A mathematical model for electrochemically active filamentous sulfate-oxidising bacteria

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A B S T R A C T
Oxygen and sulfide in ocean sediments can be consumed biologically over long spatial distances by way of filamentous bacteria in electron-conducting sheaths. To analyse observations, a mathematical model of these filamentous sulfur-oxidising bacteria was developed, including electrical conduction between reactive zones. Mechanisms include Nernst–Planck diffusion and migration of ions coupled with Ohm’s law for conduction along filaments, and metabolic activity throughout the filaments. Simulations predict outward biomass growth toward the boundaries of the sediment floor and top surface, resulting in two distinct zones with anode (sulfide consumption) and cathode (oxygen consumption) reactions enabled by electron conduction. Results show inward fluxes of 4.6 mmol O2/m²/d and 2.5 mmol S/m²/d, with consumption increasing with growth to final fluxes of 8.2 mmol O2/m²/d and 4.34 mmol S/m²/d. Qualitatively, the effect of varying cell conductivity and substrate affinity is evaluated. Controlling mechanisms are identified to shift from biomass limitation, to substrate limitation, and to conductivity limitations as the lengths of the filaments increase. While most observed data are reflected in the simulation results, a key discrepancy is the lower growth rates, which are largely fixed by thermodynamics, indicating that microbes may utilise secondary substrates or an alternative metabolism.

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1. Introduction

Bioelectrochemical systems (BESs) are systems whereby biological activity is coupled with external electron transfer, allowing a microbial cell to respire without a local electron acceptor or donor present in the medium. In essence, the electrode functions as the electron acceptor or donor. This mode allows the spatial decoupling of oxidation and reduction reactions in artificial systems such as the microbial fuel cell [1] or the microbial electrolysis cell [2]. However these cases form only a subset of what can be described as electroactive microorganisms (EAMs), as there is increasing evidence of such systems actually occurring in natural environments [3].

A key form of electrically active microorganism is the sulfur-oxidising filamentous bacteria recently unveiled in ocean sediment [4]. In this system, in comparison with man-made bioelectrochemical systems, microbes need to enable long-range centimetre-length conduction of electrons, rather than just short-range micrometre-length transfer to an artificial electrode. Filamentous bacteria, specifically Desulfobulbus, grown in ocean sediment, seemingly can conduct electrons over a long range between oxygen-reducing and sulfur-oxidising regions using a surrounding sheath, with the same species mediating both reactions. This is hypothesised to be enabled by conductive wires found in the sheath of the filament [4,5]. Electrochemically, this can be described as the microorganisms forming distinct anodic and cathodic zones at filament extremes, which develop as a result of the substrate and product gradients, in conjunction with growth of the bacteria.

Direct electron transfer can offer an advantage over organisms dependent upon substrate or otherwise mediated electron transfer. The key benefit is the separation of oxidative and reductive zones into regions of substrate availability, enabling growth in otherwise unfavourable areas. The presence of microorganisms in other electrochemical systems, such as microbially influenced corrosion or bioleaching of minerals, suggests that EAMs are more widespread than the already known systems of BES or filamentous bacteria in ocean sediment. However, the mechanistic principles behind EAMs are still poorly described. A model of the kinetics and cell metabolism of a single bioanode reaction has been previously proposed [6], albeit in a steady state system. Time-dependent BES models have likewise been developed, but with the metabolic pathway of the EAM either combined empirically with the potential of an anode [7], or formulated in such a way that the system of electrochemical and biological equations are entirely independent [8]. There has not been an attempt to model BES where anode and cathode regions form a continuum, which represents probably the majority

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of natural EAM systems, with the sulfide oxidising bacteria (SOB) Desulfovibulbaceae being the best characterised example. Desulfovibulbus is particularly interesting in this context, as it is a single species which operates conductively over large distances. This gives it the potential to be well characterised in terms of electrical and growth properties, provides a case where the separation of anode and cathode is continuous (e.g. along the microbial filaments), and forms an observable dynamic system [4].

This study evaluates the Desulfovibulbaceae system to develop a numerical model and to then assess the dynamics of electrochemically active microorganisms in both time and space. We propose in this model that biological anodic and cathodic zones develop through microbial growth and form a continuum and that the resultant distance between substrates, combined with an electrically active domain, allow for distinct separation of contrasting electrochemical regions. The goal of this theoretical study is to propose a set of governing mechanisms to further our understanding of this novel bioelectrochemical system, while being flexible enough to adapt to a wide range of EAMs.

2. Model description

The model is based on transport and reaction of several key solutes, with cells able to either oxidise or reduce depending on the availability of substrate, electron sinking or sourcing, and electron conduction. This allows for dynamic development of oxidative and reductive zones along the microbial filaments (Scheme 1). Electron sourcing/sinking ability is determined by a fixed intracellular mediator M (Scheme 1), which interacts with both the metabolic pathway and the conductive filament according to the local thermodynamics of the system.

