VOLUMEN 21
XXVIII Verano De la Ciencia
ISSN 2395-9797
www.jóvenesenlaciencia.ugto.mx

A simple elemental balance for the anaerobic sulfate removal by *Desulfovibrio alaskensis* 6SR

Un balance elemental simple para la remoción anaeróbica de sulfatos por *Desulfovibrio alaskensis* 6SR

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Summary

Sulfate-reducing bacteria (SRB) are currently important for the design of new technologies for bioremediation of water contaminated with heavy metals. The paper covers two areas investigated in the project for the basic design of batch bioreactors using sulfate-reducing bacterium *Desulfovibrio alaskensis* 6SR: 1) a simple theoretical and experimental study of sulfate removal in the presence and absence of chromium VI (Cr(VI)) in cultures using Postgate medium (with 30 g/L of NaCl) and the development of a simulation model to predict the dynamics of *D. alaskensis* 6SR and 2) black box stoichiometries were studied. Bacterial growth and product formation were monitored at 37 °C and pH 7.0-7.5 by measuring the time courses of the concentrations of free cells (biomass=X), substrates (lactate= L and sulfate=S), and products (acetate=A and total sulfide=H, namely, biogenic H_2S) in liquid medium under anaerobic conditions. The results can be summarized as follows. The dynamics of bioprocess variables for *D. alaskensis* 6SR on modified Postgate C medium showed a sulfate removal of 80-85% for a fermentation time of 30 hours. The maximum specific growth rate was markedly dependent on the medium Cr(VI). Its maximum growth rate was 0.55 1/h averaged over three experimental runs (n=3). In the bioprocesses without and with hexavalent chromium, a negative effect on cell growth rate of 21.6% was observed in contrast to the control. The dynamics of the bioprocess state was also affected by decreasing rates of substrate consumption and product generation observed during the exponential growth phase. The stoichiometric of the sulfate-reducing process considered the elemental balance of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S), and was proposed as a function of reaction rates r_L, r_S, and r_X and expressed as a function of yield coefficients as a function of carbon energy source Y_{Li} (i=A, H,N, W,D: A=acetate, H=sulfide, N=NH3, W=water, D=CO2). Predictions based on the analysis of black box stoichiometries indicated that overall stoichiometry for sulfate-reducing process was

$$L + Y_{LS} \cdot S + (0.20Y_{LX}) \cdot NH_3 \rightarrow Y_{LX} \cdot X + (1.15 - 2.5Y_{LS} - 1.05Y_{LX}) \cdot A + (-0.15 + 2.5Y_{LS} + 0.05Y_{LX}) \cdot CO_2 + (1.0Y_{LS}) \cdot H_2S + (0.15 + 1.5Y_{LS} + 0.45Y_{LX}) \cdot H_2O$$

Keywords: sulfate removal; sulfate-reducing bacteria; postgate medium; elemental balance.

Introduction

Water treatment is a process with operations of different types (physical, chemical, physical-chemical or biological) whose objective is the elimination and/or reduction of contamination or undesirable characteristics to obtain water appropriated for the intended use. Therefore, the water treatment process varies depending on the starting properties of the water and also its final use. Water treatment is increasingly necessary due to the scarcity of drinking water and the growing need of the world population (Xu & Chen, 2020). Currently, one of the biggest problems at an environmental level is the contamination of the world's water sources by heavy metals since they are toxic. Among the various existing methods for the control of this type of metals we can find methods such as: precipitation, oxidation-reduction, ionic exchange, filtration, electrochemical treatment, membrane technologies and recovery by evaporation, absorption and bioadsorption (C et al., 2023). Another problem is the presence of big concentrations of sulfates(Yan et al., 2023). To face this problematic, it has been proposed a biological treatment that transforms dissolved sulfate into elemental sulfur that could eventually be separated from the water, obtaining a value-added product, while simultaneously reducing the organic matter content in the wastewater (Chatla et al., 2023)

Sulfate-reducing bacteria (SRB) are a compound of microorganisms used as biological treatment tool. In general, SRB react by producing H_2S and the presence of metal ions and insoluble metal sulfides are formed and precipitated, in addition to offering other metal removal mechanisms (C et al., 2023). Species in this heterogeneous microbial assemblage grow chemoorganotrophically, using a variety of short-chain fatty acids, alcohols, hydrocarbons, and aromatic compounds as a carbon and energy source, reducing sulfate to hydrogen sulfide (Hao et al., 1996). Due to their anaerobic nature, BSR are very sensitive to aerobic media, which is why they require the absence of oxygen for their growth and a low redox potential (Tran et al., 2021).

In natural environments, it has been observed that the metabolic activity of BSRs is highest when they are forming biofilms. Some BSRs grow chemolitrophically when they use H_2 as an electron donor to reduce sulfate, and others grow autotrophically when CO_2 is used as the only carbon source. On the other hand, most of the BSRs are capable of growing in the presence of acetate as the only carbon source. Species that use acetate oxidize it to CO_2 and reduce sulfate to sulfite, as an intermediate energetic reaction, oxidation is carried out by the modified tricarboxylic acid cycle or by the acetyl-CoA pathway (Zhang et al., 2022).

Recently, new sulfate-reducing bacteria have been isolated, identified as *D. alaskensis*, which was isolated in Alaska (Feio, 2004). *D. alaskensis*, is a sulfate-reducing, gram-negative, non-spore-forming and rod-shaped bacterium, which ranges from 1 to 5 micrometers in length and 0.5 to 1.2 micrometers in width. Cells are motile by means of a single polar flagellum. The optimal growing conditions for *D. alaskensis* range between 6.5 and 8.5 in pH and between 10 ºC and 45 ºC in temperature. The concentration of salt allowed goes from 0 to 10% (w/v) NaCl. Lactate is used as the principal carbon source for this bacterium to induce its maximum growth rate, besides vitamins are not required (Feio, 2004).

This bacterium is strictly anaerobic and has sulfate-reducing activity. Reduces sulfate and sulfite to produce sulfide. During the reduction process, substrates as lactate, pyruvate and succinate are oxidized (Feio, 2004). In general, *D. alaskensis'* metabolism can be explained by the following reaction, which describes the oxidation of lactate and the synthesis of new bacterial cells (biomass).

$$\alpha_L \cdot L + \alpha_S \cdot S + \alpha_N \cdot NH_3 \rightarrow \alpha_X \cdot X + \alpha_A \cdot A + \alpha_D \cdot CO_2 + \alpha_H \cdot H_2S + \alpha_w \cdot H_2O \qquad \text{Eq. 1}$$

where L=lactate, S=sulfate, X=biomass, A=acetate, D=CO2, H=sulfide, and W=water. $\alpha_i = L, S, N, X, A, D, H, W$ are the stoichiometric coefficients.

