MODELLING OF BACTERIAL SULPHATE REDUCTION IN ANAEROBIC PONDS: KINETIC INVESTIGATIONS

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ABSTRACT

The aim of the study was first to develop a simple and practical model of anaerobic digestion including sulphate-reduction in anaerobic ponds. The basic microbiology of our model consists of three steps, namely, acidogenesis, methanogenesis, and sulphate reduction. This model includes multiple reaction stoichiometry and substrate utilization kinetics. The second aim was to determine some kinetic parameters associated with this model. The results of this study provide the values of the saturation constant for SO_4^{2-}, K_{SO4}, and the maximum specific rate of sulphate utilization for SRB, υ_{max} , in an anaerobic pond. The values of these parameters for sulfidogenic bacteria are used in the anaerobic pond model to describe the sulphate reduction processes and to evaluate the risk of odour generation.

Key words: Modelling, Sulphate-reducing Bacteria, Anaerobic Pond, Stoichiometry

INTRODUCTION

One of the best known disadvantages of Waste Stabilisation Ponds (WSPs) is the possible generation of offensive odours, often associated with the presence of hydrogen sulphide (H_2S), which itself is generated by sulphate reduction processes (Racault, 1997; Paing, 2002; Harerimana *et al.,* 2010). When dissolved oxygen and nitrate are absent from the wastewater in a WSP, sulphate-reducing bacteria (SRB) will use sulphate as an electron acceptor and wastewater organic matter as a substrate (Gloyna, 1972; Lens *et al*., 1998). Moreover, the main problems related to the sulphate reducing process are due to the generation of hydrogen sulphide. This phenomenon creates odour and corrosion problems (Sawyer *et al*., 2003). In addition, H_2S is also toxic. According to Mara (1998), odour nuisance does not occur in anaerobic ponds when the volumetric loading rate is lower than 400 g BOD $m^{-3}.d^{-1}$ and with domestic wastewaters containing less than 500 mg SO_4/l. Pescod (1996) suggests the same volumetric loading but with less than 100 mg SO_4^{2-}/l to avoid nuisance odour. These quite different recommendations are based on field observations, not on real bacterial activity measurements for the species involved in these processes. Measuring sulphate-reducing bacterial activity in anaerobic ponds will make it possible to have quantitative information on the sulphur cycle and odour production (Harerimana, 2010).

The Anaerobic Pond Model (APM) developed in our laboratory (Effebi, 2009) did not take sulphate reduction processes into account and is invalid to describe such types of dysfunction. The first aim of this study was thus to develop a structured mathematical model of sulphate reduction in anaerobic ponds. The second aim of the present work was to study kinetically the reduction of sulphate by SRB in the presence of acetate as an electron donor in batch mode.

MODEL DESCRIPTION

In anaerobic ponds treating sulphate-containing wastewaters, both sulphate reduction and methanogenesis can be the final step in the degradation process because SRB are able to use many of the intermediates formed during anaerobic digestion (Kalyuzhnyi and Fedorovich, 1998). Thus, according to the accepted APM/SR (Anaerobic Pond Model including Sulphate Reduction processes) scheme (Figure 1), the conversion process is carried out by five groups of micro-organisms: group X_1 contains all acetogenic bacteria, X_2 all acetotrophic methanogenic bacteria (MB), X_3 acetotrophic SRB, X_4 hydrogenotrophic MB, and X_5 hydrogenotrophic SRB, but only X_3 and X_5 are new compared with the anaerobic digestion model.

We extended the APM reaction sequences to allow for the sulphate reduction process by incorporating the following biochemical processes: sulphate reduction using volatile

fatty acids (VFA) (acetate equivalent) and sulphate reduction on hydrogen.
The process kinetics and stoichiometry for those biochemical reactions are given in Tables 1 (soluble components) and 2 (particulate components) in the same format as Anaerobic Digestion Model No 1 (ADM1) (Bastone *et al.*, 2002). Process S_{H2O} (H_2O) was excluded from Table 1 but implicit from the stoichiometry.