2.1. Microbial reactions

2.1.1. Catabolism

Catabolism generates the energy needed for a cell by redox reactions between an electron donor and an electron acceptor. Sulfide oxidising bacteria (SOB) can use sulfide as electron donor and oxygen as electron acceptor when both are available, according to the reaction (1):

\[
\text{HS}^{-} + 2\text{O}_2 \rightarrow \text{SO}_4^{2-} + \text{H}^+ \\
(1)
\]

The experimental data [4,5] suggest that in the filamentous SOB present in marine sediments this catabolism can be separated into half-cell reactions, each performed by different cells along the filament:

\[
\text{HS}^{-} + 4\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 9\text{H}^+ + 8\text{e}^- \quad \text{sulfide oxidation} \quad (2)
\]

\[
2\text{O}_2 + 8\text{H}^+ + 8\text{e}^- \rightarrow 4\text{H}_2\text{O} \quad \text{oxygen reduction} \quad (3)
\]

\text{Scheme 1. Sulfide oxidising bacteria (SOB) with a proposed metabolic coupling of separated aerobic and anaerobic environments in electrochemically active Desulfovibulbaceae. Local metabolism (1) of the separated redox processes involves an assumed intracellular intermediate M in two redox states, exchanging electrons (2) with a conductive sheath, and electron conduction (3) between the two zones (bottom: anodic, top: cathodic). There is one biomass sub-domain, } \Omega_{\text{bio}} \text{ containing the SOB filaments and two adjacent diffusion boundary layers without biomass: aerobic top } \Omega_{\text{bl,O2}} \text{ and sulfidic bottom } \Omega_{\text{bl,HS}}. \text{ (a) Fixed filament scenario (standard): the outer boundaries } \Gamma_{\text{bl,O2}} \text{ and } \Gamma_{\text{bl,HS}} \text{ are fixed, while the biomass boundaries } \Gamma_{\text{bio,O2}} \text{ and } \Gamma_{\text{bio,HS}} \text{ are moving. (b) Free-floating scenario (moving boundary): the outer boundaries move at the same speed with the filament tips, resulting in a constant-thickness diffusion layer.}
The electrons generated by oxidation of sulfide by cells in the sediment surface are transported via a conductive sheath to the cells near the sediment depth which perform oxygen reduction (Scheme 1).

Interaction between the two half-cell catabolic reactions can be enabled by an immobile redox mediator (redox centre, possibly a cytochrome) with two oxidation states, $M_{\text{ox}}$ and $M_{\text{red}}$ [6], which transfers electrons to and from the conductive sheath joining the chain of cells. It can be assumed that the reduced (HS\textsuperscript{−}) or oxidised (O\textsubscript{2}) substrate is being consumed in the reactions (4) and (5):

$$\text{HS}^- + 4\text{H}_2\text{O} + 8M_{\text{ox}} \rightarrow 8M_{\text{red}} + \text{SO}_4^{2-} + 9\text{H}^+ \quad \text{r}_{\text{cat}, \text{HS}} \quad (4)$$

$$2\text{O}_2 + 8\text{H}^+ + 8M_{\text{red}} \rightarrow 8M_{\text{ox}} + 4\text{H}_2\text{O} \quad \text{r}_{\text{cat}, \text{O}_2} \quad (5)$$

Michaelis–Menten equations are proposed for both catabolic reactions (4) and (5), with the addition of rate dependency to available redox mediator in one of the two forms:

$$r_{\text{cat}, \text{HS}} = \frac{v_{\text{max}}}{C_{\text{Max}} + C_{\text{Med}}} C_{\text{HS}} + K_{\text{HS}} \quad (6)$$

$$r_{\text{cat}, \text{O}_2} = \frac{v_{\text{max}}}{C_{\text{Med}} + C_{\text{Max}}} C_{\text{O}_2} + K_{\text{O}_2} \quad (7)$$

where $K_{\text{HS}}$ and $K_{\text{O}_2}$ are the half-rate coefficients, and $v_{\text{max}}$, $C_{\text{Max}}$, $C_{\text{Med}}$, and $C_{\text{O}_2}$ the specific substrate uptake rates. $C_{\text{Max}}$ and $C_{\text{O}_2}$ are soluble concentrations of sulfide and oxygen, while $C_{\text{Med}}$ and $C_{\text{Med}}$ are the concentrations of the redox centre in the two oxidation states.

As these reactions happen independently using the same mediator, the presence of both substrates effectively removes the intermediate from the reaction, and is equivalent to a dual Monod equation including oxygen and sulfide.

