

2.2. Analytical methods

Acetate was analyzed on an Agilent 6890 gas chromatograph (Wilmington, DE, USA). Gas production was measured on-line using bubble counters connected to glass U-tubes and interfaced with a data acquisition system. Gas composition was measured using a gas chromatograph (6890 Series, Hewlett Packard, Wilmington, DE). Detailed description of analytical methods can be found in Tartakovsky et al. (2008a).

2.3. Numerical methods and calculations

Integration of model equations was performed in MATLAB (The Mathworks Inc., Natick, MA, USA) using a variable order integration method for stiff differential equations (ode15s subroutine). Model parameters were estimated by minimizing the following objective function:

$$F_{obj} = \sum_{i=1}^m \frac{w_i}{n_i} \left(\sum_{j=1}^n (\bar{y}_{j,i}^{exp} - \bar{y}_{j,i}^{sim})^2 \right) \quad (1)$$

where $\bar{y}_{j,i}^{exp}$ and $\bar{y}_{j,i}^{sim}$ are the normalized experimental and simulated values of the i -th state variable at i -th sampling time, respectively; w_i is the weight constant of the i -th state variable; n is the number of measurements (samples) of the i -th state variable, and m is the number of measurable state variables ($i = 3$).

The objective function was minimized using the Nelder–Mead simplex algorithm (Nelder and Mead, 1965) implemented in the FMINS subroutine of the MATLAB Optimization Toolbox (Mathworks Inc., Natick, MA, USA).

Model outputs were compared with experimental results using the calculations of the mean squared error (MSE):

$$MSE = \frac{1}{n} \sum_{i=1}^n \left(\frac{y_i^{exp} - y_i^{sim}}{\bar{y}} \right)^2 \quad (2)$$

where $\bar{y}$ is the measured average value of a state variable during the test.

3. Results and discussion

3.1. Model formulation

The proposed model is focused on the description of microbial populations and corresponding bio-electrochemical reactions in the anodic compartment of the MFC while a non-limiting cathode

reaction rate is assumed. The charge transfer mechanism from a carbon source to anode is assumed to involve an intracellular mediator, which exists in the reduced and oxidized forms (i.e. NADH/NAD⁺). Also, extracellular electron transfer via nanowires or direct contact with the anode is assumed (Reguera et al., 2005). The model considers the existence of anodophilic (attached) and methanogenic (attached or suspended) populations and takes into account acetate as the sole carbon source. The proposed conceptual model is summarized in Fig. 2. In addition, the following assumptions were made to achieve a fast numerical solution of the model:

1. the carbon source is well distributed in the anodic compartment, therefore ideal mixing is assumed and substrate gradient in the biofilm is neglected;
2. uniform distribution of microbial populations in the anodic compartment biofilm is assumed and biomass retention due to biofilm formation is described by a two phase growth-washout model described below;
3. gas transport (e.g. oxygen, methane) through the porous cathode is neglected;
4. multiplicative Monod kinetics is used to describe growth kinetics of anodophilic microorganisms (Marcus et al., 2007a);
5. a constant pool of intracellular electron transfer mediator in a microorganism is assumed;
6. temperature and pH are considered fully controlled and kept constant.

The conceptual model can be described by the following equations representing substrate and intracellular mediator transformation by the anodophilic microorganisms as well as substrate transformation by the methanogenic microorganisms:

where S is the carbon source (acetate). M_{red} and M_{ox} are the reduced and oxidized forms of the intracellular mediator, respectively.

The dynamic mass balance equations of the model can be divided into anodic compartment balances and intracellular balances, as described below.

Fig. 2. Conceptual MFC model showing carbon source (acetate) conversion in the anodic compartment of an MFC by methanogenic (attached or suspended cells) and anodophilic (attached) microorganisms. M_{red} and M_{ox} denotes reduced and oxidized forms of an intracellular mediator, respectively.