Stoichiometry

Mathematical modelling calls for a description of the stoichiometry and kinetics of the processes involved.
By taking account of the proportions of main compounds in domestic wastewater (proteins, carbohydrates, and lipids) and the yield coefficient for a $C_5H_7O_2N$ biomass, one can define a "complex substrate" (Effebi, 2009), in this case $C_8H_{16}O_6N$. The nitrogen required for bacterial synthesis comes from the release of NH_3 during the reaction. A stoichiometric model of sulphate reduction by SRB in anaerobic ponds was developed by Harerimana *et al*. (2010). The theoretical yields Y of biomass X on substrate S used in the model are estimated from thermodynamic method according to Rittman & McCarthy (2001). Table 4 gives the values of yield Y taken for the overall reactions in the model (Table 5).

Based on the "complex substrate" composition, we developed the stoichiometry of the involved process taking account of the Y values and COD balances. To simplify the model the equations were developed to combine hydrolysis, acidogenesis, and acetogenesis.

Kinetics of sulphate reduction processes

Because of our interest in sulphate removal, the key rate equation is sulphate uptake, which is based on a multiplicative Monod approach, where both the electron donor and electron acceptor can be rate limiting:

$$\frac{dS_{SO_4^{2-}}}{dt} = \upsilon_{max} \frac{S_i}{K_S + S_i} * \frac{S_{SO_4^{2-}}}{K_{SO4} + S_{SO_4^{2-}}} * X_{SRB} \quad (1)$$

Where:

$$\upsilon_{max} = \frac{\mu_{max}}{Y} \quad (2)$$

υ_{max}: Maximum specific rate of sulphate utilization ($gSO4_{reduced}.gVSS^{-1}.d^{-1}$); K_S: saturation constant for S (g/l); X_{SRB}: sulphate-reducing bacteria (SRB) ($gVSS.\ l^{-1}$); VSS: volatile suspended solids (biomass); μ_{max}: Maximum specific growth rate of SRB, K_{SO4}: saturation constant for SO_4^{2-}.
Important for applying one of these equations is the estimation of typical model parameters such as υ_{max}, μ_{max}, and Y. These model parameters are specific and dependent on the COD sources.

MATERIALS AND METHODS

Micro-organisms and medium

Several batch experiments were carried out with different initial sulphate concentrations. The biomass inoculum was obtained from an anaerobic pond located at El Jem, Tunisia, where sulphate reduction is active. Twenty litres of anaerobic pond sewage was centrifuged at 3500 rpm for 10 minutes. The tests were conducted on a synthetic wastewater (O'Flaherty *et al.,* 1998). For all experiments, a basal medium was used in such a way that the C/N/P ratios did not constitute nutriant limitations on bacterial growth (Table 6).

Table 6. Composition of the synthetic wastewater used for growth of SRB

Component	Weight (mg)	Initial conditions
acetate	2000	Phase 1:
$NaHCO_3$	1000	Five tests
NaCl	1000	T°: 20°C
K_2HPO_4	500	pH: 7.8
NH_4Cl	1000	S_{SO4} (mg/l):
$MgCl_2.6H_2O$	300	250-3300
$CaCl_2.6H_2O$	1000	
yeast extract	1000	
ascorbic acid	1000	
resazurin	1	
trace element solution	$1ml.l^{-1}$	
deionised water	1000	
Trace element solution		Phase 2:
HCl (25%; 7.7 M)	$10ml.l^{-1}$	Four tests
$FeCl_2.4H_2O$	1500	S_{SO4} (mg/l):
$ZnCl_2$	70	400-700
$MnCl_2.4H_2O$	100	T°: 30°C
H_3BO_3	6	pH: 7.8
$CoCl_2.6H_2O$	190	
$CuCl_2.2H_2O$	2	
$(NiCl_2.6H_2O$	24	
$Na_2MoO_4.2H_2O$	36	
cysteine-HCl)	560	
deionised water	1000	