### 2.1.2. Growth

Biomass growth can be represented by an anabolic reaction in which the cell material is synthesised based on the energy produced from each substrate, an overall expression of reduced sulfide as the anabolic electron donor needs to be stoichiometrically found. By assuming that a fixed amount of Gibbs energy must be dissipated by a cell in order to produce biomass, a multiplication factor $f$ of catabolic to anabolic reactions can be calculated (i.e. the ratio needed to generate sufficient energy to drive anabolism, see [9]). For an autotrophic sulfur-oxidising system, microorganisms use reversed electron transport (RET), for which the energy dissipation can be taken as $\Delta G^{\text{Diss}}_{\text{cat}} = -3500\text{kJ/C-mol biomass}$. The multiplication factor is therefore calculated for each metabolic pathway:

$$f_{\text{HS}} = \frac{\Delta G^{\text{Max}}_{\text{cat,HS}} - \Delta G_{\text{an}}}{\Delta G^{\text{Max}}_{\text{cat}} - \Delta G_{\text{an}}} \quad (9)$$

The microbial growth rate can then be approximated by summing the two catabolic rates weighted by the catabolic multiplication factors:

$$r_{\text{X}} = \frac{1}{f_{\text{HS}}} r_{\text{cat}, \text{HS}} + \frac{1}{f_{\text{O}_2}} r_{\text{cat}, \text{O}_2} \quad (10)$$

Finally, if all chemical species contributions to the anabolic reaction are neglected (i.e., $f_{\text{HS}}$,$f_{\text{O}_2} \gg 1$), the net rates follow directly from the stoichiometry of catabolic reactions (on a molar basis):

$$r_{\text{Max}} = -8 r_{\text{cat}, \text{HS}} + 8 r_{\text{cat}, \text{O}_2} - r_{\text{e,M}} \quad (11)$$

$$r_{\text{O}_2} = -2 r_{\text{cat}, \text{O}_2} \quad r_{\text{HS}} = -r_{\text{cat}, \text{HS}} \quad r_{\text{SO}_4} = r_{\text{cat}, \text{HS}} \quad r_{\text{H}_2} = 9 r_{\text{cat}, \text{HS}} - 8 r_{\text{cat}, \text{O}_2}$$

#### 2.2. Electrochemical reactions

The fixed mediator used earlier in catabolism can also change redox states by electron transfer with the conductive sheath:

$$M_{\text{ox}} + e^- \rightarrow M_{\text{red}} \quad r_{\text{e,M}} \quad (12)$$

The Butler–Volmer rate equation is assumed to govern the electron transfer to mediator, with the equation developed similarly to [6] and derived as a single electron transfer between two redox states of the mediator:

$$i = k^0 \left( k_1 \frac{C_{\text{Max}}}{C_{\text{Med}}} - k_2 \frac{C_{\text{Med}}}{C_{\text{Max}} + C_{\text{Med}}} \right) \quad (13)$$

This defines the current density ($i$) as a function of mediator concentrations, where $k^0$ is the exchange current density, and further kinetic coefficients $k_1$ and $k_2$ are described as:

$$k_1 = \exp \left[ - \alpha \frac{F}{RT} (\phi_k - \phi_f - \Delta F^l) \right] \quad (14)$$

$$k_2 = \exp \left[ (1 - \alpha) \frac{F}{RT} (\phi_k - \phi_f - \Delta F^l) \right] \quad (15)$$

where $\alpha$ is the transfer coefficient, $\phi$ is the electrical potential in the conductive structure of the microbial filament ($\phi_k$) and electrolyte ($\phi_f$), $\Delta F$ is the standard redox potential of the mediator, and $F$ is Faraday’s constant. Finally the reaction rate of the electron transfer mediator/conductive sheath is:

$$r_{\text{e,M}} = \frac{IA}{nF} \quad (16)$$

An active surface area $A_s$ (m$^2$-cell/m$^3$-domain) allowing electrochemical reaction (12) needs thus to be calculated (see Section 2.5). While noting that $n$ is 1 in the above equation, stoichiometry is balanced throughout and any of the processes can be potentially rate limiting.

Cytochrome c, recognized as a vital component in the metabolic chain and a necessity for the transport of protons [10], and known to boost conductivity in biological nanowires [11], is assumed to be the electroactive intermediate in this model. The redox centres are assumed here to cover all surface area of the cell, and to be in full contact with the...
conductive sheath. The same type of redox centre \( (M_{\text{ox}}/M_{\text{red}}) \) was here assumed to mediate the electron transfer of both sulfide oxidation and oxygen reduction.

2.3. Chemical reactions

As buffer concentrations in ocean sediment are unknown or largely variable, acid–base equilibria are solved with the aim of qualitative analysis. Included in the acid–base equilibria are water \( (H_2O = OH^- + H^+) \), carbon-dioxide \( (CO_2 = HCO_3^- + H^+) \), and hydrogen sulfide \( (H_2S = HS^- + H^+) \), with equilibrium constants \( K_{GICO}, K_{HICO} \) and \( K_{HS} \). The equilibrium equations are solved using fast-kinetic differential equations as for [12], allowing calculation of local pH distribution within the system [13]. Correction for ion activity and ion pairing are not included, justified by the lack of divalent species in the model, the limited pH variation modelled, and the limited ion pairs available.

2.4. Balance equations

2.4.1. Geometry, domains, phases

The model assumes only one-dimensional gradients along the depth of marine sediment. Conductive filamentous bacteria are also assumed to run along the domain length, effectively as porous electrodes with the anodic ends toward the ocean floor and the cathodic ends nearer the air/liquid interface at the top of the sediment.