3.2. Anodic compartment material balances

For a continuous flow MFC with biofilm retention the influent and effluent flow rates are equal and the following substrate and microorganisms material balances can be written:

$$\frac{dS}{dt} = -q_a x_a - q_m x_m + D(S_0 - S) \quad (6)$$

$$\frac{dx_a}{dt} = \mu_a x_a - K_{d,a} x_a - \alpha_a D x_a \quad (7)$$

$$\frac{dx_m}{dt} = \mu_m x_m - K_{d,m} x_m - \alpha_m D x_m \quad (8)$$

where S and S_0 is the substrate concentration and the influent substrate concentration, respectively (mg-S L^{-1}); x_a and x_m is the concentration of anodophilic and methanogenic microorganisms, respectively (mg-x L^{-1}); t is the time (d); q_a and q_m is the substrate consumption rates by anodophilic and methanogenic microorganisms, respectively ($\text{mg-S mg-x}^{-1} \text{d}^{-1}$); μ_a , μ_m are the growth rates (d^{-1}); D is the dilution rate ($D = F_{in} V^{-1}$), F_{in} is the incoming flow (L d^{-1}), V is the anodic compartment volume (L); K_d is the decay rate (d^{-1}); and α_a , α_m are the dimensionless biofilm retention constants.

Biofilm formation and retention was simulated using a two-phase biofilm growth model. It was assumed that in the growth phase no biofilm washout occurs so that a batch reactor balance can be used. In the stationary phase an equilibrium between biofilm growth and washout is reached when biofilm approaches its steady state thickness (Tartakovsky et al., 2008b; Wanner and Gujer, 1986). Therefore, biofilm washout is equal to net biofilm growth, i.e. a CSTR reactor balance can be used. This two-phase biofilm model is described by CSTR material balances with a biomass retention parameter α defined as follows (Tartakovsky et al., 2008b):

$$\alpha = \left(\frac{1 + \tanh[K_x(x_a + x_m - X_{MAX})]}{2} \right) \quad (9)$$

where K_x is the steepness factor [L mg-x^{-1}]; and X_{MAX} is the maximal attainable biomass concentration [mg-x L^{-1}].

Visual inspection of the MFCs at the end of each test showed that at least some anaerobic sludge used for MFC inoculation remained in the anodic compartment. To account for the existence of these suspended methanogenic microorganisms, different X_{MAX} values were used for anodophilic and methanogenic populations with $X_{max,m} > X_{max,a}$.

The methane production rate was assumed to be directly proportional to the amount of substrate consumed by methanogenic microorganisms, hence

$$Q = Y_{CH_4} q_m x_m V \quad (10)$$

where Y_{CH_4} is the methane yield ($\text{mL-CH}_4 \text{ mg-S}^{-1}$).

3.3. Intracellular material balances

Since the intracellular mediator exists either in its oxidized or reduced form and a constant pool of the mediator per anodophilic microorganism is assumed, the following balance equations can be written:

$$M_{Total} = M_{red} + M_{ox} \quad (11)$$

$$\frac{dM_{ox}}{dt} = -Y q_a + \gamma \frac{I_{MFC}}{mF} \frac{1}{V x_a} \quad (12)$$

where M_{ox} is the oxidized mediator fraction per anodophilic microorganism [mg-M mg-x^{-1}]; M_{red} is the reduced mediator fraction per each anodophilic microorganism [mg-M mg-x^{-1}]; M_{Total} is the total mediator fraction per microorganism [mg-M mg-x^{-1}]; Y is the mediator yield [mg-M mg-S^{-1}]; I_{MFC} is the MFC current [A]; F is

the Faraday constant [A d mole^{-1}]; γ is the mediator molar mass [$\text{mg-M mol}_{\text{med}}^{-1}$]; and m is the number of electrons transferred per mol of mediator [$\text{mol-e}^{-} \text{mol}_{\text{med}}^{-1}$].

In Eq. (12), the rate of oxidized mediator formation from the reduced form is described by Faraday's law, while the consumption rate corresponds to carbon source consumption by the anodophilic microorganisms according to Eq. (3).

