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Mathematical modeling of competition and coexistence of sulfate-reducing bacteria, acetogens, and methanogens in multispecies biofilms

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ABSTRACT

This work presents an integrated mathematical model able to simulate the physical, chemical, and biological processes prevailing in a sulfate-reducing biofilm under dynamic conditions. The model includes sulfate reduction by complete and incomplete sulfate-reducing bacteria (SRB); lactate removal by sulfate reduction and by acetogenic bacteria and acetate consumption via methanogenesis. Numerical integration based on the method of characteristics has been developed. The major problem of sulfate-reducing fixed-growth reactors is the formation of undesired bacterial species, which compete for space and substrate within the biofilm with SRB. The effect of COD/SO_4^{2-} ratio on the reactor performances in terms of bacterial species distribution and substrate diffusion trends in the biofilm has been assessed. The simulation results reveal a stratification of microbial activities in biofilm reflecting the different ecological niches created by substrate gradients.

Keywords: Sulfate-reducing biofilms; Microbial competition; Mathematical model; Method of characteristics

1. Introduction

High Sulfate Containing Wastewaters (HSCWs) are generated from various industrial activities such as pulp and paper industries, mining and mineral processing, production of explosives, scrubbing of flue gases, food processing, and petrochemical industries [1]. These anthropogenic activities have contributed to local imbalances in the natural sulfur cycle, resulting in acidification, leaching of toxic metals, elevated sulfate levels in natural waters, potential production of corrosive and toxic sulfide, emissions of SO_2, H_2S, and odorous volatile sulfur compounds, cat clays, and heavy metal release upon oxygen exposure of sediments after dredging [2]. HSCWs often contain

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elevated concentrations of metals (iron, aluminum and manganese, and other heavy metals) and metalloids, deriving from the mining and processing of metal ores and coals, which increase the complexity of the degradation routes [3,4]. During the last years, numerous psychochemical and biological techniques have been investigated for the neutralization and removal of metals and sulfate from wastewaters. Two main categories can be individuated: passive and active processes. Passive treatment processes commonly replace the conventional neutralization techniques involving the addition of a chemical-neutralizing agent. Passive treatment processes require less energy and chemicals, and relatively low maintenance costs. Among these, it is possible to enumerate natural wetlands, aerobic and anaerobic wetlands, and open limestone channels. Although the passive processes are considered low-cost treatment technologies, their efficiency is not very high compared with very expensive surface requests in terms of land. Active treatment processes are, instead, much more efficient. The treatment efficiency is improved through the application of energy, chemical, and biological agents. Active treatment processes require certainly higher maintenance cost and manpower when compared to passive processes, but these costs are offset by the high treatment efficiency. Technologies, such as reverse osmosis, ion exchange, limestone and chemical neutralization, and active biological treatment, represent typical examples of active treatment processes. In particular, active biological sulfate removal from HSCWs represents a valid and cost-effective alternative to the costly and sometimes complex physicochemical sulfate removal methods. Biological sulfate removal can be accomplished in two steps: a dissimilatory sulfate reduction to sulfide performed by sulfate-reducing bacteria (SRB), followed by sulfide removal through partial oxidation to sulfur or precipitation of heavy metals sulfide. The dissimilatory sulfate reduction can take place in methanogenic or sulfidogenic bioreactors. The production of sulfide has been shown to be inhibitory for anaerobic digestion. As a consequence, many studies have been carried out to assess the sulfide toxicity, individuate the most suitable strategies to prevent it and steer the competition between SRB, acetogenic, and methanogenic micro-organisms in the direction of methanogenesis. On the other hand, sulfidogenesis can be seen as an ideally suited process to remove both sulfate and heavy metals from HSCWs and the interest in the application of this process as the main step for the biological treatment of specific waste streams from chemical, mining, and galvanic industries as well as scrubbing water for flue-gas desulfurization has been growing [5].