Two types of sub-domains are defined in the one-dimensional model; the biomass and the boundary layer. There is one biomass sub-domain, \( \Omega_{\text{bio}} \), and two adjacent boundary layers: top (aerobic) \( \Omega_{\text{bio,O}} \) and bottom (sulfidic) \( \Omega_{\text{bio,HS}} \) (Scheme 1). The mass transfer boundary layers involve only diffusional transport of chemical species and separate the biomass from the two substrate reservoirs.

One can imagine two distinct situations for the boundary layers: fixed or changing thickness. In a fixed thickness situation the boundary layers are assumed to span a certain distance from the biomass and to move as the biomass grows (Scheme 1). In the second situation the total domain length is fixed and the boundary layers begin far apart from the initial biomass, which will grow toward the reactant pools (thus decreasing the boundary layer thickness, Scheme 1).

Solid (biomass) and liquid (pore water) phases exist in the system and overlap in the biomass domain.

2.4.2. Biomass growth

Filaments in the system grow outward with a velocity \( u \) due to biomass generation with the rate \( r_X \). The change in filament boundary layers is governed by advection and growth equations [14] in a total biomass balance:

\[
\frac{dC_X}{dt} = \frac{d(C_i u)}{dx} + r_X. \quad (16)
\]

The assumption has been made that biomass only grows, with biomass decay currently not considered for the purposes of this model. Further, the density of biomass in the sediment \( C_X \) was assumed to be constant.

To allow growth toward both oxygen and sulfide, two options were considered: (i) a seed point (biomass inoculum) which is spatially fixed, and (ii) a free-floating filament. The ability of the filament to grow freely in either direction enables preferential asymmetric growth. The net growth velocity is split into velocities toward the substrate boundaries, \( H_2S \) \( (u_{\text{H2S}}) \) and \( O_2 \) \( (u_{\text{O2}}) \) (Scheme 1). A linear profile is applied to the growth reactions in Eq. (16) so that:

\[
\frac{du_{\text{H2S}}}{dx} = \frac{r_X}{C_X} (1-x^*), \quad \frac{du_{\text{O2}}}{dx} = \frac{r_X}{C_X} x^* \quad (17)
\]

where \( x^* = \frac{u_{\text{H2S}}}{u_{\text{H2S}} + u_{\text{O2}}} \) is the normalised position to allow for moving boundaries.

The boundary conditions used to solve Eq. (17) are \( u_{\text{H2S}} = 0 \) at \( \Gamma_{\text{bio,O}} \) and \( u_{\text{O2}} = 0 \) at \( \Gamma_{\text{bio,HS}} \).

Velocities are then set so that the filaments grow with rates \( u_{\text{H2S}} \) at \( \Gamma_{\text{bio,O}} \) and \( u_{\text{O2}} \) at \( \Gamma_{\text{bio,HS}} \), allowing expansion of the domain \( \Omega_{\text{bio}} \) and leading to a problem with moving boundaries:

\[
\frac{dx}{dt} = u_{\text{H2S}} \quad \frac{dx}{dt} = u_{\text{O2}}. \quad (18)
\]

2.4.3. Chemical species in liquid phase

Dissolved chemical components \( S_i \) (neutral \( i = CO_2, O_2, H_2S \) and ions \( i = HS^-, HCO_3^- \)) exist in the liquid phase, each with a concentration \( C_i \). The Nernst–Planck equation, coupled with the electroneutrality condition, governs the transport and reaction of molecules in aqueous solution, covering the whole computational domain:

\[
\frac{dC_i}{dt} = \frac{d}{dx} \left( D_i \frac{dC_i}{dx} + z_i \rho \frac{d\phi}{dx} \right) + r_i \sum z_i C_i = 0 \quad (19)
\]

where \( z_i \) is the charge of the ion, \( D_i \) is the diffusion coefficient, and \( \phi \) is a variable liquid potential. Net component rates \( r_i \) include microbial and chemical reactions in the biomass and only the chemical reactions in the boundary layers.

In the standard case, boundary conditions are of fixed reactant concentrations, assuming infinite sulfidic and oxic reservoirs (bulk liquid). In the aerobic bulk liquid \( (\Gamma_{\text{bl,O}}) \) the dissolved oxygen concentration corresponds to a partial pressure of one atmosphere of air. The anaerobic bulk liquid \( (\Gamma_{\text{bl,HS}}) \) has a fixed total concentration of \( HS^- \). All other concentrations are in chemical equilibrium with a pH of 7. Flux and concentration continuity is assumed for internal boundaries \( \Gamma_{\text{bio,HS}} \) and \( \Gamma_{\text{bio,O}} \) (Scheme 1). A reference electrical potential was assumed on the bulk liquid boundaries, \( \phi = 0 \) at \( \Gamma_{\text{bl,O}} \) and \( \Gamma_{\text{bio,HS}} \). Initial conditions for sulfide and oxygen are zero, while remaining species have the same concentration as the anaerobic bulk liquid.