3.4. Kinetic equations

For anodophilic bacteria, the growth rate was assumed to be limited by concentrations of substrate and oxidized form of the mediator, while for methanogenic microorganisms the growth rate was limited only by the substrate concentration. Also, the substrate consumption rates by each microorganism were assumed to be proportional to corresponding growth rates. By using multiplicative Monod kinetics the following equations can be written:

$$\mu_a = \mu_{\max,a} \frac{S}{K_{S,a} + S} \frac{M_{ox}}{K_M + M_{ox}} \quad (13)$$

$$\mu_m = \mu_{\max,m} \frac{S}{K_{S,m} + S} \quad (14)$$

$$q_a = q_{\max,a} \frac{S}{K_{S,a} + S} \frac{M_{ox}}{K_M + M_{ox}} \quad (15)$$

$$q_m = q_{\max,m} \frac{S}{K_{S,m} + S} \quad (16)$$

where $\mu_{\max}$ is the maximum growth rate [d^{-1}]; $q_{\max}$ is the maximum substrate consumption rate [$\text{mg-S mg-x}^{-1} \text{d}^{-1}$]; and K is the half-saturation (Monod) constant [mg-S L^{-1} or mg-M L^{-1}].

3.5. Electrochemical equations

MFC voltage can be calculated using theoretical values of electrode potentials and subtracting ohmic, activation, and concentration losses. By applying Ohm's law to compute the MFC current and ohmic losses ($\eta_{ohm} = I_{MFC} R_{int}$), the balance can be expressed as:

$$I_{MFC} R_{ext} = E_{thermo} - I_{MFC} R_{int} - \eta_{conc} - \eta_{act} \quad (17)$$

where η_{act} is the activation over-potential [V]; η_{conc} is the concentration over-potential [V]; E_{thermo} is the thermodynamic MFC maximum voltage [V]; R_{ext} is the external resistance [Ω]; R_{int} is the internal resistance [Ω];

The concentration losses can be calculated using the Nernst equation assuming the reference concentration as the total mediator concentration (Marcus et al., 2007a):

$$\eta_{conc} = \frac{RT}{F} \ln \left(\frac{M_{red}^{Reference}}{M_{red}} \right) = \frac{RT}{F} \ln \left(\frac{M_{Total}}{M_{red}} \right) \quad (18)$$

where R is the ideal gas constant [$\text{J K}^{-1} \text{mol}^{-1}$], and T is the MFC temperature [K].

The activation losses in Eq. (17) can be calculated by the Butler–Volmer equation. These losses mainly occur at high external resistance (Fuel Cell Handbook (2005)), yet have little influence on the MFC operation under optimal or close to optimal electrical load, i.e. when the external resistance is close to the internal resistance value (Logan, 2008). Therefore, activation losses are expected to be low, so that the Butler–Volmer equation can be approximated by the following equation (Newman and Thomas-Alyea, 2004):

$$\eta_{act} = \frac{I_{MFC}}{A_{Sur} i_{0,ref}} \frac{RT}{mF} \left(\frac{M_{red}}{M_{ox}} \right) \quad (19)$$

where $i_{0,ref}$ is the exchange current density for mediator oxidation in reference conditions [A m^{-2}]; A_{Sur} is the anode surface area [m^2].

MFC current can be calculated by combining Eqs. (17)–(19) and replacing E_{thermo} in Eq. (17) with an experimentally measured value of open circuit voltage (E_{OCV}). As shown in Cannarozzo et al. (2007), the concentration losses can be more accurately represented by addition of boundary conditions at high current densities. To avoid the discontinuity when integrating model equations the boundary conditions were replaced with a Monod-like term, which limits calculated MFC current at low values of M_{red} :

$$I_{MFC} = \frac{(E_{OCV} - \eta_{conc} - \eta_{act})}{(R_{ext} + R_{int})} \frac{M_{red}}{\varepsilon + M_{red}} \quad (20)$$

where ε is the constant [mg-M mg-x^{-1}]; $\varepsilon \sim 0$.

Notably, if activation losses are neglected a simplified expression for current calculation is obtained:

$$I_{MFC} = \frac{(E_{OCV} - \eta_{conc})}{(R_{ext} + R_{int})} \frac{M_{red}}{\varepsilon + M_{red}} \quad (21)$$

3.6. Parameter estimation and model revision

Experimental results obtained in MFC-1 and MFC-2 tests were used to estimate model parameters, while the two remaining data sets (MFC-3 and MFC-4) were reserved for model validation. Importantly, MFC-1 was inoculated with heat-treated sludge. The heat-treatment procedure deactivated methanogenic microorganisms in the inoculum. Consequently, kinetic parameters of the anodophilic microorganisms were estimated using this data set by setting the methanogenic growth rate to zero.