Another important characteristic of this bacterium is the metallic resistance, in the absence of oxygen, most of the microorganisms respire using metal ions and small molecules. *D. alaskensis* has been proved to have a higher metallic resistance with respect to other sulfate-reducing microorganisms. Their reducing-sulfate metabolism, the ability to secrete extracellular polymeric substances and to present other type of metallic resistance mechanisms allows at *D. alaskensis* to be considered as a biological agent for the removal and recovery of metals(Diao et al., 2023). In 2022, *D. alaskensis* strain G20 was reported to generate biogenic Pd nanoparticles to catalyze the Sonogashira cross-coupling of phenylacetylenes and aryl iodides in membrane-associated micelles (Era et al., 2022).

Another strain of biotechnological interest is *D. alaskensis* 6SR, which was isolated from a biofilm formed inside an oil pipeline in southeastern Mexico (Neria-González et al., 2006). Most of the sulfate-reducing bacteria reported are not able to tolerate high concentrations of metal ions, however *D. alaskensis* 6SR tolerates higher concentrations compared to the other species of the same genus. In 2014, experimental studies were performed to evaluate the high capacity of cadmium removal by *D. alaskensis* 6SR, finding a fast production of extra polymeric substances (associated to cadmium removal) (López Pérez et al., 2015). The bacterium was able to remove 99.9% of cadmium at the tested concentration (170 mg/L). Also, the use of biogenic hydrogen sulfide produced by *D. alaskensis* 6SR to reduce hexavalent chromium (Cr(VI)) has been reported (Peña-Caballero et al., 2016).

The integration of both *D. alaskensis'* 6SR characteristics (sulfate-reducing and heavy metal removal) makes this strain interesting to study. Therefore, the objective of this article is to analyze the stoichiometric reaction of the sulfate removal bioprocess in anaerobic cultures of the bacterium *D. alaskensis* 6SR by means of an elemental matter balance for carbon, hydrogen, oxygen, nitrogen and sulfur, respectively, C, H, O, N and S. Many studies have addressed the kinetics and stoichiometry of the microbial sulfate reduction by various kinds of SRB, as reviewed by (Okabe et al., 1992). For this study, an average elemental composition for the biomass ($X = CH_{1.8}O_{0.5}N_{0.2}$) is considered to express the reaction as a function of yields Y_{ij}:

$$L + Y_{LS} \cdot S + Y_{LN} \cdot NH_3 \rightarrow Y_{LX} \cdot X + Y_{LA} \cdot A + Y_{LD} \cdot CO_2 + Y_{LH} \cdot H_2S + Y_{LW} \cdot H_2O \quad \text{(Eq. 1)}$$

Theoretical considerations (mathematical description)

Black box stoichiometries

Stoichiometry for sulfate-reducing process, according with dissimilatory sulfate reduction by sulfate-reducing bacteria can be represented by a pair of chemical equations that describe the oxidation of lactate and the synthesis of bacterial cells (see Eq. (2)) and Equations 3 to 5 describes the oxidation of lactate (Okabe et al., 1992):

$$\underbrace{\overset{C,H,O,N,S,P\ldots}{\widetilde{S_i}}}_{i=1,2,\ldots,M} \xrightarrow{r_X=\mu X} \underbrace{\overset{C,H,O,N,S,P\ldots}{\widetilde{X}}}_{j=1,2,\ldots,L} + \underbrace{\overset{C,H,O,N,S,P\ldots}{\widetilde{P_j}}}_{k=1,2,\ldots,N} \quad \text{(Eq. 2)}$$

Where S_i=substrate, X =biomass, P_j= product.

Now, in the case of sulfate reducing bacteria, to oxidize the source of carbon and energy, it is oxidized in the presence of sulfate (Okabe et al., 1992). This reaction is summarized in Equation 5 (Nielsen, 2001).

Table 1. *Chemical equation for the full sulfate reducing process*

Energy ($\Delta G° = -34.2\ KJ/Mol\ e^-$)	
$\alpha_L \cdot L + \alpha_S \cdot S \rightarrow \alpha_A \cdot A + \alpha_D \cdot CO_2 + \alpha_H \cdot HS_2$	Eq. 3
Synthesis	
$\alpha_L \cdot L + \alpha_N \cdot NH_3 \rightarrow \alpha_X \cdot X + \alpha_w \cdot H_2O$	Eq. 4
Overall stoichiometry	
$\alpha_L \cdot L + \alpha_S \cdot S + \alpha_N \cdot NH_3 \rightarrow \alpha_X \cdot X + \alpha_A \cdot A + \alpha_D \cdot CO_2 + \alpha_H \cdot H_2S + \alpha_w \cdot H_2O$	Eq. 5

Where L= Lactate, S= Sulfate, N= Ammonia, X = Biomass, A=Acetate, H=Sulfide, D=Carbon dioxide, W=water and, respectively, α_L, $\alpha_{S,}$ α_N, α_X, α_A, α_D, α_H, and α_w the stoichiometric coefficients.

All the chemical formulae in Equation 1 (lactate, sulfate, biomass, acetate, sulfide, carbon dioxide, water) are known and assumed as constant, and the eight unknown stoichiometric coefficients α_L, $\alpha_{S,}$ α_N, α_X, α_A, α_D, α_H, and α_w can be calculated from four elemental balances for C, H, N, and O or five elemental balances for C, H, N, O, and S and two measured variables. This is in accordance with the degrees of freedom (DF) in Equation 6.

$$DF = \underbrace{number\ of\ variables}_{\alpha_L,\alpha_{S,}\ \alpha_N,\alpha_X,\alpha_A,\alpha_D,\alpha_H,\text{and } \alpha_w} - \underbrace{number\ elemental\ balances}_{C,H,N,O,S} \quad \text{(Eq. 6a)}$$

or

$$DF = 8 - 5 = 3 \quad \text{(Eq. 6b)}$$

Remark 1. The stoichiometric relationships ($r_l, l = 1 + M + N$) are then used to calculate various rates: $\alpha_L \Longrightarrow r_L$, $\alpha_S \Longrightarrow r_S$, $\alpha_N \Longrightarrow r_N$, $\alpha_X \Rightarrow r_X$, $\alpha_A \Rightarrow r_A$, $\alpha_D \Rightarrow r_D$,$\alpha_H \Rightarrow r_H$, and $\alpha_W \Rightarrow r_w$, where r_L, r_S, r_N,r_X, r_A, r_H, r_D, and r_w are conversion rates for lactate, sulfate, biomass, acetate, sulfide, carbon dioxide, and water, respectively:

$$DF = \underbrace{number\ of\ variables}_{r_L,r_S,r_X,r_A,r_H,r_D,\text{and } r_w} - \underbrace{number\ elemental\ balances}_{C,H,N,O,S} \quad \text{(Eq. 7)}$$

Remark 2. For Equations 7, a velocity vector ($r \in \mathbb{R}^{1+M+N}$) can be expressed as follows.

$$r = [r_L \quad r_S \quad r_N \quad r_X \quad r_A \quad r_H \quad r_D \quad r_W]^T \in \mathbb{R}^{1\times 8} \qquad \text{(Eq. 8)}$$

Observation 1. Equation 8 defines the first term to the right of equality in Equation 7 and the second term is defined by **Remark 3** in Equation 9.