Biological sulfate reduction is mediated by heterotrophic or autotrophic SRB, which are able to reduce sulfate to sulfide in the presence of a carbon source (CO_2, acetate, lactate, propionate, etc.). Different types of carbon can be used as energy sources; most of the substrates are typical fermentation products or intermediate breakdown products of larger molecules [6]. A minimum chemical oxygen demand (COD) to sulfate mole ratio of 0.67 is required for achieving theoretically possible removal of sulfate [7]. Lens et al. [1] reported that SRB are very diverse in their carbon source utilization and metabolic activities. The availability of carbon and energy source provides the energy for the growth and maintenance of SRB. SRB carry out sulfate reduction based on the following reaction [8]:

$$SO_4^{2-} + 8e^- + 4H_2O \rightarrow S^{2-} + 8OH^- \quad (1)$$

In most cases, the electron donor and carbon source are the same compound. However, when hydrogen is used as the electron donor, CO_2 can be used as carbon source by SRB. The selection of the electron donor depends on the ability of SRB to utilize the substrate, its costs per unit of reduced sulfate, the availability in sufficient quantities, and the remaining pollution load of the additive in the waste stream [1,6]. The choice of a suitable carbon source and electron donor for this process is still a challenge. SRB can be classified into two groups based on their functional ability to oxidize the organic compounds completely to CO_2—SRB completely oxidizers ($SRB_{(C)}$)—or incompletely to acetate and CO_2—SRB incompletely oxidizers ($SRB_{(I)}$).

Postgate [9] indicated that lactate offers potential advantages as carbon source and electron donor in the sulfate reduction process. Lactate can be used by many SRB species; its oxidation results in high biomass yield and high alkalinity production. However, the potential accumulation of acetate in the effluent due to the incomplete oxidation of lactate to acetate and CO_2 represents the main disadvantage of using lactate as carbon source. For this inconvenience, a large amount of lactate is needed to achieve complete reduction of sulfate, contributing to increase the costs of bioreactors' performance. In addition, due to the release of acetate, the COD of the effluent stream increases. The incomplete oxidation of carbon sources to acetate can be attributed to the lower value of free energy for the oxidation of acetate to carbon dioxide, which prevents further oxidation of acetate to carbon dioxide [9]. Furthermore, the presence of acetate and lactate can allow the development of both methanogenic archaea (MA) and acetogenic bacteria (AB) that

can ferment lactate, resulting in the production of acetate. Due to their kinetic properties, high levels of lactate encourage the growth of AB. On the other hand, lactate oxidation becomes dominant under conditions of lactate limitation and excess sulfate [10]. Indeed, in an investigation based on a full-scale anaerobic digester [11], lactate oxidizers were shown to have lower K_s and μ_{max} values than lactate fermenters.

Numerous reactor designs dedicated to biological sulfate reduction have been reported [6]. They can be classified into two main groups: (i) suspended growth reactors that involve the growth of planktonic bacteria such as batch reactors, baffled reactors, up-flow anaerobic sludge bed reactors, and gas-lift reactors; and (ii) attached growth reactors that involve a bacterial biomass attached to media (biofilm), i.e. fixed-bed reactors or fluidized bed reactors. Various immobilized biomass reactors have gained increasing attention due to the advantages of displacing biomass in biofilms. Bacteria growing in biofilms cannot be washed out with the water flow. This allows to retain the biomass within the reactor and therefore to operate at shorter hydraulic retention time. Maximal biomass retention is desirable for process stability and minimal sludge production. Moreover, the high biomass retention and concentration characterizing biofilm reactors strongly affects the achievable loading rates, with the possibility of obtaining high treatment efficiencies [6]. In addition, biofilms show good tolerance for shocks of hydraulic and organic loading and can allow treating contemporary different pollutants, thanks to niche differentiation.

Biological sulfate reduction in anaerobic fixed-growth reactors has been investigated extensively at lab scale. In designing these biofilm reactors, in predicting their behavior under different operating conditions and in understanding the complex microbial relations existing in anaerobic environments in the presence of sulfate, mathematical modeling seems to be essential. Indeed, mathematical models can be used to estimate parameters that cannot be observed directly in experiments and develop an online control strategy. Therefore, the use of mathematical modeling clearly benefits engineers, designers, and operators [12,13].