Estimation of model parameters was carried out in several steps. First, Fisher information matrixes (FIM) were used to select identifiable parameters. Because of the limited number of measurable state variables FIM analysis showed that several model parameters cannot be estimated with an acceptable accuracy. The identifiable parameters were $\mu_{\max,a}$, $q_{\max,a}$, Y , $\mu_{\max,m}$, and $q_{\max,m}$. With a 95% confidence level, the intervals of confidence found for these parameters were 3.01%, 0.11%, 0.91%, 3.4%, and 3.75%, respectively. Other model parameters were chosen based on available literature data. In particular, all half-rate constants were not identifiable, since to be identified with a reasonable level of confidence these parameters require experimental data acquired in a broad range of substrate concentrations, including tests at low substrate concentration, which were unavailable. Therefore, half-rate constants were chosen based on available literature values. Once the identifiable model parameters were selected, the parameter estimation procedure was carried out by minimizing the objective function defined in Eq. (1) using experimentally measured values of acetate concentration, MFC output voltage, and methane production.

First parameter estimation attempts were carried out using MFC-1 data set. Large discrepancies between the experimental and simulated values of the MFC voltage were obtained as one can see from the analysis of MSE values presented in Table 2. MFC startup appeared to be most difficult to model, since for the first 15–20 days of the experiment the simulated output voltage was significantly higher than the measured values. This difference

Table 2

A comparison of mean squared errors (MSE) calculated for MFC-1 data set at different calculation methods of E_{OCV} , R_{int} , and η_{act} .

State variable	$E_{OCV} = \text{const}$ $R_{int} = \text{const}$ $\eta_{act} = 0$	$E_{OCV} = \text{const}$ Eq. (22) for R_{int} $\eta_{act} = 0$	Eq. (23) for E_{OCV} $R_{int} = \text{const}$ $\eta_{act} = 0$	Eq. (23) for E_{OCV} Eq. (22) for R_{int} $\eta_{act} = 0$	Eq. (23) for E_{OCV} Eq. (22) for R_{int} Eq. (19) for η_{act}
Voltage	0.43	0.37	0.27	0.14	0.09
Substrate	0.36	0.36	0.44	0.44	0.45

was attributed to the observed variations in the values of E_{OCV} and R_{int} estimated during the MFC experiments. Indeed, during the first days of MFC-1 operation E_{OCV} and R_{int} were estimated at 0.38 V and 175 Ω respectively, while after 17 days of operation these values stabilized at 0.66 V and 25 Ω . These results agree with the observations of Aelterman et al. (2008), which also demonstrate improved MFC performance after the start-up period.

To improve model accuracy during the start-up period the E_{OCV} and R_{int} values were linked to the concentration of anodophilic microorganisms. This agrees with the known dependence of cell voltage and internal resistance on the catalyst load (Chaparroa et al., 2009). The following dependence was used to describe the observed changes of internal resistance:

$$R_{int} = R_{MIN} + (R_{MAX} - R_{MIN})e^{-K_R x_a} \quad (22)$$

where R_{MIN} is the lowest observed internal resistance [Ω], R_{MAX} is the highest observed internal resistance [Ω], K_R is the constant, which determines the curve steepness [$L\ mg^{-1}$];

Similarly, MFC open circuit voltage was observed to increase during the start-up period. Cathode and anode open circuit potential (OCP) measurements against an Ag/AgCl reference electrode showed that the cathode OCP remained at 140–160 mV throughout the tests, while the anode OCP became more negative. This trend was attributed to anode colonization by anodophilic microorganisms, i.e. increasing biocatalyst load, and described by the following dependence:

$$E_{OCV} = E_{MIN} + (E_{MAX} - E_{MIN})e^{\frac{1}{K_x x_a}} \quad (23)$$

where E_{MIN} is the lowest observed E_{OCV} value [V] and E_{MAX} is the highest observed E_{OCV} value [V]. The R_{min} , R_{max} , E_{min} , and E_{max} values were obtained from the polarization tests. Parameters K_x and K_R were identified using available voltage measurements during the first 20 days of MFC-1 operation.