2.4.4. Chemical species in solid phase

The redox mediator (redox centre) is immobile as it is assumed to be fixed to the outside of the cell, in contact with the sheath. In the biomass sub-domain, the mediator is described by the summation of all relevant reaction rates (Eq. (11)), as well as the movement of the sheath described by Eq. (17):

\[
\frac{dC_{\text{M,red}}}{dt} + (u_{\text{H2S}} + u_{\text{O2}}) \frac{dC_{\text{M,red}}}{dx} = r_{\text{M,red}}. \quad (20)
\]

The mediator concentration is initially defined as existing in equal parts oxidised and reduced forms, from a total concentration \( C_{\text{M,T}} \) calculated as a fraction \( \delta \) of biomass concentration \( C_X \):

\[
C_{\text{M,T}} = \delta \frac{C_X}{C_{\text{M,red}}} - C_{\text{M,red}}. \quad (21)
\]

2.4.5. Electron conduction in solid phase

Electrons are transferred in the biomass domain according to electrical conduction. Stationary one-dimensional Ohm’s law defines the current density \( i \) using the biomass domain conductivity \( \sigma \) and the gradient of electrical potential \( \phi \) along the sheath:

\[-\sigma \frac{d\phi}{dx} = i \]
Table 1
Model parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
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<td><strong>Microbial rate parameters</strong></td>
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<tr>
<td>(K_{\text{H}_2\text{S}})</td>
<td>Half rate coefficient for sulfide</td>
<td>(10 \times 10^{-6})</td>
<td>mol/L</td>
<td>Chosen</td>
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<tr>
<td>(K_{\text{O}_2})</td>
<td>Half rate coefficient for oxygen</td>
<td>(10 \times 10^{-6})</td>
<td>mol/L</td>
<td>Chosen</td>
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<tr>
<td>(v_{\text{H}_2\text{S}})</td>
<td>Growth rate for sulfide</td>
<td>0.0023</td>
<td>mol/m³/s</td>
<td>Calculated, [19]</td>
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<tr>
<td>(v_{\text{O}_2})</td>
<td>Growth rate for oxygen</td>
<td>0.0023</td>
<td>mol/m³/s</td>
<td>Calculated, [19]</td>
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Generation and consumption of electrons gives the change in current over the biomass length. The current source is defined using the Butler–Volmer Eq. (13) leading to the electron balance:

\[ \rho \frac{d^2 \phi}{dx^2} + r_{\text{M,F}} = 0. \]  

(21)

Both ends of the filaments are considered to be electrically insulated (i.e., \(d\phi/dx = 0\)).

2.5. Parameters

The model parameters are listed in Table 1. To calculate the biomass growth, a constant biomass concentration was assumed based on a cell concentration in the sediment of \(4 \times 10^{13}\) cells/m² [4]. Assuming the cells are approximately 3 μm long with a diameter of 1 μm, a density close to water and a molecular weight of 25 g/C-mol, the biomass concentration becomes \(C_X = 3.8 \text{ C-mol/m}^3\).

From experimental data the conductivity of ocean sediment containing conductive filaments ranged from 0.2 to 80 μS/cm, depending upon pyrite addition [18]. A value of biomass conductivity has thus been estimated as 10 μS/cm. Making the assumption that the cell wall is entirely electroactive, an active specific area can be calculated as \(A_c = 377 \text{ m}^2/\text{cell/m}^3\)-domain.

Eqs. (6), (7) and (13) were defined independently of the total concentration of mediator so that the effect of \(\alpha\) on kinetics is taken into account by the coefficients. As limited data is available, 0.1% of biomass fraction has been assumed to be cytochrome c. This leads to a mediator concentration \(C_{\text{M,T}} = 3.8 \times 10^{-3}\) mol/L.

Electrochemical rate parameters for cytochrome-silver have been identified [16,17] as \(k_0 = 8.1 \times 10^{-4}\) cm/s and \(\alpha = 0.5\). Converting units to the \(k_0^\prime\) used in the model leads to \(k_0^\prime = 8.1 \times 10^{-4}\) F/M\(C_X\) ≈ 3 A/m².

Fitted data of sulfide oxidisers show a maximum growth rate \(v_{\text{max}} = 0.22 \text{ h}^{-1}\) [19]. In order to convert this into a substrate consumption rate, we used \(v_{\text{max}} = u D_{\text{max}} f c_X\) with an average \(f_i\) taken to allow for a constant, \(v_{\text{max}}^\prime\), giving \(f_i\) as approximately 10 (where \(i = \text{O}_2\) or HS).

2.6. Model solution

COMSOL Multiphysics (COMSOL 4.4, Comsol Inc., Burlington, MA, www.comsol.com) was used with one-dimensional Nernst–Planck and Transport of Dilute Species interfaces, as well as Partial Differential Equation modules for conduction, in a finite-element approach with moving mesh to account for the moving boundaries. The mesh size was defined to be initially 1 μm for the biomass sub-domain, dilating at a growth rate of 1.1 toward the domain boundaries. The system of model equations was solved using the backwards Euler approach to time-stepping. For parametric sweeps, COMSOL was linked to
MATLAB (MATLAB 2012a, MathWorks, Natick, MA, www.mathworks.com) and the data exported for plotting. The model solution was stopped when the biomass entirely filled the domain.