The scope of this work is to evaluate the SRB growth in multispecies biofilms by modeling the competition between the different bacterial groups involved in the lactate metabolism under biosulfidogenic conditions. In particular, this work is aimed at evaluating the dynamical response of the model under established boundary conditions assessing the effect of different COD/SO_4^{2-} ratios on microbial population shifts. The numerical simulations have been obtained with great accuracy by the method of characteristics. Simulation results show that the model can predict the short-term responses of biofilm performance to substrate variations in the bulk liquid as well as the long-term development of film thickness and microbial species.

2. Statement of the problem

Sulfate-reducing applications usually utilize mixed cultures comprising of SRB and anaerobic fermentative micro-organisms such as methanogens and acetogens [6]. To perform a complete reduction of sulfate to sulfide, SRB have to effectively compete with the other anaerobic bacteria for the available organic substrate. The presence of sulfate seems to be crucial in this competition. As stated in [14], the degradation of organic matter in sulfate-reducing environments is different from the degradation in methanogenic environments. Macromolecules, such as proteins, polysaccharides, and lipids, are hydrolyzed by hydrolytic bacteria. Subsequently, the monomers, amino acids, sugars, and fatty acids are fermented by fermentative bacteria into a range of fermentation products such as acetate, propionate, butyrate, lactate, and hydrogen. In the presence of sulfate, SRB consume these fermentation products. However, in the absence of sulfate, hydrogen, and acetate, the acetate having been produced directly by fermentation or indirectly by acetogenesis, are consumed by the methanogens.

Among simple organic substrates, SRB have been demonstrated to use lactate, ethanol, methanol, acetate, proprionate, and butyrate [15]. Lactate can support the growth of a wide spectrum of SRB, encouraging microbial diversity and consequent treatment system resilience [16]. Lactate can be metabolized via fermentation or sulfate-reducing oxidation or both by a wide range of micro-organisms. Lactate fermentation is the anaerobic degradation of lactate, independent of sulfate reduction [17]. Lactate is oxidized either incompletely or completely in the presence of sulfate by a diverse range of SRB strains [18]. According to [15], only particular species of SRB are able to oxidize lactate to CO_2, whereas others oxidize lactate to acetate and very few can use acetate as carbon source. Besides the limited capability of SRB to degrade it, acetate can accumulate in solution even if other micro-organisms, such as methanogens, are present.

Competition between the different microbial groups depends on the kinetic properties of the interacting micro-organisms such as the maximum specific growth rate (μ_{max}) and substrate affinity (K_s) [16]. Extensive experimental efforts have been devoted to

the kinetic study of lactate metabolic pathway under biosulfidogenic conditions in chemostat cultures [16–18]. In these studies, the effects of different sulfate concentrations, lactate concentrations, and volumetric loading rates on the kinetics of lactate utilization and the stoichiometry of biological sulfate reduction have been investigated.

In the case of immobilized biomass reactors, the competition between the different microbial groups is regulated not only by kinetic properties and dilution rates, but substrate diffusion and niche differentiation have been found to have a crucial role in dictating lactate utilization pathway.

In this work, the dynamics of the anaerobic sulfate reduction in a multispecies biofilm are discussed. Chemical, physical, and biological transient processes are analyzed. In particular, the model takes into account the bioprocess pathways reported in Fig. 1.

The model can simulate the activities of microorganisms living in a sulfate-reducing multispecies biofilm and evaluate the interactions between the related processes: lactate and acetate consumption, sulfate reduction, bacterial growth and decay. Three reacting components are simultaneously considered: lactate, sulfate, and acetate.

The proposed model takes into account the growth of two types of SRB classified into two groups based on their functional ability to oxidize the lactate completely to carbon dioxide ($LDSRB_{(C)}$) or incompletely to acetate and carbon dioxide ($LDSRB_{(I)}$).

The presence of lactate in an anaerobic environment allows the development of AB with the production of acetate and hydrogen. The undesired acetate production by both incomplete SRB and AB allows the growth of MA that produce methane as a final metabolic product. Inert residues (Inert), deriving from microbial biomass decay, are also taken into account. AB compete for space and lactate with SRB, while MA compete only for space.

The growth of these micro-organisms is favored by the formation of zones in biofilm characterized by different substrate concentration levels.