To evaluate the impact of the proposed dependencies on model accuracy the parameter estimation procedure was repeated. First, Eq. (22) was introduced while keeping E_{OCV} constant. Then Eq. (23) was introduced while keeping R_{int} constant and finally both

Eq. (22) and (23) were used. The results presented in Table 2 clearly show that the accuracy of voltage prediction was improved. In addition, model outputs with activation losses calculated according to Eq. (19) were compared with a simplified electrochemical balance, which neglects activation losses (Eq. (21)). No significant difference in model accuracy was obtained (Table 2) thus confirming that the activation losses can be neglected. Consequently, electrochemical balance Eq. (20) was used in all following calculations.

Once model structure was refined by including Eqs. (22) and (23) and parameters related to anodophilic microorganisms were identified based on MFC-1 data set, the remaining identifiable parameters related to methanogenic activity ($\mu_{max,m}$ and $q_{max,m}$) were identified using the MFC-2 data set. The resulting values of model parameters are given in Table 3. The estimated values of $\mu_{max,m}$ and $q_{max,m}$ were within the range of parameter values used in ADM1 (Batstone et al., 2000). Also, Table 3 contains values of non-identifiable model parameters that were chosen based on the literature review.

The total mediator fraction per microorganism was assumed to be 5% (Table 3) to avoid numerical problems in the model solution. Although this value was arbitrary, it did not affect model outputs since Eq. (18) uses a M_{Total}/M_{red} ratio.

A comparison of model outputs with acetate and voltage measurements during MFC-1 and MFC-2 tests is shown in Fig. 3(a–d). Each simulation required less than 2 s on a PC with 2.99 GHz dual core processor. Model outputs closely follow experimentally measured acetate concentrations at low and moderate acetate loads. A larger discrepancy was observed at the highest influent acetate concentration around day 40. This was attributed to a power failure during this operating period, which likely led to an overestimation of the influent acetate concentration. Voltage predictions (Fig. 3b and d) agreed well with the experimental measurements, both during normal MFC operation and during polarization tests. In more detail voltage changes during the polarization test performed on day 47 are shown in Fig. 3e and the predicted changes in M_{red} and M_{ox} during this test are shown in Fig. 3f. This simulation suggests that MFC operation in the open circuit mode at the begin-

Table 3
Model parameters. Non-identifiable model parameters were selected based on modeling results of Marcus et al. (2007b), Batstone et al. (2000), and Tartakovsky et al. (2008b).

Parameter	Description	Value	Dimension	Notes
F	Faraday constant	96,485	A s mole ⁻¹	Physical constant
R	Ideal gas constant	8.314472	J K ⁻¹ mol ⁻¹	Physical constant
T	MFC temperature	298.15	K	Physical constant
Y	Yield in Eq. (12)	22.75	mg-M mg-S ⁻¹	Estimated
Y_{CH_4}	Methane yield	0.3	mL-CH ₄ mg-S ⁻¹	Assumed
$q_{max,a}$	Maximum anodophilic reaction rate	8.48	mg-S mg-x ⁻¹ d ⁻¹	Estimated
$q_{max,m}$	Maximum methanogenic reaction rate	8.20	mg-S mg-x ⁻¹ d ⁻¹	Estimated
$\mu_{max,a}$	Maximum anodophilic growth rate	1.97	d ⁻¹	Estimated
$\mu_{max,m}$	Maximum methanogenic growth rate	0.1	d ⁻¹	Estimated
$K_{S,a}$	Half-rate constant of anodophilics	20	mg-S L ⁻¹	Assumed
$K_{S,m}$	Half-rate constant of methanogens	80	mg-S L ⁻¹	Assumed
m	Electrons transferred per mol of mediator	2	mole ⁻¹ mol _{mediator} ⁻¹	Assumed
γ	Mediator molar mass	663,400	mg-M mol _{mediator} ⁻¹	Assumed
M_{Total}	Mediator fraction	0.05	mg-M mg-x ⁻¹	Assumed
K_M	Mediator half-rate constant	$0.2^* M_{Total}$	mg-M L ⁻¹	Assumed
$K_{d,a}$	Decay rate of anodophilic microorganisms	$0.02^* \mu_{max,a}$	d ⁻¹	Assumed
$K_{d,m}$	Decay rate of methanogenic microorganisms	$0.02^* \mu_{max,a}$	d ⁻¹	Assumed
$X_{max,a}$	Anodophilic biofilm space limitation	512.5	mg-x L ⁻¹	Assumed
$X_{max,m}$	Methanogenic biofilm space limitation	525	mg-x L ⁻¹	Assumed
K_x	Parameter in Eq. (9)	0.04	L mg-x ⁻¹	Estimated
R_{MIN}	Minimum internal resistance	25	Ω	Measured
R_{MAX}	Maximum internal resistance	2000	Ω	Measured
E_{MIN}	Minimum E_{OCV}	0.01	V	Measured
E_{MAX}	Maximum E_{OCV}	0.61–0.66 [*]	V	Measured
K_R	Parameter in Eqs. (22) and (23)	0.006	L mg-x ⁻¹	Estimated