3. Results

3.1. Growth and conductivity

3.1.1. Separation of anodic and cathodic bioconversion zones

The model simulates the development of spatial separation of anodic (sulfide oxidation) and cathodic (oxygen reduction) microbial activity into the expected centimetre-length filaments. Initially sulfide oxidation takes place in a narrow region of the sediment (Fig. 1.A1), demonstrating the behaviour shown by non-electrochemically active sulfide-oxidising bacteria, albeit with a temporary limitation of oxygen availability. Separation into electroactive zones occurs once the biomass layer has grown thick enough to prevent substrates from penetrating to the other side of the filaments (Fig. 1.A2), with the sharp drop in concentration showing a diffusion-limiting mechanism. Outwards growth finally slows after an increase in electrical resistance and a decrease in biological activity, creating a smoother gradient of substrates (Fig. 1.A3) and a greater pH imbalance (alkaline in the oxygen-reducing zone and acid in the sulfide-oxidation region). These concentration and pH profiles describe qualitatively the experimental observations of [4,5].

The calculated current density and catabolic reaction profiles over the sediment height show the link between production, transport and consumption of electrons during the biomass growth for the filament-containing region $\Omega_{bio}$. Initially, current density is very low with no separation (Fig. 1.B1), increasing with development into discrete spatial zones (Fig. 1.B2), and increasing even further as the electrochemical zones separate into longer filaments (Fig. 1.B3). This is because as the filaments extend, conduction starts to become limited and hence active zones increase in length. Current production in the sulfidic zone leads to a current increase toward the top up to a maximum value (when sulfide is depleted), while the current drops continuously in the aerobic zone where electrons are consumed by O$_2$ reduction metabolism. Without metabolic activity in the middle zone, current is only conducted,

Fig. 1. Development of spatial separation of anodic and cathodic activity in the sediment containing Desulfobulbus filaments, at (1) 1 d, (2) 25 d, and (3) 80 d. (A) Simulated profiles of total H$_2$S, O$_2$ (top x-axis) and pH (bottom x-axis) over the sediment depth. The growing biomass domain is shown as a green line on the y-axis. (B) Current density distribution (top x-axis) and catabolic rates $r_{cat,HS}$ and $r_{cat,O2}$ (bottom x-axis) along the biomass layer. (C) Mediator concentrations (Mox and Mred, bottom x-axis) and the developed electric potential along the biomass layer (top x-axis). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
without consumption or production of electrons through catabolic activity. This is shown as the flat line between 6 and 12 mm along the filaments (Fig. 1.B3). Note that the charge conservation within the biomass domain implies that the overall electron production equals overall consumption rate, so that \( \int (\Gamma_{\text{cat,HS}} - \Gamma_{\text{cat,O2}}) \, dx = 0 \). Voltage in the filament develops similarly to current. The potential in the conductive biological material is proportional to the conductivity of the filament, in this case a total drop from one end of the filament to the other displays 300 mV (Fig. 1.C3). Additionally, the redox state of the electron mediators can be estimated (Fig. 1.C3), going from a fully oxidised mediator \( (\text{Mox dominating}) \) in the aerobic region to fully reduced electron (\( \text{Mred} \) dominating) in the anaerobic region.

### 3.1.2. Initial biomass positioning

The initial biomass position can greatly change the dynamics of zone separation, but not the overall growth or boundary velocity. Diffusion limitations can be seen if the initial seed lies outside the optimal position (Fig. 2.A2,3), whereas if growth starts where standard sulfide oxidisers would reside a conductive/biological limitation begins to occur (Fig. 2.A1). Nevertheless, similar filament lengths of 6 to 7 mm are attained in all cases after 50 days. The filaments grow faster toward oxygen when placed close to the sulfide source (Fig. 2.B2). The opposite is noticed when the seed is situated near the oxygen source (Fig. 2.B3), but the tip growth rates are very similar when the biomass is seeded in the optimal position (Fig. 2.B1). Remarkably, after more than 100 days the same growth rates have been reached in all three cases. The fact that the growth rate is not greatly affected by the initial biomass positioning suggests that energy loss due to conduction is minimal.

### 3.1.3. Effect of boundary conditions

The standard conditions used in the numerical simulations implied a floating filament strand, able to freely move in both directions while growing. Conversely, the filament may be fixed in one point in the sediment, with the strand remaining stationary but its tips growing. A further comparison of the standard case (i.e. constant sulfide concentration on the \( \Gamma_{\text{in,HS}} \) boundary and floating filament) can be made with the case of a constant flux of sulfide through the bottom boundary \( \Gamma_{\text{in,HS}} \), which was matched to the values described in [4].