Experimental procedure

The study was performed at 20°C in Phase 1 and at 30°C in Phase 2. In Phase 1 of the study, five reactors were fed with the same level of acetate (2000 $mg.l^{-1}$) but different levels of sulphate, i.e., 257 $mg.l^{-1}$ for reactor A; 644 $mg.l^{-1}$ for reactor B; 934 $mg.l^{-1}$, for reactor C; 1432 $mg.l^{-1}$ for reactor D; and 3255 $mg.l^{-1}$ for reactor E.

In Phase 2 of the study, four reactors were fed with the same level of acetate (2000 $mg.l^{-1}$) but different levels of sulphate, i.e., 422 $mg.l^{-1}$ for reactor A; 494 $mg.l^{-1}$ for reactor D; 664 $mg.l^{-1}$ for reactor C; and 700 $mg.l^{-1}$ for reactor D. 125 ml serum vials were used and filled with 100 ml of sample, leaving a headspace of 25 ml. The pH of all media was set to a value above 8. Nitrogen was bubbled through the sample for at least 5 minutes after the addition of sodium acetate and potassium sulphate to ensure that the wastewater sample and headspace were free from oxygen that would otherwise inhibit the sulphate reduction processes. The water samples in the reactors were continuously mixed by magnetic stirrer. Syringes were used to withdraw 4 ml of liquid samples. The samples were immediately filtered through a 0.45 µm membrane filter. Sulphate was analysed by ion chromatography. VSS and chemical oxygen demand (COD) were determined by standard methods (1998).

RESULTS

The sulphate concentrations in each test were determined over the duration of the test. Typical variations are shown in Figure 2.

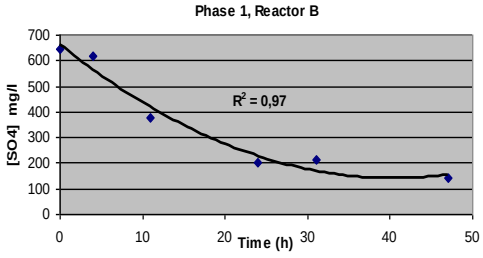

Figure 2. Sulphate utilization for SRB growth

Sulphate depletion data were used to estimate the kinetic parameters. Assuming the biomass concentration X_{SRB} is constant and $S_i >> K_i$, equation (1) can be transformed into equation (3):

$$\upsilon_{SO4} = \frac{1}{X_{SRB}} \frac{dS_{SO_4^{2-}}}{dt} = \upsilon_{max} \frac{S_{SO_4^{2-}}}{K_{SO4} + S_{SO_4^{2-}}} \qquad (3)$$

From equation (3): $\upsilon_{SO4} = \frac{1}{X_{SRB}} \frac{dS_{SO_4^{2-}}}{dt}$ and the sulphate consumption data $\Delta S_{SO_4^{2-}}$ recorded at fixed time intervals Δt, assuming the biomass concentration X_{SO4} was constant, the data for sulphate-reducing activity (υ_{SO4}) as a function of the initial sulphate concentration (SO_4^{2-}) are obtained, as can be seen from Figure 3.