Remark 3. For elemental balancing in matrix E. Knowing the composition of lactate, sulfate, ammonia, biomass, acetate, sulfide, carbon dioxide, water allows the following "steady state " balances of the elements in matrix E ($E \in \mathbb{R}^{5\times(1+M+N)}$) (Villadsen et al., 2011):

$$E \cdot r = [e_{ij}][r_{ij}] = [e_{ij}]\begin{bmatrix} r_L \\ r_S \\ r_N \\ r_X \\ r_A \\ r_D \\ r_H \\ r_W \end{bmatrix} = 0 \qquad \text{(Eq. 9)}$$

or using Equation 5 and data in Table 2

Table 2. *Component properties and rate designations*

Compound	Chemical formula	C-mol basis	Conversation rate
Lactate (L)	$C_3H_8O_3$	$CH_{\frac{8}{3}}O$	r_L
Sulfate (S)	$NaSO_4$	$NaSO_4$	r_S
Ammonia(N)	NH_3	NH_3	r_N
Biomass (X)	$CH_{1.8}O_{0.5}N_{0.5}$	$CH_{1.8}O_{0.5}N_{0.5}$	r_X
Acetate (A)	$C_2H_4O_2$	CH_2O	r_A
Sulfide (H)	H_2S	H_2S	r_H
Carbon dioxide (D)	CO_2	CO_2	r_D
Water (W)	H_2O	H_2O	r_W

Developing the matrix product in Equation. 9, we have (Nielsen & Villadsen, 1992; Villadsen et al., 2011):

$$E \cdot r = \begin{bmatrix} 1.0 & 0.0 & 0.0 & 1.0 & 1.0 & 1.0 & 0.0 & 0.0 \\ 2.6 & 0.0 & 3.0 & 1.8 & 2.0 & 0.0 & 2.0 & 2.0 \\ 1.0 & 4.0 & 0.0 & 0.5 & 1.0 & 2.0 & 0.0 & 1.0 \\ 0.0 & 0.0 & 1.0 & 0.2 & 0.0 & 0.0 & 0.0 & 0.0 \\ 0.0 & 1.0 & 0.0 & 0.0 & 0.0 & 0.0 & 1.0 & 0.0 \end{bmatrix}\begin{bmatrix} r_L \\ r_S \\ r_N \\ r_X \\ r_A \\ r_D \\ r_H \\ r_W \end{bmatrix} = 0 \qquad \text{(Eq. 10)}$$

Where $E \in \mathbb{R}^{5\times 8}$; $r \in \mathbb{R}^{8\times 1}$ and the elemental matrix (E) is composed by c-mol basis (see Table 2)

Remark 4. If we consider Equation 5, vector Equation 8 can be expressed in velocities that can be measured ($r_m = measured\ rates$) and velocities that are not measurable ($r_c = nonmeasured\ rates$) as following,

$$E \cdot r = [E_m \quad E_c]\begin{bmatrix} r_m \\ r_c \end{bmatrix} = 0 \qquad \text{(Eq.11)}$$

Where E_m is the measurable elemental matrix and E_c is the calculable elementary matrix.

Observation 2. For Equation 11 there are two solutions for vector r_c (Nielsen & Villadsen, 1992; Villadsen et al., 2011):

$$r_c = -(E_c)^{-1} \cdot E_m \cdot r_m \quad \text{(Eq.12)}$$

With E_c, a square matrix $(n \times n)$

And

$$r_c = -((E_c)^T \cdot E_c)^{-1} \cdot (E_c)^T \cdot E_m \cdot r_m \quad \text{(Eq.13)}$$

With $((E_c)^T \cdot E_c)$, a square matrix $(n \times n)$

Finally, the vector r_m is obtained by experimental measurable data and then is used to calculate those rates present in r_c, using information of the matrix $E = [E_m \quad E_c]$ for the reaction in Equation 5 considering Definition 1:

Definition 1: $\alpha_L \Longrightarrow r_L$, $\alpha_N \Longrightarrow r_N$, $\alpha_X \Rightarrow r_X$, $\alpha_A \Rightarrow r_A$, $\alpha_D \Rightarrow r_D$,$\alpha_H \Rightarrow r_H$, and $\alpha_W \Rightarrow r_w$.

From Equation 10, these are 5 equations with 8 unknowns (vector $r \in \mathbb{R}^{8\times1}$). Then three additional equations are provided by three reaction kinetic relationships $(\mu_{max} \prod f(\cdot))X)$.

Methods and mathematical methods

Cultivation of *Desulfovibrio alaskensis* 6SR. The sulphate-reducing bacteria were grown at 37 °C in 500 mL glass bioreactor (Evelsa-México) containing 300 mL autoclaved medium, as described previously (Peña-Caballero et al., 2016). The medium had the following composition (per litre distilled water): $0.5\ g\ K_2PO_4$; $1.0\ g\ NH_4Cl$; $0.06\ g\ MgK_2SO_4 \cdot 7H_2O$; $30\ g\ NaCl$; $0.06\ g\ CaCl_2 \cdot H_2O$; $1.0\ g$ yeast extract; $1.0\ g$ sodium citrate; $6\ mL\ (60\%\ w/w)$ sodium lactate; $4.5\ g\ Na_2SO_4$. The medium pH was adjusted to 7.0 with NaOH (0.5 M). A sulfate reducing batch bioreactor was initiated by inoculating *D. alaskensis* 6SR cells into the liquid medium, and this point was taken as zero time. The initial cell concentration in the medium ranged from 100 to 110 mg/L $(OD_{580}\ between\ 0.35\ and\ 0.4)$ and the initial medium pH was 7.0. In all experiments the initial concentrations of sulfate in the medium were 500 mg/L. Before autoclaving, the medium was flushed with nitrogen (100 mL/min) to remove dissolved oxygen in medium and the head space. Samples from the cultures were taken anaerobically. Sulfate in the medium was measured by the turbidimetric method based on the precipitation of barium. Also, the production of sulfide was measured by a colorimetric method. The OD reading for cell growth was transformed to dry weight (concentration) through a standard growth curve.

Black box stoichiometries

In relation to the r_m vector, their velocities are to be known and related to the following material balances for a batch reactor if the culture is axenic with *D. alaskensis* 6SR (see Equation 2):

$$r_{S_i} = \frac{d[S_i]}{dt} = f_{S_j}(X, S_i, P_j);\ S_i = S_i(t=0) = S_i(t=t_0) = S_i(0) \quad \text{(Eq.14)}$$

$$r_X = \frac{d[X]}{dt} = f_X(X, S_i, P_j);\ X = X(t=0) = X(t=t_0) = X(0) \quad \text{(Eq.15)}$$

$$r_{p_i} = \frac{d[P_j]}{dt} = f_{P_i}(X, S_i, P_j);\ P_j = P_j(t=0) = P_j(t=t_0) = P_j(0) \quad \text{(Eq.16)}$$

Where $S_i(0)$, $X(0)$ and $P_j(0)$ are the initial conditions at $t = 0$, respectively, for substrates, biomass, and products. And $f_{S_j}(X, S_i, P_j)$, $f_X(X, S_i, P_j)$, and $f_{P_i}(X, S_i, P_j)$ are non-linear functions for the substrates, biomass, and products, respectively.