Fixing the centre of the filaments has little effect in the standard case with constant substrate concentrations on the boundaries, as separation of electrochemical zones quickly occurs and thus any growth is equally split; as such, results are not shown here. As the initial position is already in the optimal zone where the diffusion limitations of sulfide and oxygen are equal, rapid movement toward one substrate is not required, and thus the floating scheme offers no advantage. In systems with dynamic boundary conditions, or where there are heavily thermodynamically-unbalanced substrates, the floating scheme may be more advantageous than having the filaments fixed in a point.

Using a constant flux of sulfide on the boundary only marginally reduced overall growth, but it shifted the active zone closer toward the boundary (Fig. 3.B). This occurred to such a degree because the filaments grow faster toward the source of limiting substrate but, due to a fixed flux, the rate at which sulfide enters the system stays constant. The total flux of sulfide was taken as 0.003 mol/m²/d, an approximation based on experimental data [4]. Although for short filaments this provided little difference, in later filament growth stages the assumed constant flux led to a substantially lower concentration at the boundary. This suggests that the parameters used in the model may lead to a higher consumption rate than experimentally found or that, in the experimental system, consumption of reactant decreases toward the boundary.

### 3.2. Parameter sensitivity and controlling mechanisms

#### 3.2.1. Electrical sensitivity

Since the electrical conductivity of Desulfobulbas filaments is not exactly known, a model-based evaluation of its effects on electrochemical activity and microbial growth is justified. Electroactive zones were defined to allow for the illustration of the separation of biological activity. These were defined as regions of biomass where substrate concentration (oxygen or total sulfide) is greater than 5 \( \mu \)M.

The distance between anodic and cathodic zones grows with regards to electrical conductivity of the filament sheath (Fig. 4.B). Overall biomass growth, displayed as biomass layer length or filament length, decreases at intermediate conductivities (1 \( \mu \)S/cm) (Fig. 4.A, C) due to electrical energy losses. At high conductivity values, the loss is negligible, whereas at low conductivity, no separation occurs. However, biomass growth appears mostly unaffected by conductivity (Fig. 4.A), as the microbial energy limitations come more from diffusion losses than from electrochemical losses. Total separation between zones is heavily dependent upon conductivity, with almost no separation occurring at values lower than 100 \( \mu \)S/cm.

The rate at which cytochrome c can gain or lose electrons has a large effect on separation in electrochemical zones, but not on growth (Fig. 5). This is nearly identical to the results obtained in conductivity sensitivity simulations, showing that these parameters are highly linked.

#### 3.2.2. Biological sensitivity

The overall biomass growth rate and biomass length were not affected by substrate affinity, as the half-saturation coefficient \( K_{m} \) does not contribute to thermodynamic loss (Fig. 6.A). However, a high value of \( K_{m} \) corresponds to slower reaction rate and thus more substrate being able to diffuse into the biomass layer (Fig. 6.B), thus showing less separation of electroactive zones (Fig. 6.A).

### 4. Discussion

#### 4.1. Model outcomes and parameter selection

Results from the model show strong and fundamental similarities to observational data published [4,5], suggesting that the proposed mechanism of direct electron conduction is a key feature of such a system. The sulfide and oxygen concentration profiles over the sediment depth can be used to verify model results. Once verified, the model can lead to other information currently unattainable by experiments. These include voltage and current profiles, which allow the identification of electroactive zones and the generation of current throughout
the biomass. Interestingly, limiting mechanisms for the conversion rate vary depending upon the life cycle of the filaments, starting with biological (too little biomass), then diffusive (too slow substrate transport) and finally conductive limitations (too high electrical resistance) with increasing length of the filaments. This raises the questions of how filamentous sulfide-oxidizing microorganisms have constructed the sheath, and is thickness determined based on interaction between conductivity and optimal length?

A result of a mechanistic model of this level is that parameter identifiability is limited. While the model does qualitatively display many of the system features as observed in the experimental data of [4], many of the parameters needed to be estimated from first principles or analogous systems. With the unknown nature of the metabolic pathways in Desulfobulbaceae, coupled with the splitting of the metabolic pathway into half reactions of electron donor and acceptor, the Monod equation parameters become difficult to identify.

Sheath conductivity is a highly important parameter, and was chosen to lie within the bounds discovered in recent experimental data [18], where conductivity in a sediment was between 0.2 and 80 μS/cm. By calculating the cell fraction in the domain, a conversion factor of $\phi_{cell/domain} = 9.42 \times 10^{-5} \text{ mcell/m}^3$ can be estimated, meaning that filament or cell conductivity must be 100 mS/cm to allow for approximately 10 μS/cm domain conductivity. This high sheath conductivity has been assumed in the standard case as otherwise electron transport would be too limited to allow for the development of cathodic and anodic regions. However, the conductivity of microbial nanowires isolated from Geobacter sulfurreducens has until now only been reported as up to 5 mS/cm [20]. While this is much lower than 100 mS/cm, the SOB filaments form a system which has

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**Fig. 4.** Zone separation length and length of biomass region $\Omega_{bio}$: (A, B) in time; (C) as a function of conductivity (μS/cm), at 50 d.