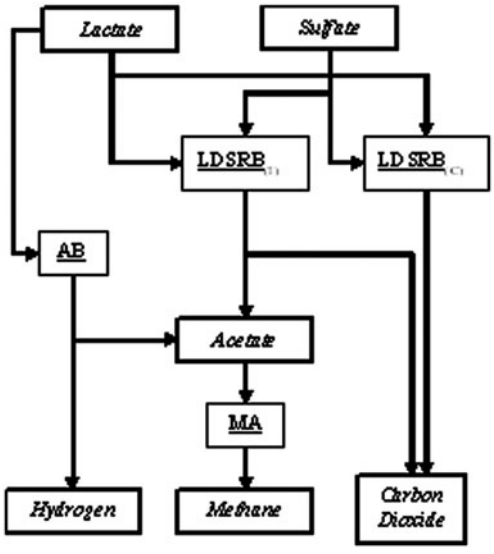

Fig. 1. Main pathways of the biological process.

3. The mathematical model

The biofilm growth is governed by the following equations [19–21]:

$$\frac{\partial X_i}{\partial t}+\frac{\partial}{\partial z}(uX_i)=\rho_i r_{M,i}(z,t,\mathbf{X},\mathbf{S}),\quad 0\le z\le L(t),\quad t>0,\quad i=1,2,3,4,5, \tag{2}$$

$$\frac{\partial u}{\partial z}=\sum_{i=1}^{5} r_{M,i}(z,t,\mathbf{X},\mathbf{S}),\quad 0<z\le L(t),\quad t>0, \tag{3}$$

where $X_i=\rho_i f_i(z,t)$ denotes the concentration of the microbial species and inert residues; f_i denotes the volume fraction of microbial species $i=1,2,3,4,5$; ρ_i is the density assumed constant; $u(z,t)$ is the velocity of the microbial mass displacement with respect to the biofilm support interface; and the term $r_{M,i}\ (z,t,\mathbf{X},\mathbf{S})$ represents the biomass growth rate; $\mathbf{X}=(X_1,X_2,X_3,X_4X_5)$ and $\mathbf{S}=(S_1,S_2,S_3)$.

The net biomass growth rates are given by

$$r_{M,1}=(\mu_1-K_{d,1})X_1, \tag{4}$$

$$r_{M,2}=(\mu_2-K_{d,2})X_2, \tag{5}$$

$$r_{M,3}=(\mu_3-K_{d,3})X_3, \tag{6}$$

$$r_{M,4}=(\mu_4-K_{d,4})X_4, \tag{7}$$

while for inert residues

$$r_{M,5}=K_{d,1}X_1+K_{d,2}X_2+K_{d,3}X_3+K_{d,4}X_4, \tag{8}$$

where μ_1,μ_2,μ_3 and μ_4 are the biomass growth rates for biomass X_1,X_2,X_3 and X_4. $K_{d,1},K_{d,2},K_{d,3}$ and $K_{d,4}$ are the decay-inactivation rates for the single microbial species.

The biomass growth rates are given by:

$$\mu_1 = \mu_{\max,1}\frac{S_1}{K_{1,1}+S_1}\frac{S_2}{K_{1,2}+S_2}, \tag{9}$$

$$\mu_2 = \mu_{\max,2}\frac{S_1}{K_{2,1}+S_1}\frac{S_2}{K_{2,2}+S_2}, \tag{10}$$

$$\mu_3 = \mu_{\max,3}\frac{S_2}{K_{3,2}+S_2}, \tag{11}$$

$$\mu_4 = \mu_{\max,4}\frac{S_3}{K_{4,3}+S_3}, \tag{12}$$

where $\mu_{\max,i}$ is the maximum growth rate for biomass i; and $K_{i,j}$ is the half saturation constant for substrate j (S_1, S_2, S_3) of biomass i.