In each of these differential equations (Equations 14, 15, and 16), the rate of change of biomass (X) and products formations (P_j) and substrate consumption (S_i) may be related to biomass (X), substrates (S_i) and products (P_j)) concentrations in using the appropriate functional forms, for example in (Peña-Caballero et al., 2016) report the use of functions given in Table 3 for sulfate reducing bacteria *D. alaskensis* 6SR.

Table 3. *Model equations*

$r_X = \frac{d[X]}{dt}$	$r_{S_i} = \frac{d[S_i]}{dt}$	$r_{p_i} = \frac{d[P_j]}{dt}$	Model
$\left(\mu_{max}\prod f(\cdot)\right)X$	$\left(\mu_{max}\prod f(\cdot)\right)X$	$\left(\mu_{max}\prod f(\cdot)\right)X$	(Eq.17)
$(\mu - k_d)\cdot X$	$-\frac{\mu\cdot X}{Y_i}$	$+\frac{\mu\cdot X}{Y_i}$	in this work

Definition 2: If we consider the balances for a batch reactor with unstructured kinetics (see Table 3), the equations are transformed to the following (Peña-Caballero et al., 2016):

$$r_{S_i} = \frac{d[S_i]}{dt} = \left(\mu_{max}\prod f(\cdot)\right)X;\ S_i(0) \qquad \text{(Eq.19)}$$

$$r_X = \frac{d[X]}{dt} = \left(\mu_{max}\prod f(\cdot)\right)X;\ X(0) \qquad \text{(Eq,20)}$$

$$r_{p_i} = \frac{d[P_j]}{dt} = \left(\mu_{max}\prod f(\cdot)\right)X;\ P_j(0) \qquad \text{(Eq.21)}$$

Results and discussion

Sulfate reduction: basic kinetics and stoichiometry

To establish the reaction in Equation 1 and 5 with Definition 1 relatives to the vector r_m, we redefine the reaction as follows:

Overall stoichiometry

$$r_L\cdot L + r_S\cdot S + r_N\cdot NH_3 \rightarrow r_X\cdot X + r_A\cdot A + r_D\cdot CO_2 + r_H\cdot H_2S + r_w\cdot H_2O \qquad \text{(Eq.22)}$$

Now we consider the matrix equality in Equation 10 as follows:

$$\begin{gathered} 1\cdot r_L + 1\cdot r_X + 1\cdot r_A + 1\cdot r_D = 0 \\ 2.6\cdot r_L + 3\cdot r_N + 1.8\cdot r_X + 2\cdot r_A + 2\cdot r_H + 2\cdot r_W = 0 \\ 1\cdot r_L + 4\cdot r_S + 0.5\cdot r_X + 1\cdot r_A + 2\cdot r_D + 1\cdot r_W = 0 \\ 1\cdot r_N + 0.2\cdot r_X = 0 \\ 1\cdot r_S + 1\cdot r_H = 0 \end{gathered} \qquad \text{(Eq. 23)}$$

These are 5 equations with 8 unknowns (vector r). Then three additional equations are provided by three reaction kinetic relationships (cases a), b), c) in Table 4). But if we decide to measure four of the eight conversion rates, then we will have the case d) (see Table 4). Table 4 shows four cases for the selection of the vector $r_m \in \mathbb{R}^{3\times 1}$ to calculate the stoichiometry of the reaction in Equation 1 for batch cultures of *D. alaskensis* 6SR.

Table 4. *Specific measurable rates (r_m) for the bioprocess of oxidation of lactate to acetate by D. alaskensis 6SR (see stoichiometry of sulfate reducing bacteria in Equations 1 and 5)*

Case study	Matrix size $\boldsymbol{r_m}^T$	Matrix size $\boldsymbol{r_c}$	Matrix size $\boldsymbol{E_m}$	Matrix size $\boldsymbol{E_c}$
a)	$[r_L \quad r_X \quad r_H]$	(5×1)	(5×3)	(5×5)
b)	$[r_N \quad r_X \quad r_H]$	(5×1)	(5×3)	(5×5)
c)	$[r_L \quad r_X \quad r_D]$	(5×1)	(5×3)	(5×5)
d)	$[r_L \quad r_X \quad r_D \quad r_H]$	(4×1)	(5×4)	(5×4)

So, Table 5 shows the elemental material balances for the four cases. Note that the vector r_c is related as a function of the vector r_m as follows for each case study: $r_c = f(r_L, r_X, r_H)$, $r_c = f(r_L, r_X, r_N)$, $r_c = f(r_L, r_X, r_D)$, and $r_c = f(r_L, r_X, r_H, r_D)$, respectively, a), b), c), and d) (Peña-Caballero et al., 2016).

Table 5. *Elemental balancing for carbon, hydrogen, oxygen, nitrogen, and sulfur for each molecule in the reaction of sulfate reducing bacteria D. alaskensis 6SR in Equation 5)*

Case (see Table 4)	Elemental balancing
For the carbon balance	$1 \cdot r_L + 1 \cdot r_X + 1 \cdot r_A + 1 \cdot r_D = 0$
For the oxygen balance	$2.6 \cdot r_L + 3 \cdot r_N + 1.8 \cdot r_X + 2 \cdot r_A + 2 \cdot r_H + 2 \cdot r_W = 0$
For the nitrogen balance	$1 \cdot r_N + 0.2 \cdot r_X = 0$
For the sulfur balance	$1 \cdot r_S + 1 \cdot r_H = 0$

The importance of the equations (elemental balancing) in Table 5, is to calculate the velocity vector $r_c = f(r_m)$ knowing the velocity of the vector r_m (Nielsen & Villadsen, 1992; Villadsen et al., 2011).

According, with results for the cases a), b), c), and d) in Table 4, the solution of the system of algebraic equations are given in Table 6.

Table 6. *Algebraic solution for the case studies a). b), c), and d) in the reaction of sulfate reducing bacteria D. alaskensis 6SR.*