^{*} Average OCP values after first 20 days of MFC operation were 0.66, 0.66, 0.61, and 0.61 V for MFC-1 to MFC-4, respectively.

Fig. 3. Comparison of model outputs with experimentally measured values of acetate and output voltage during MFC-1 (a and b) and MFC-2 (c and d) operation. MFC-2 output voltage during the polarization test at day 47 (indicated by an arrow in panel d) and predicted values of M_{red} and M_{ox} during this polarization test are shown in panels e and f, respectively.

ning of the polarization tests resulted in M_{red} accumulation. Progressive decrease of R_{ext} during the test led to a low level of M_{red} .

Statistical analysis of model outputs is provided in Table 4. Large MSE values were obtained for methane due to a poor fit between the predicted and measured methane production rates (Table 4). This discrepancy can be explained by the low accuracy of methane measurements. Because of the small volume of the anodic compartment (50 mL), methane production rates were between 0.5 and 10 mL d⁻¹. Measuring such small flow rates presented a technical challenge resulting in a large standard deviation of the measurements. Also, we hypothesize that at least part of the methane produced in the anodic compartment was not accounted for because its diffusion through the cathode.

In more details, model accuracy can be estimated from the analysis of Fig. 3e, which compares model outputs and measured values of MFC-2 voltage during the polarization test conducted at day 47 of MFC-2 operation. To evaluate model accuracy in a broad R_{ext} range, R_{ext} was changed between 10 k Ω and 15 Ω during this polarization test. Also, the predicted values of the oxidized and reduced form of the intracellular mediator during this polarization

test are shown in Fig. 3f. As expected, open circuit MFC operation leads to an increase in the reduced mediator form. A decrease in R_{ext} value during the polarization test increases anodophilic activity thus leading to an increase in M_{ox} . After the polarization test, R_{ext} was maintained at 15 Ω for 24 h, which explains low level of M_{red} and low output voltage after the polarization test. R_{ext} was changed to 35 Ω one day later (not shown).

In the absence of more detailed information, the internal mediator molar mass (γ) and the number of electrons transferred per mol of mediator (m) were assumed to be equal to that of NADH

Table 4

MSE calculated by comparing experimental results and model outputs for parameter estimation (MFC-1, 2) and model validation (MFC-3, 4) data sets.

State variable	MFC-1	MFC-2	MFC-3	MFC-4
Voltage	0.14	0.11	0.036	0.032
Substrate	0.44	0.87	0.065	0.053
Methane	n/a	23.47	1.00	0.027

n/a, Not available.

(Table 3). However, the electron transfer process is believed to be more complex than the NADH/NAD⁺ cycle. Since γ , m and Y (yield) values are included in Eq. (12), the parameter estimation procedure aimed at estimating Y might account for the arbitrary choice of γ and m without affecting model predictions.