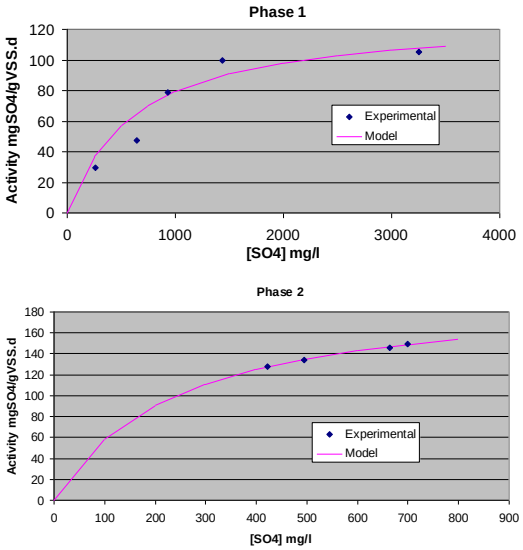

Figure 3. Sulphate-reducing activity for various sulphate concentrations in Phases 1 and 2 (COD acetate = 2133mgCOD.l^{-1}; T° = 20 and 30°C)

- ***Linearization***

Inverting equation (3) gives:

$$\frac{1}{\upsilon_{SO4}} = \frac{K_{SO4}}{\upsilon_{max}.S_{SO_4^{2-}}} + \frac{1}{\upsilon_{max}} \qquad (4)$$

Multiplying equation (4) by $S_{SO_4^{2-}}$ produces equation (5):

$$\frac{S_{SO_4^{2-}}}{\upsilon_{SO4}} = \frac{K_{SO4}}{\upsilon_{max}} + \frac{S_{SO_4^{2-}}}{\upsilon_{max}} \qquad (5)$$

which leads to the familiar Langmuir plot for the estimation of K_{SO4} and υ_{max} (Figure 4).

Therefore, a Langmuir plot (Doran, 1995) of $\frac{S_{SO_4^{2-}}}{\upsilon_{SO4}}$ versus $S_{SO_4^{2-}}$ gives a straight line with slope $\frac{1}{\upsilon_{max}}$ and intercept $\frac{K_{SO4}}{\upsilon_{max}}$ (Figure 4).

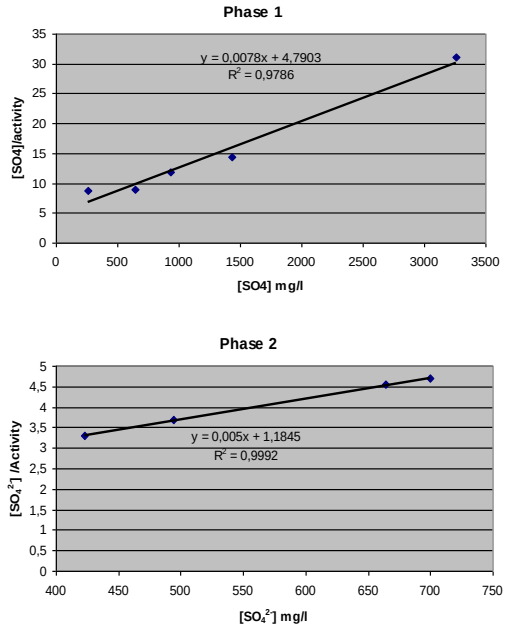

Figure 4. Estimation of K_{SO4} and υ_{max} for sulphate in Phases 1 and 2

Based on these graphs, the results of the tests are shown in Table 7 (X is here the total VSS):

Table 7. K_{SO4} and υ_{max} values for SRB in Phases 1 (20°C) and 2 (30°C)

	$\frac{1}{\upsilon_{max}}$	$\frac{K_{SO4}}{\upsilon_{max}}$	υ_{max} mgSO4/gVSS.d	K_{SO4} mg/l
Phase 1	0,0078	4,8	128	614
Phase 2	0,005	1,2	200	240

DISCUSSION AND CONCLUSIONS

The calculated K_{SO4} values for Phases 1 and 2 are 614 and 240 mg/l, respectively. The saturation constant's values decreased as temperature increased. Using data from a batch system, Characklis and Marshall (1989) likewise reported an increase in K_{SO4} with a temperature increase.

The values of K_{SO4} in this study are higher than the values reported in the literature, which range from 27 to 125 mg/l (Ingvorsen, 1984). This means that the sulphate reduction processes in stabilisation ponds are slower to reach their maximum process rates than the processes in other anaerobic reactors. In our case the biomass was not pure strains of SRB species.