The diffusion of substrates is governed by the equations:

$$\frac{\partial S_j}{\partial t} - D_j\frac{\partial^2 S_j}{\partial z^2} = r_{S,j}(z,t,\mathbf{X},\mathbf{S}),\quad 0<z<L(t),\quad 0<t\le T,\quad j=1,2,3, \tag{13}$$

where D_j denotes the diffusivity coefficient and $r_{S,j}(z,t,\mathbf{X},\mathbf{S})$ the net conversion rate of substrate j, expressed by:

$$r_{S,1} = -1,5\frac{(1-Y_{1,2})}{Y_{1,2}}\mu_1 - 1,5\frac{(1-Y_{2,2})}{Y_{2,2}}\mu_2, \tag{14}$$

$$r_{S,2} = -\frac{1}{Y_{1,2}}\mu_1 - \frac{1}{Y_{2,2}}\mu_2 - \frac{1}{Y_{3,2}}\mu_3, \tag{15}$$

$$r_{S,3} = -0,8\frac{(1-Y_{2,3})}{Y_{2,3}}\mu_2 - 0,8\frac{(1-Y_{3,3})}{Y_{3,3}}\mu_3 - \frac{1}{Y_{4,3}}\mu_4, \tag{16}$$

where $Y_{i,j}$ denotes the yield for biomass i on substrate j.

The following initial-boundary conditions will be considered for Eqs. (2,3,13):

$$X_i(z,0) = \varphi_i(z),\quad u(0,t) = 0,\quad 0\le z\le L_0,\quad t\ge 0,\quad i=1,2,3,4,5, \tag{17}$$

$$S_j(z,0) = S_{0j}(z),\quad 0\le z\le L_0,\quad j=1,2,3, \tag{18}$$

$$\frac{\partial S_j}{\partial z}(0,t) = 0,\quad S_j(L(t),t) = G_j(t),\quad t>0,\quad i=1,2,3. \tag{19}$$

The functions $\varphi_i(z)$ represent the initial concentrations of biomass i, the functions $S_{0j}(z)$ represent the initial substrate concentrations into biofilm, and $G_j(t)$ represent the values assumed by substrates S_j at the biofilm–bulk liquid interface.

The free boundary evolution is governed by the following ordinary differential equation:

$$\dot{L}(t) = u(L(t),t) - \sigma(L(t),t),\quad t>0, \tag{20}$$

with the following initial condition:

$$L(0) = L_0, \tag{21}$$

where L_0 denotes the initial biofilm thickness and $\sigma(L(t),t)$ represents the velocity at which biomass is exchanged between biofilm and bulk liquid [19], the expression used in this work is:

$$\sigma(L(t),t) = \lambda L^2(t) \tag{22}$$

The qualitative analysis of system (2,3,13) developed in [20] was based on the characteristics method. The numerical method proposed in [22] has been applied. The procedure can be briefly summarized as follows: from the initial-boundary conditions, u is computed; then L, $\boldsymbol{X}$, and $\boldsymbol{S}$ are computed in this order; next the computational process is repeated and the solution at the final time is obtained. Numerical integration of the system (2,3,13,17–21) has been performed using original software.

The schematic representation of the microbial process is reported in Table 1.

4. Results and discussion

The mathematical model proposed in this paper has been applied to simulate the sulfate reduction process in a multispecies biofilm with an initial thickness of 300 μm. The initial conditions and biological parameters, used in the model, are reported in Table 2. The model has been addressed to evaluate the microbial structure of a sulfate-reducing biofilm as affected by changing COD to sulfate ratios.

The model assumes appropriate boundary conditions for the biological process modeled. An initial arbitrary biomass distribution has been adopted in order to evaluate the dynamical response of the model as the system tends to reach an equilibrium that is not affected by the initial conditions. The microbial equilibrium is only governed by the boundary conditions, as for each dynamical model. Kinetic and

Table 1
Petersen Matrix of the proposed model

	Components →	1	2	3	1	2	3	4	5	
	Process ↓	S_1	S_2	S_3	X_1	X_2	X_3	X_4	X_I	Rate [gCOD $m^{-3} d^{-1}$]
1	Sulfate reduction by X_1	$-1,5\frac{(1-Y_{1,2})}{Y_{1,2}}$	$-\frac{1}{Y_{1,2}}$		1					$\mu_1 X_1$
2	Sulfate reduction by X_2	$-1,5\frac{(1-Y_{2,2})}{Y_{2,2}}$	$-\frac{1}{Y_{2,2}}$	$0,8\frac{(1-Y_{2,3})}{Y_{2,3}}$		1				$\mu_2 X_2$
4	Uptake of Lactate by X_3		$-\frac{1}{Y_{3,2}}$	$0,8\frac{(1-Y_{3,3})}{Y_{3,3}}$			1			$\mu_3 X_3$
5	Uptake of Acetate by X_4			$-\frac{1}{Y_{4,3}}$				1		$\mu_4 X_4$
6	Decay of X_1				−1				1	$Kd_1 X_1$
7	Decay of X_2					−1			1	$Kd_2 X_2$
8	Decay of X_3						−1		1	$Kd_3 X_3$
9	Decay of X_4							−1	1	$Kd_4 X_4$
		Sulfate	Lactate	Acetate	$LDSRB_{(C)}$	$LDSRB_{(I)}$	AB	MA	Inert	