3.7. Model validation

The model was validated using MFC-3 and MFC-4 tests that were not used in the parameter estimation procedure. Importantly, MFC-3 was operated at external resistances, which were overall higher in comparison to those used during MFC-2 operation as described in Table 1. MFC-4 was operated at a low external resistance of 5 Ω , which was much lower than external resistance values used in other tests. Therefore experimental results used for model validation were acquired using different operating conditions thus allowing for model predictive capacity validation. When validating the model, parameters were kept unchanged except for OCV values. These values were estimated for each MFC using polarization

curves. Fig. 4 shows a comparison of model predictions with the results obtained in MFC-3 and MFC-4 tests.

For MFC-3, a satisfactory agreement was obtained between measured and predicted output voltage and effluent acetate (Fig. 4a, c, and e). In part this may be due to the similarity between the operation conditions of MFC-2 and MFC-3, since both MFCs had their external resistance periodically adjusted during the length of the experiment so that R_{ext} remained above the estimated R_{int} value.

As mentioned above MFC-4 was operated at R_{ext} much below the estimated R_{int} value for this MFC. Therefore, a comparison between experimental results and model outputs (Fig. 4b, d, and f) for MFC-4 confirmed the predictive capacity of the model. Both experimental measurements and model predictions showed low voltage and low power output due to low external resistance.

A comparison of predicted and measured Coulombic efficiencies and averaged methane production values are presented in Fig. 5. This comparison was based on MFC operation at pseudo steady-state conditions, e.g. the biomass growth period (first 25 days of MFC operation) and MFC operation at high acetate load were ex-

Fig. 4. Comparison of model outputs with experimentally measured values MFC-3 (a, c and e); MFC-4 (b, d and f). Polarization curves acquired on day 30 of MFC-3 operation (digital resistor with a step of 2.5 Ω) and on day 28 of MFC-4 operation (manual resistor control) are shown in panels e and f (days are also indicated by arrows in panels c and d). In panels a and b, 'eff' denotes effluent.

Fig. 5. Average Coulombic efficiency (a) and average methane production (b). Data acquired during the biofilm growth phase (first 25 days) and MFC operation at a high acetate load were excluded from calculation.

cluded from consideration. Yet, variations in influent acetate concentration resulted in relatively large standard deviations. As discussed above, large standard deviations of methane flow measurements were attributed to difficulties in measuring small amounts of methane produced in the anodic compartment and due to possible losses resulting from methane diffusion through the porous cathode. More accurate methane measurements can potentially be made by significantly increasing the anodic compartment volume and measuring the methane percentage in the cathodic chamber off-gas. Nevertheless, the link between Coulombic efficiency and methane production can be clearly observed, as the highest Coulombic efficiency was observed in MFC-1, which had no methanogens, and in MFC-4, which was operated at $R_{ext} = 5 \Omega$. Coulombic efficiency was lower and methane production was higher in MFC-2 and MFC-3, which were operated at higher R_{ext} values.

Statistical analysis of parameter estimation and validation results was performed by comparing MSE values. Analysis of MSE calculations presented in Table 4 show that model validation produced MSE values that are smaller than those obtained in the parameter estimation procedure. In part this difference can be explained by a larger number of voltage measurements during MFC-1 and MFC-2 startup (Fig. 3b and d), where model predictions are less accurate. Nevertheless, it also confirms excellent predictive capacity of the model. In particular, the model was able to predict MFC dynamics observed in the MFC-4 test, which was operated outside the range of operating conditions used in the parameter estimation procedure.

3.8. The influence of external resistance and organic load on microbial populations

The four MFCs used for model identification and validation were operated at various organic loads and external resistances as outlined in Table 1. A comparison of MFC performances suggested that both of these operating parameters significantly affected MFC power output. Obviously, it was maximized when R_{ext} was equal or slightly above the internal resistance value (Aelterman et al., 2008; Woodward et al., 2009). By periodically acquiring polarization curves and adjusting R_{ext} to maintain it about 5–10 Ω above R_{int} during MFC-1 and MFC-2 operation, volumetric power output was maintained at 35–50 mW L_a⁻¹. This dependence was adequately simulated by the model as can be seen from the analysis of mean squared errors in Table 4.