When the temperature was increased from 20°C to 30°C, the maximum specific rate of sulphate utilization increased from 128 to 200 mg $SO_{4reduced}.gVSS^{-1}.d^{-1}$. This increase in maximum specific rate with increasing temperature has also been reported by Moosa *et al.* (2004). The υ_{max} values obtained for this work compare well with those reported in the literature. For instance, the υ_{max} values reported by Patidar *et al*., (2004) for bioreduction of sulphate vary between 40 and 190 mg $SO_{4reduced}.gVSS^{-1}.d^{-1}$.

In conclusion, the results of this study have established the values of K_{SO4} and υ_{max} for SRB in an anaerobic pond. These parameters of sulfidogenic bacteria will be used in the Anaerobic Pond Model to describe the sulphate reduction processes and to evaluate the risk of odour generation.

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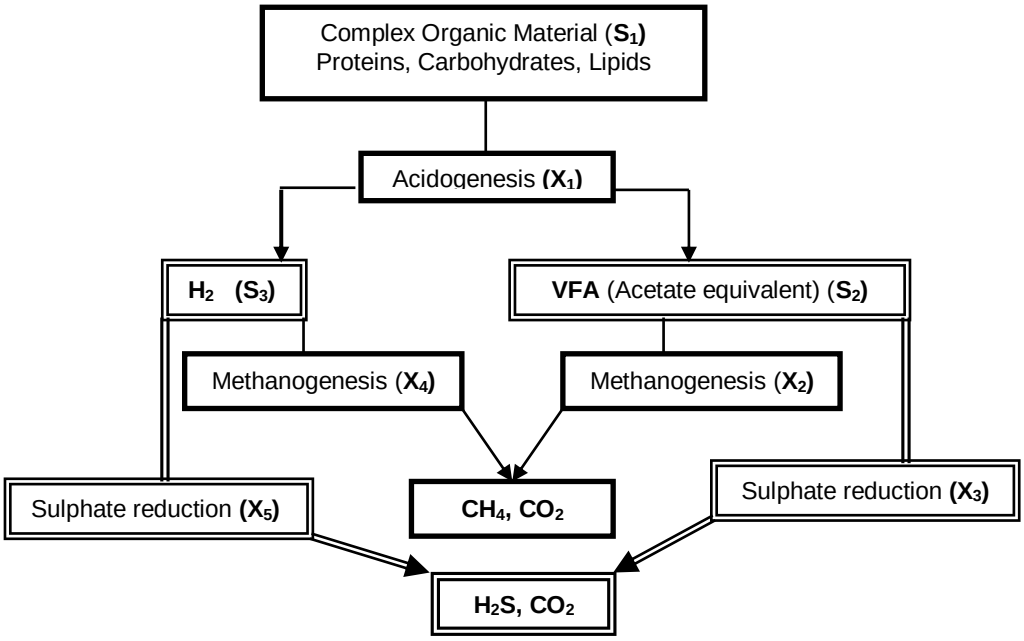

Figure 1. Flow chart of APM with sulphate reduction processes

Table 1. Sulphate reduction extension for APM (soluble components)

	Component i	**3**	**4**	**5**	**7**	**8**	**9**	**Rate (ρ_j, kg COD.m^{-3}.d^{-1})**
j	**Process**	S_{AC}	S_{so4}	S_{H2S}	S_{IC}	S_{IN}	S_{H+}	
6	Uptake of acetate by SRB	-1	Y_3 - 1	1 - Y_3	2 - 2Y_3	-0.4Y_3	2.4 Y_3 -2	$\upsilon_{\max} \frac{S_{SO4}}{K_{S04}+S_{SO4}} \frac{S_{ac}}{K_{ac}+S_{ac}} X_3$
7	Uptake of H_2 by SRB	-1	Y_5 - 1	1 - Y_5	-2Y_5	-0.4Y_5	2.4 Y_5 - 2	$\upsilon_{\max} \frac{S_{SO4}}{K_{S,S04}+S_{SO4}} \frac{S_{h2}}{K_{S,h2}+S_{h2}} X_5$
		Acetate (kgCOD m^{-3})	Sulphate (kgCOD m^{-3})	Total sulphide (kgCOD m^{-3})	Inorganic carbon gas (kmoleC m^{-3})	Inorganic nitrogen (kmoleN m^{-3})	Proton (kgCOD m^{-3})	