Table 2
Operational parameters used for model simulation

Parameter	Unit	Set A	Set B	Set C
COD concentration	mg COD l^{-1}	0.2	0.3	0.1
Sulfate concentration	mg l^{-1}	0.1	0.2	0.2
Time simulation	d	10	10	10
Initial biofilm thickness	μm	300	300	300
Initial volume fraction of $LDSRB_{(C)}$	–	0.3	0.3	0.3
Initial volume fraction of $LDSRB_{(I)}$	–	0.3	0.3	0.3
Initial volume fraction of AB	–	0.2	0.2	0.2
Initial volume fraction of MA	–	0.2	0.2	0.2
Initial volume fraction of Inert	–	0	0	0
Shear constant	$m^{-1} d^{-1}$	2000	2000	2000

stoichiometric parameters, and diffusion coefficients reported in Table 3 have been adopted.

Figs. 2–4 show the results of model simulations, named respectively set A, set B, and set C, performed to assess the COD/SO_4^{2-} ratio effect on the reactor performances in terms of bacterial volume fractions (Figs. 2(B), 3(B), and 4(B)) and concentration trends of substrates (Figs. 2(A), 3(A), and 4(A)) within biofilm for a 10-d simulation. COD/SO_4^{2-} ratios in the range 0.5–2 have been investigated.

The simulations have been performed to evaluate the dynamical response of the biofilm in terms of volume fractions of bacteria and concentration trends of substrates. In particular, the results show the model capability to reveal the microbial stratification in the biofilm and evaluate the effect of substrate diffusion on biomass growth.

Figs. 2–4 show biomass distribution and substrate concentration trends at 0.5 (Fig. 2), 1.5 (Fig. 3), and 2 (Fig. 4) COD/SO_4^{2-} ratio.

As shown in Figs. 2(B), 3(B), and 4(B), after 10 d, the biomass stratification appears visible: $LDSRB_{(C)}$ and $LDSRB_{(I)}$ prevail in the outer layer of biofilm, where sulfate and lactate remain abundant. In the deepest zone of the biofilm, characterized by a low level of sulfate and lactate, due to substrate diffusion coupled with microbial consumption, the MA compete for space with other microbial species. Indeed, the MA are mostly present in the deepest zone of the biofilm, where the optimal conditions for their growth are established.

With a COD/SO_4^{2-} ratio of 0.5 (Fig. 2), the AB are present at the inner layer of the biofilm, where the concentration of sulfate is lower, while both $LDSRB_{(C)}$ and $LDSRB_{(I)}$ are found to be predominant at the outmost layer of the biofilm. In particular, $LDSRB_{(C)}$ and $LDSRB_{(I)}$ represent the most abundant species in the biofilm showing that in the presence of excess sulfate, the quantitative oxidation of lactate to acetate or CO_2 coupled to sulfate reduction is the dominant reaction.