Case (see Table 4)	$\boldsymbol{r_m}^T$	$\boldsymbol{r_c}$	Elemental balancing
a)	$[r_L \quad r_X \quad r_H]$	$\boldsymbol{r_c} = f(r_L, r_X, r_H)$	$r_S(r_H) = -1 \cdot r_H$ $r_N(r_X) = -0.2 \cdot r_X + 0 \cdot r_H$ $r_A(r_L, r_X, r_H) = -1.15 \cdot r_L - 1.05 \cdot r_X - 2.5 \cdot r_H$ $r_D(r_L, r_X, r_H) = 0.15 \cdot r_L + 0.05 \cdot r_X + 2.5 \cdot r_H$ $r_w(r_L, r_X, r_H) = -0.15 \cdot r_L + 0.45 \cdot r_X + 1.5 \cdot r_H$
b)	$[r_N \quad r_X \quad r_H]$	$\boldsymbol{r_c} = f(r_N, r_X, r_H)$	Linearly dependent system, no real solution
c)	$[r_L \quad r_X \quad r_D]$	$\boldsymbol{r_c} = f(r_L, r_X, r_D)$	$r_S(r_L, r_X, r_D) = 0.06 \cdot r_L + 0.02 r_X - 0.4\, r_D$ $r_N(r_X) = -0.2 \cdot r_X$ $r_A(r_L, r_X, r_D) = -1 \cdot r_L - 1 \cdot r_X - 1 \cdot r_D$ $r_H(r_L, r_X, r_D) = -0.06 \cdot r_L - 0.02 \cdot r_X + 0.4 \cdot r_D$ $r_w(r_L, r_X, r_D) = -0.24 \cdot r_L + 0.42 \cdot r_X + 0.6 \cdot r_D$
d)	$[r_L \quad r_X \quad r_D \quad r_H]$	$\boldsymbol{r_c} = f(r_L, r_X, r_D, r_H)$	$r_S = f(r_L, r_X, r_D, r_H) = 0.0615 \cdot r_L + 0.0205 \cdot r_X - 0.4103 \cdot r_D + 0.0256 \cdot r_H$

VOLUMEN 16
XXVII Verano De la Ciencia
ISSN 2395-9797
www. jóvenesenlaciencia.ugto.mx

$$r_N = f(r_L, r_X, r_D, r_H) = -0.0231 \cdot r_L - 0.2077 \cdot r_X + 0.1538 \cdot r_D - 0.3846 \cdot r_H$$

$$r_A = f(r_L, r_X, r_D) = -1 \cdot r_L - 1 \cdot r_X - 1 \cdot r_D$$

$$r_W = f(r_L, r_X, r_D, r_H) = -0.2615 \cdot r_L + 0.4128 \cdot r_X + 0.7436 \cdot r_D - 0.359 \cdot r_H$$

Formulation of the kinetic equations for lactate, biomass and sulfide as measured rates

For the case a), the relation between r_x and r_s, i.e., biomass formation rate and substrate uptake rate is given in Table 3, according to Monod type equation for the uptake of lactate by *D. alaskensis* 6SR (Monod, 1949). In this work, we are interested in the growth of the microorganism at its maximum cell growth rate (μ_{max}), so the Equations 19, 20 and 21 take the following form (see Figure 1):

$$r_{S_i}(t) = \frac{d[S_i(t)]}{dt} = -\frac{\mu_{max} \cdot X(t)}{Y_i};\ S_i(0) \quad \text{(Eq.24)}$$

$$r_X(t) = \frac{d[X(t)]}{dt} = (\mu_{max} - k_d) \cdot X(t);\ X(0) \quad \text{(Eq.25)}$$

$$r_{p_i}(t) = \frac{d[P_j(t)]}{dt} = \frac{(\mu_{max} - k_d) \cdot X(t)}{Y_i};\ P_j(0) \quad \text{(Eq.26)}$$

Now, integrating with respect to the initial conditions Equations 24, 25 and 26, the mathematical functions ($X(t)$, $S(t)$, and $P(t)$) for the process variables are obtained in Equations 27, 28, and 29:

$$\int_{X(0)}^{X} \frac{d[X(t)]}{X(t)} = \int_{t=t_0}^{t=t} (\mu_{max} - k_d) \cdot dt \quad \text{(Eq.27)}$$

$$\int_{S_i(0)}^{S_i(t)} d[S_i(t)] = \int_{t=t_0}^{t=t} -\frac{(\mu_{max} - k_d)}{Y_j} \cdot X(t)dt \quad \text{(Eq.28)}$$

$$\int_{P_i(0)}^{P_i(t)} d[P_i] = \int_{t=t_0}^{t=t} \frac{(\mu_{max} - k_d)}{Y_i} \cdot X(t)dt \quad \text{(Eq.29)}$$

So, the solution of the equations for the r_m vector (measured rates) can be used to calculate the velocities in the r_c (nonmeasured rates) vector (see Table 7).

Tabla 7. *Explicit model equations for liquid phase cultivation of batch cultivation for D. alaskensis 6SR.*

Substance	Notation (g/L)	Balance equation
Lactate (substrate)	$L(t)$	$L(t) = L(0) - \frac{(\mu\max - \mathrm{Kd})}{\frac{Y_l}{X}} X(t_0)\left[e^{(\mu max - Kd)t} - 1\right]$
Sulfate (substrate)	$S(t)$	$S(t) = S(0) - \frac{(\mu\max - \mathrm{Kd})}{\frac{Y_s}{X}} X(t_0)\left[e^{(\mu max - Kd)t} - 1\right]$

Ammonia (substrate)	$N(t)$	$N(t) = N(0) - \frac{(\mu\max - \mathrm{Kd})}{\frac{Y_N}{X}} X(t_0)\left[e^{(\mu max - Kd)t} - 1\right]$
Biomass (product)	$X(t)$	$X(t) = X(t_0)[e^{(\mu max - Kd)t}]$
Acetate (product)	$A(t)$	$A(t) = A(0) + \frac{(\mu\max - \mathrm{Kd})}{\frac{Y_A}{X}} X(t_0)\left[e^{(\mu max - Kd)t} - 1\right]$
Sulfide (product)	$H(t)$	$H(t) = H(0) + \frac{(\mu\max - \mathrm{Kd})}{\frac{Y_H}{X}} X(t_0)\left[e^{(\mu max - Kd)t} - 1\right]$
Carbon dioxide (product)	$D(t)$	$D(t) = D(0) + \frac{(\mu\max - \mathrm{Kd})}{\frac{Y_D}{X}} X(t_0)\left[e^{(\mu max - Kd)t} - 1\right]$
Water (product)	$W(t)$	$W(t) = W(0) + \frac{(\mu\max - \mathrm{Kd})}{\frac{Y_W}{X}} X(t_0)\left[e^{(\mu max - Kd)t} - 1\right]$

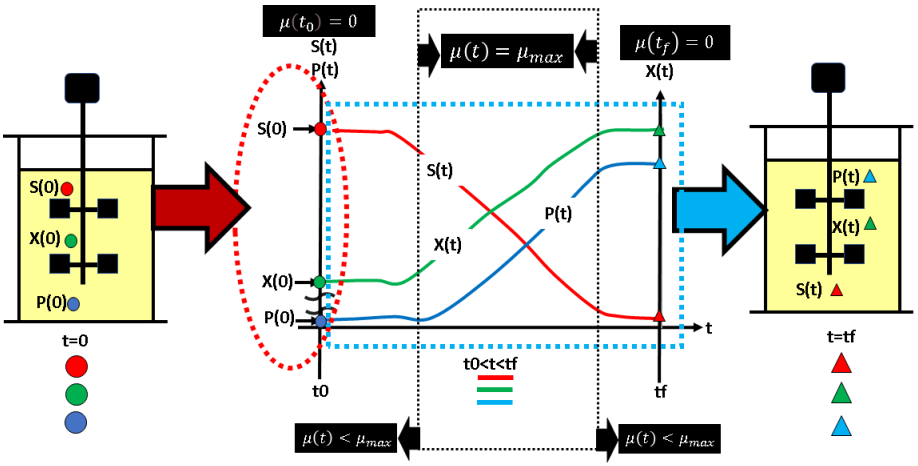

Figure 1. Schematic diagram of a stirred biorreactor and related model variables for this study.