In addition to this immediate impact of R_{ext} on MFC power output, it was hypothesized that R_{ext} might also influence the growth and distribution of microbial populations in the anodic compartment. Indeed, the model assumes that the growth rate of anodophilic microorganisms depends on the concentration of oxidized form of the intracellular mediator (Eq. (10)). In turn, the M_{ox} con-

centration depends on the R_{ext} value such that for the same carbon source concentration a faster growth rate of anodophilic microorganisms is expected for a MFC with lower external resistance. Overall, MFC operation at an optimal ($R_{ext} = R_{int}$) or below optimal resistance is expected to maximize growth of anodophilic microorganisms.

Acetate concentration in the anodic compartment is another factor affecting the distribution of anodophilic and methanogenic microorganisms. Since Monod-like kinetics is used to describe growth of both populations, the growth rates are maximized at high organic loads. Based on the experimental evidence (Marcus et al., 2007a) a smaller value of the half-rate constant is assigned to anodophilic microorganisms (Table 2). Thus, the outcome of the competition between anodophilic and methanogenic populations and therefore MFC power output is expected to be dependent on both acetate concentration and external resistance values.

To analyze the impact of these two operating conditions on MFC performance, the model was integrated for a period of 200 days to obtain a steady state solution and the predicted power density was calculated. In these calculations the influent acetate concentration was varied between 10 to 800 mg L⁻¹ and R_{ext} was varied between 10 and 800 Ω . The resulting 3D plot is presented in Fig. 6a. It confirms that both parameters strongly influence the power output and suggests a range of acceptable operating conditions. In particular, a significant decrease in power output is predicted both at very low and very high acetate concentrations. The former can be explained by combined limitation of the growth rate of anodophilic microorganisms by low concentrations of acetate and M_{ox} , while the later can be attributed to increased growth rate of the methanogens. Interestingly, the model analysis predicted high concentrations of anodophilic microorganisms for R_{ext} values that are equal or less than the MFC internal resistance. This result appears to be counterintuitive, as MFC power output is maximized at $R_{ext} = R_{int}$, while a sharp drop is observed if R_{ext} is below its optimal value (Fig. 6b). However, this model prediction was confirmed in MFC-4 test where R_{ext} was kept below R_{int} , yet high activity of the anodophilic populations was confirmed in the polarization tests. These polarization tests showed that as soon as the MFC external resistance is increased to its optimal value, the power output increases to values comparable to those of MFC-1 and MFC-2, which were always operated close to optimal R_{ext} (Fig. 6b).

Overall, model analysis suggested that MFC operation under non-optimal conditions (i.e. high external resistance and high organic load) not only leads to a loss in power output, but also favors growth of methanogenic microorganisms accompanied by an increased acetate utilization for methane production. This conclusion is in agreement with the results of Aelterman et al. (2008).

Although anodophilic populations are not affected at low R_{ext} values, if one tries to operate an MFC at low R_{ext} to maximize anodophilic growth and the R_{ext} is set below the MFC's internal resis-

Fig. 6. (a) Predicted steady state power output as a function of operating conditions (acetate concentration and R_{ext}) and (b) comparison of power outputs during polarization curves obtained for MFC-1, MFC-2 (close to optimal external resistance), and MFC-4 (low external resistance) tests.

tance the power production is extremely affected, as shown in Fig 6a where even a small difference between R_{int} and R_{ext} leads to large power losses. To ensure both high power output and stability of anodophilic populations, periodic adaptation of electric load (external resistance) might be required. This task can be best accomplished by a real time control algorithm, which seeks to maximize power output based on voltage or current measurements. Such maximum power point tracking (MPPT) algorithms are widely used in photovoltaic cell operation and after some adaptation can be used in MFC operation (Woodward et al., 2009). Also, model-based optimization algorithms can be developed.

4. Conclusion

This study presents a fast-convergence two-population model of an MFC. The accuracy of model predictions is improved by proposing a dependence of electrochemical parameters (E_{OCV} and R_{int}) on anodophilic biomass density. The accuracy of the parameter estimation procedure and the predictive capacity of the model is confirmed using two independent data sets. Model analysis demonstrates the influence of organic load and R_{ext} on MFC power output and long-term performance. It is demonstrated, that R_{ext} should be at least periodically adjusted to avoid proliferation of methanogens. Overall, this model provides a convenient tool for off-line process optimization and real-time process control.

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