Table 3
Kinetic, Stoichiometric, and diffusion coefficients used in the model

Symbol	Definition	Value	Units	Reference
$\mu^{max}_{LDSRB_{(C)}}$	Maximum specific growth rate of $LDSRB_{(C)}$	4.9	d^{-1}	[23]
$\mu^{max}_{LDSRB_{(I)}}$	Maximum specific growth rate of $LDSRB_{(I)}$	4.9	d^{-1}	[23]
μ^{max}_{AB}	Maximum specific growth rate of AB	2.88	d^{-1}	Adapted from [24]
μ^{max}_{MA}	Maximum specific growth rate of MA	8	d^{-1}	[24]
$Y_{LDSRB_{(C)},Lac}$	Yield of $LDSRB_{(C)}$ on Lactate	0.12	g COD g^{-1} COD	[23]
$Y_{LDSRB_{(I)},Lac}$	Yield of $LDSRB_{(I)}$ on Lactate	0.12	g COD g^{-1} COD	[23]
$Y_{AB,Lac}$	Yield of AB on Lactate	0.04	g COD g^{-1} COD	adapted from [24]
$Y_{MA,Ace}$	Yield of MA on Acetate	0.05	g COD g^{-1} COD	[24]
$K^{LDSRB_{(C)}}_{S,Lac}$	Half-saturation coefficient of $LDSRB_{(C)}$ on Lactate	0.015	g COD l^{-1}	[23]
$K^{LDSRB_{(I)}}_{S,Lac}$	Half-saturation coefficient of $LDSRB_{(I)}$ on Lactate	0.015	g COD l^{-1}	[23]
$K^{LDSRB_{(C)}}_{S,SO_4}$	Half-saturation coefficient of $LDSRB_{(C)}$ on Sulfate	0.00045	g l^{-1}	[23]
$K^{LDSRB_{(I)}}_{S,SO_4}$	Half-saturation coefficient of $LDSRB_{(I)}$ on Sulfate	0.00045	g l^{-1}	[23]
$K^{AB}_{S,Lac}$	Half-saturation coefficient of AB on Lactate	11	g COD l^{-1}	adapted from [24]
$K^{MA}_{S,Ace}$	Half-saturation coefficient of MA on Acetate	0.15	g COD l^{-1}	[24]
$K_{d_{LDSRB_{(C)}}}$	Decay constant of $LDSRB_{(C)}$	0.004	d^{-1}	[23]
$K_{d_{LDSRB_{(I)}}}$	Decay constant of $LDSRB_{(I)}$	0.004	d^{-1}	[23]
$K_{d_{AB}}$	Decay constant of AB	0.002	d^{-1}	Adapted from [24]
$K_{d_{MA}}$	Decay constant of MA	0.002	d^{-1}	[24]
D_{Lac}	Lactate diffusion coefficient in biofilm	7.32×10^{-5}	$m^2\ d^{-1}$	[22]
D_{SO_4}	Sulfate diffusion coefficient in biofilm	9.80×10^{-5}	$m^2\ d^{-1}$	[22]
D_{Ace}	Acetate diffusion coefficient in biofilm	8.35×10^{-5}	$m^2\ d^{-1}$	[22]

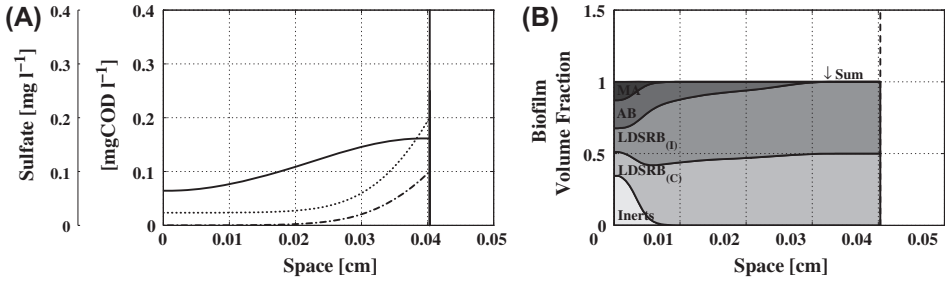

Fig. 2. Substrate trends in the biofilm (A) and bacterial volumetric fractions (B) in the biofilm for a COD/SO_4^{2-} ratio = 0.5. Dotted line: sulfate concentration; dashdot line: COD; continuous line: acetate concentration.

A similar result has been achieved in [25], where the authors experienced high participation of SRB, and in particular of $LDSRB_{(I)}$, on COD removal in a down-flow fluidized bed reactor. Concerning sulfate reduction, it is possible to note that sulfate is not completely depleted within biofilm, probably due to the presence of incomplete oxidizers, while lactate–COD concentration drops to zero in 200 μm (Fig. 2(A)). As shown in Fig. 2(B), methanogenesis and acetogenesis are not completely suppressed; however, the volume fraction of AB is sensitively reduced with respect to the initial condition and this trend is expected to exacerbate with time. On the other hand, the formation of a zone in the inner part of biofilm characterized by abundance of acetate and lack of lactate–COD could support the methanogenic metabolism allowing the methanogens to remain present in the biofilm. According to the experience of [15], acetate production can be recognized as the rate-limiting step in such a sulfate-reducing process.