**Fig. 5.** Zone separation length and length of biomass region $\Omega_{bio}$ as a function of the rate coefficient $k^0$ for electron transfer between mediator and the conductive structure, at 50 d.

**Fig. 6.** (A) Zone separation length and length of biomass region $\Omega_{bio}$ as a function of half-saturation coefficient $K_m$. (B) Substrate concentration and pH profiles over the sediment depth for $K_m = 10^{-3}$ mol/L, at 50 d.
biologically developed enhanced conductivity and the sheaths have a very pronounced morphological feature of *Desulfobulbaceae*. Measurements of the conductivity are essential for further development of quantitative insight in the electrochemical conversions in the sediments. It is also possible that the saline environment allows for much better chemical sheath conductivity than would be achieved in other systems, as the increased ionic conductivity facilitates counter-ion diffusion.

While the experimental system was found to separate the electrochemical zones by 12 to 19 mm after an incubation period of three to four weeks [4], it took as much as 12 weeks to achieve the same result in the model. Given the mechanism of growth—the dissipation of energy—there is little room for inaccuracy. This difference in yield can be analysed by comparing energy input or substrate boundary flux. Experimentally determined substrate consumption rates, driven by electrical conduction, were for Aarhus Bay 3 mmol O₂/m²/d and 0.6 mmol S/m²/d, and for Aarhus Harbour 19 mmol O₂/m²/d and 1.34 mmol S/m²/d [5]. This demonstrates a stoichiometric imbalance of oxygen to sulfide of 2.5:1 and 7:1 for Aarhus Bay and Aarhus Harbour respectively, which is suggested to be balanced by oxidation of organic carbon present in the sediment [5]. In comparison, the model predicted a time-increasing inward flux, with initial fluxes of 4.6 mmol O₂/m²/d and 2.5 mmol S/m²/d, and final fluxes of 8.2 mmol O₂/m²/d and 4.34 mmol S/m²/d. While more sulfide is being consumed in the model, less oxygen is, and therefore a much reduced growth rate is seen. More likely, the higher growth in the sediments is the result of other substrates used by the bacteria providing additional energy and carbon source to the bacteria, further supporting the argument that sulfide alone is not able to support the growth seen in the biomass [5].

4.2. Biological advantage of filamentous growth

As previously hypothesised [4], a clear advantage of conductive filamentous bacteria can be seen by the outcompeting of electrically inactive biomass. Further, the filament can grow directly toward the source of substrate, reducing diffusion limitations and thus maximising the energy source.

The rate of biological growth can also be enhanced with the mechanisms described by this model. In the absence of chemotaxis, a regular biofilm attached to sediment would be limited in the speed with which it could grow toward the food source. A filament has the advantage of more rapid growth due to the preferential direction in which new cells are produced. In this way, every cell that is able to metabolise substrate and divide can do so, each pushing the filament closer to the food source, as has been previously discussed in [21] and modelled in [22].

4.3. Possible model extensions

No organic substrates have been included in this model, even though these may be available in the sediment and contribute as alternative carbon or energy sources. The model could therefore be expanded to include heterotrophic activity. Moreover, other inorganic substrates may be important in the sediment, and may be acting as a source of electrons. Using the mechanism of splitting metabolism into half reactions with an intermediate, further substrates may be seamlessly included.

Multiple-dimension models (i.e., 2-d or 3-d) are also possible based on this method, as the flow of current depends upon substrate availability and so the system adapts to any environment regardless of the number of dimensions the simulation runs in. In this sense, individual-based colony formation models including filamentous groups such as developed in [22,23] or [24] could also be beneficial.

While the model has been used for the small system of filamentous ocean bacteria, the principle equations can be applied to a variety of systems, for instance:

- An environment where substrate limitation occurs, as in the case of a batch reactor or a sequencing batch reactor. An ‘untherered’ biomass can grow more easily toward the substrates, and adapt rapidly to varying conditions.
- Systems of many metabolites or impure feed streams that have few electron acceptors. Using split metabolic reactions could lead to a faster, more accurate model.
- A single-cell bioelectrochemical system where substrate crossover occurs, as the electron sink is then split between either an electrode or the interior of the cell; using this model the split would be calculated using thermodynamic favourability.
- Microbiologically influenced corrosion, and other such systems of electroactive microorganisms.
- Besides the simple conductive electron transfer mechanism used, mediation or external electron acceptors and donors could be implemented.

5. Conclusions

Basic diffusive and electrochemical mechanisms can describe the complex system which enables growth from a single-celled stage to a filament with self-determined anodic and cathodic zones existing in a continuous domain. The proposed model reflects in-situ chemical and physical observation [4] with electrochemically-active sulfide-oxidising microorganisms. While sulfide and oxygen gradients are well described, the modelled growth rates are much lower than experimentally seen. Thus these bacteria must either have a higher growth yield than simulated, or they supplement growth with alternative electron donors, or they use organic carbon for cell synthesis.

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References