Model for the ideal Sulfate-reducing bioreactor (a basic bioreactor design)

Cultures of *D. alaskensis* 6SR without the presence of hexavalent chromium

With the information in Table 7, the dynamics of the bioprocess variables is calculated, i.e., the evolution of the variables over time in the exponential growth phase for the bacteria *D. alaskensis* 6SR. The solution of the functions for $X(t)$, $S(t)$, $H(t)$, $L(t)$, $A(t)$ as a function of time at $t = 0\ h$ and $t = 30\ h$, i.e. $\mu(t) < \mu_{max}$ (see Figure1), with the following parameters (stoichiometric parameters and kinetic parameters): $\mu_{max} = 0.1014\ 1/\mathrm{h}$, $k_\mathrm{d} = 0.0027\ 1/\mathrm{h}$, $\frac{1}{\mathrm{Y_{x/S}}} = 7.4443\ \mathrm{g/g}$, $\frac{1}{\mathrm{Y_{x/H}}} = 1.7068\ g/\mathrm{g}$, $\frac{1}{\mathrm{Y_{x/L}}} = 14.3198\ \mathrm{g/g}$, $\frac{1}{\mathrm{Y_{x_{1/A}}}} = 9.6588\ \mathrm{g/g}$ (see Table 5 and Equation 30), are shown in Figures 2 and 3. It can be observed that when growing the bacterium *D. alakensis* 6SR at maximum cell growth rate (μ_{max}), a fast dynamics of the bioprocess is reached with a sulfate removal of 80% with respect to the initial concentration (biomass (X) 110.5 mg/L, sulfide (H) 30 mg/L, lactate (L) 4640 mg/L, sulfate (S) 3065 mg/L, and acetate (A) 0 mg/L with 30 g/L).

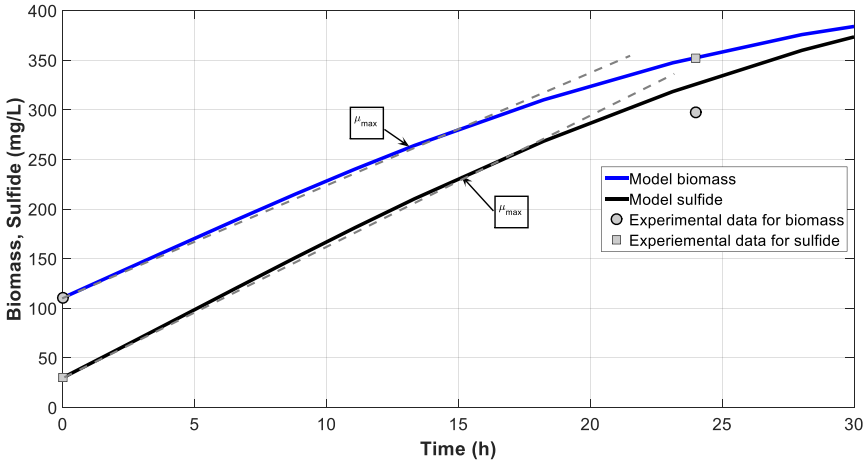

Figure 2. The experimental data (symbols) and the kinetic model predictions, drawn as curves for functions X(t), S(t) and H(t), respectively, biomass and sulfide (see Table 5).

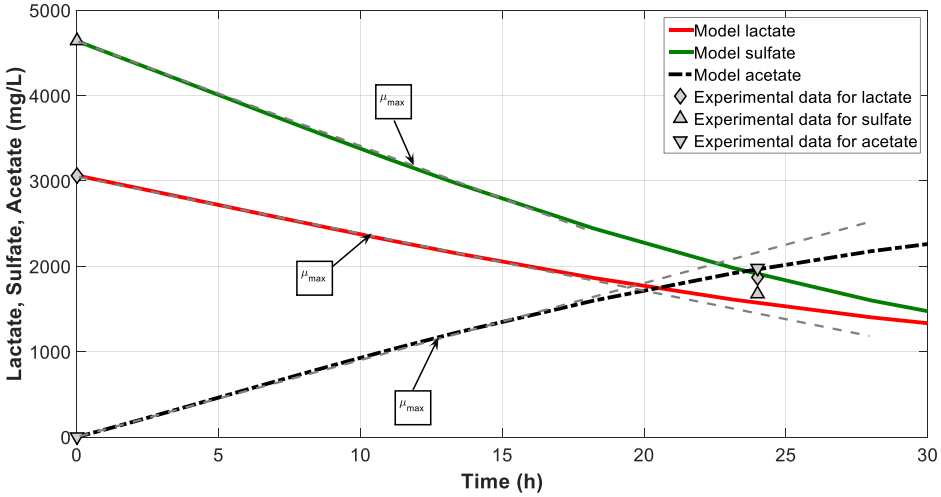

Figure 3. The experimental data (symbols) and the kinetic model predictions, drawn as curves for functions S(t), L(t), and A(t), respectively, sulfate, lactate, and acetate (see Table 5).

According to the results of the experimental yields ($Y_{x_{/S}}0.1343 g/g$, $Y_{x_{/H}} = 0.5859 g/g$, $Y_{x_{/L}} = 0.0698\ g/g$, $Y_{x_{1/A}} = 0.1035 g/g$) for the sulfate removal bioprocess by *D. alaskensis* 6SR, the production of carbon dioxide (D(t) see Table 5) and water (W(t) see Table 5) can be observed (see stoichiometric reaction in equation 1 and 5 and Definition 1) using information in Equations 10 to 13. According with the balances for the anaerobic sulfide fermentation (see Table 2), on a c-mol basis the element balances are (see Eq. (10):

$$E \cdot r = [E_m \quad E_c]\begin{bmatrix} r_m \\ r_c \end{bmatrix} = [E_m \quad E_c]\begin{bmatrix}\begin{bmatrix} r_X \\ r_S \\ r_L \\ r_A \end{bmatrix} \\ \begin{bmatrix} r_N \\ r_D \\ r_H \\ r_W \end{bmatrix}\end{bmatrix} = 0 \qquad \text{(Eq.30)}$$

or

$$r_c = \underbrace{\begin{bmatrix} -r_N \\ r_A \\ r_D \\ r_H \\ r_W \end{bmatrix}}_{nonmeasured\ rates} = \begin{bmatrix} 0.0 & 0.0 & -0.20 \\ -1.15 & 2.5 & -1.05 \\ 0.15 & -2.5 & 0.05 \\ 0.0 & -1.0 & 0.0 \\ -0.15 & -1.5 & 0.45 \end{bmatrix} \cdot \underbrace{\begin{bmatrix} -r_L \\ -r_S \\ r_X \end{bmatrix}}_{measured\ rates} \quad \text{(Eq.31)}$$