Table 2. Sulphate reduction extension for APM (particulate components)

	Component i	**13**	**14**	**Rate (ρ_j, kg COD.m^{-3}.d^{-1})**
j	**Process**	X_3	X_5	
6	Uptake of Acetate by SRB	0.4Y_5		$\upsilon_{\max} \frac{S_{SO4}}{K_{S04}+S_{SO4}} \frac{S_{ac}}{K_{ac}+S_{ac}} X_3$
7	Uptake of H_2 by SRB		0.4Y_3	$\upsilon_{\max} \frac{S_{SO4}}{K_{S,S04}+S_{SO4}} \frac{S_{h2}}{K_{S,h2}+S_{h2}} X_5$
12	Decay of X_3	-1		$K_{dec,X3}.X_3$
13	Decay of X_5		-1	$K_{dec,X5}.X_5$
		Acetotrophic SRB (kg COD m^{-3})	Hydrogenotrophic SRB (kg COD m^{-3})	

Table 3. Kinetic parameters and rates used in the model

Symbol	Description	Units
μ_{max}	Monod maximum specific growth rate	d^{-1}
υ_{max}	Monod maximum specific uptake rate	gCOD_S. gCOD_X^{-1}. d^{-1}
Y_s	Yield of biomass on substrate	gCOD_X. gCOD_S^{-1}
$K_{S,process}$	Half saturation value of substrate	gCOD_S. l^{-1}
$K_{SO4,process}$	Half saturation value of sulphate	gSO_4^{2-}. l^{-1}
k_{dec}	First order decay rate	d^{-1}
ρ_j	Kinetic rate of process j	gCOD_S. l^{-1}. d^{-1}
Y_{SO4}	Yield of biomass on sulphate	gCOD_X. gSO_{42-}

Table 4: True yield Y estimated from thermodynamic method

Organism Types	X_1	X_2	X_3	X_4	X_5
Y (gCOD_X.gCOD_S^{-1})	0.14	0.05	0.08	0.08	0.05

Table 5. Metabolic stoichiometric reactions involved in the APM/SR

Uptake of complex organic material by X_1

$$C_8H_{16}O_6N + 2.476H_2O \rightarrow 0.231C_5H_7O_2N + 2.838CH_3COOH + 2.838H_2 + 1.169CO_2 + 0.769NH_3$$

Uptake of acetate by X_2

$$CH_3COOH + 0.02NH_3 \rightarrow 0.02C_5H_7O_2N + 0.95CH_4 + 0.95CO_2 + 0.06H_2O$$

Uptake of acetate by X_3

$$CH_3COOH + 0.92SO_4^{2-} + 0.032NH_3 + 1.84H^+ \rightarrow 0.032C_5H_7O_2N + 0.92H_2S + 1.84CO_2 + 1.8H_2O$$

Uptake of H_2 by X_4

$$H_2 + 0.008NH_3 + 0.27CO_2 \rightarrow 0.008C_5H_7O_2N + 0.23CH_4 + 0.524H_2O$$

Uptake of H_2 by X_5

$$H_2 + 0.95SO_4^{2-} + 0.02NH_3 + 1.9H^+ + 0.1CO_2 \rightarrow 0.02C_5H_7O_2N + 0.95H_2S + 3.96H_2O$$