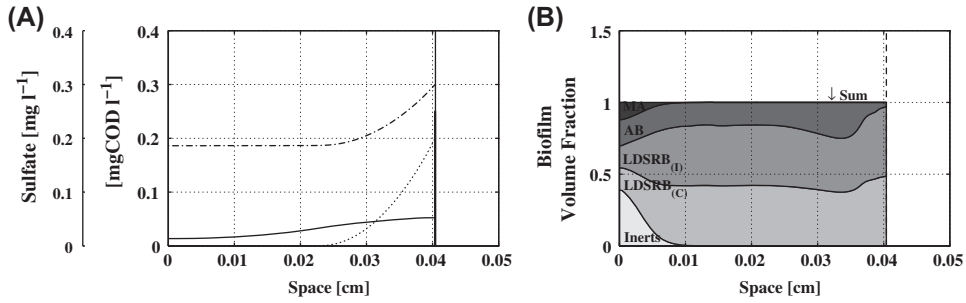

Fig. 3. Substrate trends in the biofilm (A) and bacterial volumetric fractions (B) in the biofilm for a COD/SO_4^{2-} ratio = 1.5. Dotted line: sulfate concentration; dashdot line: COD; continuous line: acetate concentration.

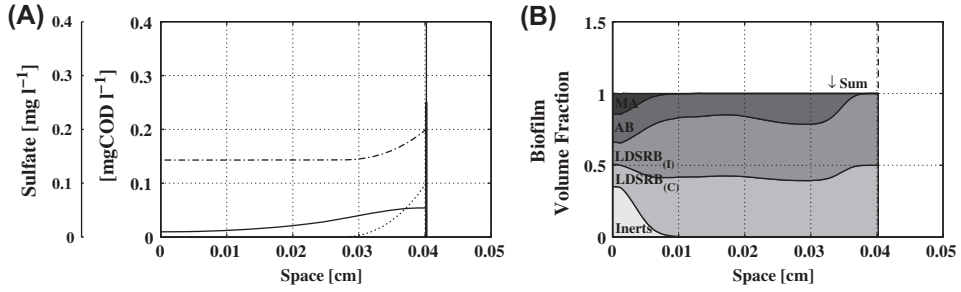

Fig. 4. Substrate trends in the biofilm (A) and bacterial volumetric fractions (B) in the biofilm for a COD/SO_4^{2-} ratio = 2. Dotted line: sulfate concentration; dashdot line: COD; continuous line: acetate concentration.

In Figs. 3 and 4, the response of the multispecies biofilm to the increasing COD/SO_4^2 ratio is shown. As experienced in [15], the excess of lactate over sulfate continuously guaranteed the required carbon source for SRB to reduce sulfate to sulfide. The exposure to higher COD/SO_4^2 ratios was enough for the development of substantial sulfidogenesis leading to sulfate depletion (Figs. 3(A) and 4(A)). In this condition, acetogens do not experience competition for the remaining COD; therefore, the area of acetogenic within the biofilm becomes broader at increasing COD/SO_4^{2-} ratios (Figs. 2(B), 3(B), and 4(B)). This occurs since the increase of the COD load results in a higher lactate concentration throughout the biofilm thickness. A similar shift in microbial population has been found in a continuously stirred tank reactor fed with a COD/SO_4^2 ratio of 1.94 [26].

5. Conclusions

A mathematical model, which is able to simulate the physical, chemical, and biological processes prevailing in a multispecies sulfate-reducing biofilm under dynamic conditions, has been presented. Special attention has been given to the competition between sulfate reduction, acetogenesis, and methanogenesis in the biological system. The effects of the variations of the operational conditions in terms of COD/SO_4^{2-} ratio on the bacterial competition can be properly predicted with this model, which thus can be used for process optimization and control. The simulation results confirm that COD/sulfate ratio represents a crucial variable in the optimization of lactate utilization via oxidation in preference to fermentation and in the maximization of the efficiency of biological sulfate reduction.

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