These are 5 equations with 5 unknowns (see Eq x). We will change then in dependencies of r_X, r_S, r_L, and r_A only:

$$\begin{gathered} -r_N = -0.20 r_X \\ r_A = 1.15 r_L - 2.5 r_S - 1.05 r_X \\ r_D = -0.15 r_L + 2.5 r_S + 0.05 r_X \\ r_H = 1.0 r_S \\ r_w = 0.15 r_L + 1.5 r_S + 0.45 r_X \end{gathered} \quad \text{(Eq. 34)}$$

Definition 2 Stoichiometric parameters. These define the stoichiometric relationships in the reactions or biological activity :

$$Y_{ji} = \left| \frac{r_i}{r_j} \right| \quad \text{(Eq. 35)}$$

Here with $j = L$ and $i =$ X, S, A, H, D, and W, respectively, biomass, sulfate, acetate, sulfide,

Now, considering the Definition 1 Eq. 34 yields

$$\begin{gathered} Y_{LN} = 0.20 Y_{LX} \\ Y_{LA} = 1.15 - 2.5 Y_{LS} - 1.05 Y_{LX} \\ Y_{LD} = -0.15 + 2.5 Y_{LS} + 0.05 Y_{LX} \\ Y_{LH} = 1.0 Y_{LS} \\ Y_{LW} = 0.15 + 1.5 Y_{LS} + 0.45 Y_{LX} \end{gathered} \quad \text{(Eq. 36)}$$

Remark 5. These equations are derived without any knowledge about the reactions that take place in the bioreactor.

Overall stoichiometry

$$\begin{aligned} L + Y_{LS} \cdot S + (0.20 Y_{LX}) \cdot NH_3 \\ \rightarrow Y_{LX} \cdot X + (1.15 - 2.5 Y_{LS} - 1.05 Y_{LX}) \cdot A + (-0.15 + 2.5 Y_{LS} + 0.05 Y_{LX}) \cdot CO_2 \\ + (1.0 Y_{LS}) \cdot HS_2 + (0.15 + 1.5 Y_{LS} + 0.45 Y_{LX}) \cdot H_2O \end{aligned} \quad \text{(Eq.37)}$$

Equation 37 defines the analysis of black box stoichiometries for sulfate-reducing process without Cr(VI).

Cultures of *D. alaskensis* 6SR in the presence of hexavalent chromium (Cr(VI)

Now, in this section presents numerical results based on experimental observations to model the response of anaerobic culture variables for the sulfate-reducing bacterium *D. alaskensis* 6SR growing in Postgate C medium in the presence of hexavalent chromium (Cr(VI)=10 mg/L). In particular, the dynamics of the culture in the maximum exponential growth phase is analyzed, since the highest percentage of substrate consumption and production of biomass and products has been experimentally observed in this phase (see zone for $\mu(t) < \mu_{max}$ in Figure 1). Note the negative and positive slope for substrate consumption and production of products such as biomass and other products, respectively, red line and green and blue lines in zone $\mu(t) < \mu_{max}$of the Figure 1.

Now, considering a fast dynamic for lactate and sulfate consumption, and consequently sulfide and acetate production, an approximation to the process can be proposed by simplifying the number of variables, for example to three, for biomass, sulfate and hydrogen sulfide (see Figure 4). Figure 5 shows the response of the variables for the control cultures and with the presence of hexavalent chromium (Cr(VI)). It can be observed that the presence of chromium in the cultures (10 mg/L using K2Cr2O7) affects the maximum specific growth rate (control $\mu_{max}^{control} = 0.0848\ 1/h$ and with chromium $\mu_{max}^{Cr(VI)} = 0.0658\ 1/h$) and the rate cell death are $K_d^{control} = 0.0085\ 1/h$ and with chromium $k_d^{Cr(VI)} = 0.0058\ 1/h$. This effect has been reported for other sulfate-reducing bacteria by (Gu et al., 2021; Peña-Caballero et al., 2016). Finally, the stoichiometric relationships are

$\frac{1}{Y^{control}{}_{X/S}} = 15.9173\ g/g$ and $\frac{1}{Y^{Cr(VI)}{}_{X/S}} = 20.2596\ g/g$ and $\frac{1}{Y^{Control}{}_{X/H}} = 1.9183\ g/g$ and $\frac{1}{Y^{Cr(VI)}{}_{X/H}} = 2.5690\ g/g$. The dotted lines in Figure 5 represent the maximum specific speed for cell growth ($\mu_{max}^{control}$ and $\mu_{max}^{Cr(VI)}$) for the three variables a) biomass, b) sulfate and c) sulfide with and without the presence of hexavalent chromium. The slope of the lines is clearly seen, being for all cases a lower slope for the cultures with the presence of hexavalent chromium in contrast to the cultures without the presence of chromium, i.e., the control. However, for the control bioprocess, biomass production is affected, i.e., by the presence of biogenic H_2S in the culture media at t>50 h. For sulfate-reducing bacteria, the negative effect of hydrogen sulfide, as described previously (Dordević et al., 2021; Reis et al., 1992) (see biomass and sulfide in Figure 4).

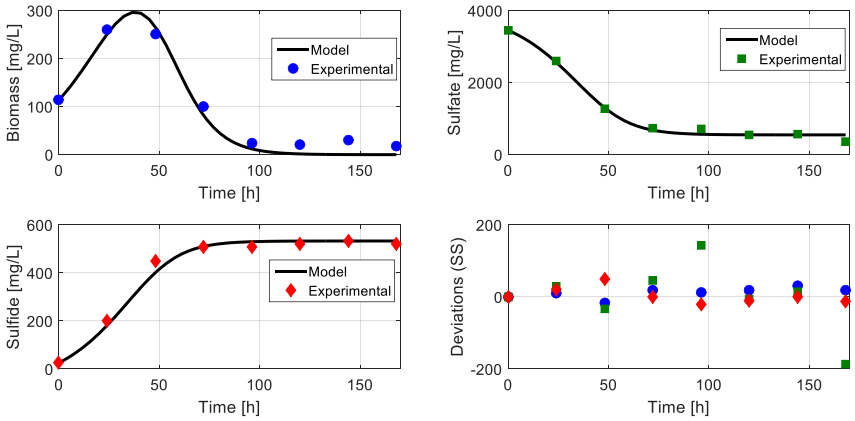

Figure 4. Substrate consumption (sulfate) and product formation (biomass and sulfide) during batch growth of *D.alaskesnsis* 6SR on sulfate and lactate at pH 7.0 and 30 g/L of NaCl, with an initial cell concentration of 1.2101 :(0) biomass (●); (■) sulfate; (♦) sulfide.

The sulfide formed exists in the liquid phase in any of three forms: H_2S, HS^-, and S^{2-}, and at equilibrium, the concentration of molecular H_2S in the bulk liquid is given by the following Equation

$$[H_2S] = \frac{[H_2S] + [HS^-] + [S^{2-}]}{1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}} \quad \text{(Eq. 38)}$$

Where K_1 and K_2 denote the first and second dissociation equilibrium constants for dissolved H_2S. Finally, for sulfate-reducing process is a well-mixed batch reactor of constant volume, the desorption rate dissolved molecular H_2S from liquid medium is zero $N_A = 0$, and can be related to its microbial formation rate through the material balance on sulfide species with Equation 39,

$$\frac{d}{dt}\{[H_2S] + [HS^-] + [S^{2-}]\} \triangleq \frac{d}{dt}[S]_T = \frac{\gamma}{Y_S}\mu_{max}X - N_A \quad \text{(Eq. 39)}$$

or $N_A = 0$

$$\frac{d}{dt}[S]_T = \frac{\gamma}{Y_S}\mu_{max}X \quad \text{(Eq. 40)}$$

Where γ the stoichiometric coefficient for the microbialformation of total sulfide, which is based on the consumption of sulfate, $[S]_T = [H_2S] + [HS^-] + [S^{2-}]$ is the total sulfide concentration in the liquid phase, and N_A is the desorption rate dissolved molecular H_2S from liquid medium (see Figure 4).

For the case of the bioprocess with and without the presence of chromium, it is not possible to determine Equation 1 and 5 with the experimental observations (see Figure 5), i.e., measured rates, because the matrix E_c has no inverse, i.e., it has a row of null values.

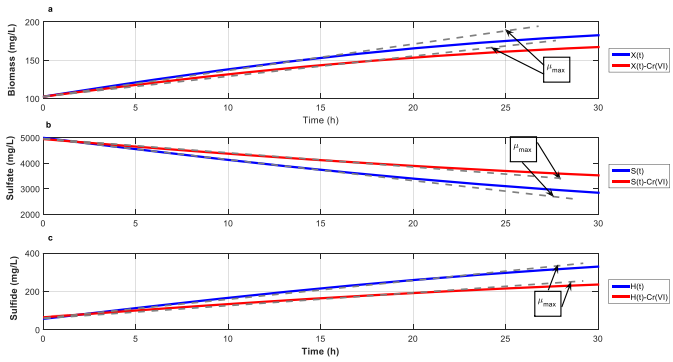

Figure 5. a)Biomass (mg/L), b) sulfate (mg/L), and sulfide (mg/L) with (control) and without Cr(VI).

In this case it is possible to design the vector r_m substituting r_S by r_D, this is feasible due to the ease of measuring CO2 in the bioreactor exhaust gases. Finally, considering that for the bioprocess of *D. alaskensis* 6SR with the presence of Cr(VI), the vector r_m with elements r_X, r_S and r_H, i.e., $r_m = [r_X \quad r_S \quad r_H](measured\ rates)$ and considering the definition in Equation 11, it is concluded that the elementary matrix E_c, corresponding to the vector $r_c = [r_L \quad r_N \quad r_A \quad r_D \quad r_w](nonmeasured\ rates)$, is as follows:

$$E \cdot r = [E_m \quad E_c]\begin{bmatrix} r_m \\ r_c \end{bmatrix} = \begin{bmatrix} E_m & \begin{bmatrix} 1.0 & 0.0 & 1.0 & 1.0 & 0.0 \\ 2.6 & 3.0 & 2.0 & 0.0 & 2.0 \\ 1.0 & 0.0 & 1.0 & 2.0 & 1.0 \\ 0.0 & 1.0 & 0.0 & 0.0 & 0.0 \\ 0.0 & 0.0 & 0.0 & 0.0 & 0.0 \end{bmatrix} \end{bmatrix} \begin{bmatrix} \begin{bmatrix} r_S \\ r_X \\ r_H \end{bmatrix} \\ \begin{bmatrix} r_L \\ r_N \\ r_A \\ r_D \\ r_w \end{bmatrix} \end{bmatrix} = 0 \Rightarrow r_c = -(E_c)^{-1} \cdot E_m \cdot r_m \quad \text{(Eq. 41)}$$

therefore, with $r_m$it is not possible to obtain the model in Equation 1 or 5 because the matrix ${E_c}^{-1}$does not exist. But the stoichiometry of the sulfate-reducing process can be obtained if r_m contains the elements r_X, r_S, and r_L, i.e., $r_m = [r_X \quad r_S \quad r_L](measured\ rates)$ and $r_c = [r_H \quad r_N \quad r_A \quad r_D \quad r_w](nonmeasured\ rates)$:

$$E \cdot r = [E_m \quad E_c]\begin{bmatrix} r_m \\ r_c \end{bmatrix} = \begin{bmatrix} E_m & \begin{bmatrix} 0.0 & 1.0 & 1.0 & 0.0 & 0.0 \\ 3.0 & 2.0 & 0.0 & 2.0 & 2.0 \\ 0.0 & 1.0 & 2.0 & 0.0 & 1.0 \\ 1.0 & 0.0 & 0.0 & 0.0 & 0.0 \\ 0.0 & 0.0 & 0.0 & 1.0 & 0.0 \end{bmatrix} \end{bmatrix} \begin{bmatrix} \begin{bmatrix} r_L \\ r_S \\ r_X \end{bmatrix} \\ \begin{bmatrix} r_L \\ r_N \\ r_A \\ r_D \\ r_w \end{bmatrix} \end{bmatrix} = 0 \quad \text{(Eq. 42)}$$

Overall stoichiometry +Cr(VI)

$$L + Y_{LS}^{Cr} \cdot S + \left(0.20 Y_{LX}^{Cr}\right) \cdot NH_3 \rightarrow Y_{LX}^{Cr} \cdot X + \left(1.15 - 2.5 Y_{LS}^{Cr} - 1.05 Y_{LX}^{Cr}\right) \cdot A + \left(-0.15 + 2.5 Y_{LX}^{Cr} + 0.05 Y_{LX}^{Cr}\right) \cdot CO_2 + \left(1.0 Y_{LS}^{Cr}\right) \cdot HS_2 + \left(0.15 + 1.5 Y_{LS}^{Cr} + 0.45 Y_{LX}^{Cr}\right) \cdot H_2O \quad \text{(Eq 43)}$$

Conclusions

The dynamic rate of biomass, sulfide, acetate during the batch growth of *D.alaskensis* 6SR on sulfate and lactate at 37 °C and pH 7.0 with and without Cr(VI) was studied. A generic model for the stoichiometric reaction (black box model) of the batch reductive sulfate process in the presence and absence of haxavalent chromium was presented as a function of elemental balance (C, H, O, N and S) for *D. alaskesnis* 6SR considering a vector of measurement rates with carbon source (lactate=L); electron acceptor (sulfate=S); and biomass production (X) considering that $X = CH_{1.8}O_{0.5}N_{0.2}$ on a C-mol basis. Finally, the availability of a stoichiometric model for *D. alaskensis* 6SR could be used to estimate simplified metabolic pathways for the sulfate-reducing bioprocess.

Acknowledgment

To the science summer program of the University of Guanajuato. Project: "Dynamic Analysis of Biological Systems of Academic and Industrial Importance of the DCSI of the CCS of the UG and Design of nonlinear estimators with application to biological systems". No.: CIDSC-3291201.

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