Modelling of bacterial sulphate reduction in anaerobic ponds: Kinetics 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 stoichiometrics and kinetics parameters associated to this model. The results of this study provide the values of 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 K_{SO4} calculated at 20°C and 30°C are 614 mg/l and 240 mg/l respectively. When the temperature was increased from 20°C to 30°C, the maximum specific rate of sulphate utilization increased from 128 to 200 $mgSO4_{reduced}.gVSS^{-1}.d^{-1}$. The values of those parameters of sulfidogenic bacteria are used implementation of the Anaerobic Pond Model, to describe the sulphate reduction processes and to evaluate the risk of odour generation in a second paper.

Index Terms— Modelling, Sulphate-Reducing Bacteria, Anaerobic Pond, Stoichiometry, kinetics, Stoichiometry, odour.

1 INTRODUCTION

One of the most known disadvantages of Waste Stabilisation Ponds (WSP) is possible offensive odours generation, often associated with the presence of hydrogen sulphide (H_2S), itself generated by sulphate reduction processes [1], [2], [3]. When dissolved oxygen and nitrate in wastewater of WSP are absent, sulphate-reducing bacteria (SRB) will use sulphate as electron acceptor and of wastewater organic matter as substrate [4], [5]. Moreover, the main problems related to the sulphate reducing process are due the generation of hydrogen sulphide. This phenomenon creates odour and corrosion problems [6]. In addition H_2S is also toxic. According [7], odour nuisance does not occur in anaerobic pond when volumetric loading rate is lower than 400 g BOD m-3 d-1. and with domestic wastewaters containing less than 500 mg SO_4^{2-}/l. Pescod [8] suggested the same volumetric loading but with less than 100 $mgSO_4^{2-}$/l, to avoid nuisance odour. These quiet different recommendations are based on field observations and not on real measurements of the bacteria activity involved in these processes. Measuring the activity of the sulphate-reducing bacteria in the anaerobic pond will make it possible to have quantitative information on the sulphur cycle and the odours production [3]. The Anaerobic Pond Model (APM) developed by [9] did not take into account sulphate reduction processes, and is invalid to describe such type of dysfunction. The first aim of this study was to develop a structured mathematical model of sulphate reduction in anaerobic ponds and to estimate the true yield values from thermodynamic method. The second aim of the present work was to study kinetically the reduction of sulphate by SRB in presence of acetate as electron donor in batch mode.

2 MODEL DESCRIPTION

In anaerobic pond treating sulphate-containing waste waters, both sulphate reduction and methanogenesis can be the final step in the degradation process, because SRB are able of using many intermediates formed during anaerobic digestion [10]. Thus according to the accepted APM/SR (Anaerobic Pond Model including Sulphate Reduction processes) scheme (fig. 1), the conversion process is carried out by five groups of microorganisms: the group X_1 contains all acetogenic bacteria, X_2, all acetotrophic methanogenic bacteria (MB), X_3 acetotrophic SRB, X_4 hydrogenotrophic MB, X_5 hydrogenotrophic SRB but only X_3 and X_5 groups are new compared to the anaerobic digestion model.

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Extension of the APM reaction sequences for the sulphate reduction process was done by incorporation of the following biochemical processes: the sulphate reduction using volatile fatty acids VFA (Acetate equivalent) and sulphate reduction on hydrogen.

The process kinetic and stoichiometry for those biochemical reactions are given in table 1 (soluble components) and table 2 (particulate components) in the same format as Anaerobic Digestion Model no 1 (ADM1) [11]. The Table 3 lists the Kinetics parameters and rates used in the model.

Process S_{H2O} (H_2O) was excluded from table 1, but implicit from the stoichiometry.

2.1 Stoichiometry

Mathematical modelling needs a description of the stoichiometry and of the kinetic of the processes involved.

By taking account of the proportion 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", in this case $C_8H_{16}O_6N$ [12]. The nitrogen required for bacterial synthesis comes from the release of NH_3 during reaction. A stoichiometric model of sulphate reduction by SRB in Anaerobic Pond was developed by [3]. The theoretical yields Y of biomass X on substrate S used in the model are estimated from thermodynamic method according [13] (Table 4). Based on the "complex substrate" composition, stoichiometry of the involved process were developed taking into account the Y values and balances on COD. To simplify the model, the equations were developed to combine hydrolysis, acidogenesis and acetogenesis (Table 5).

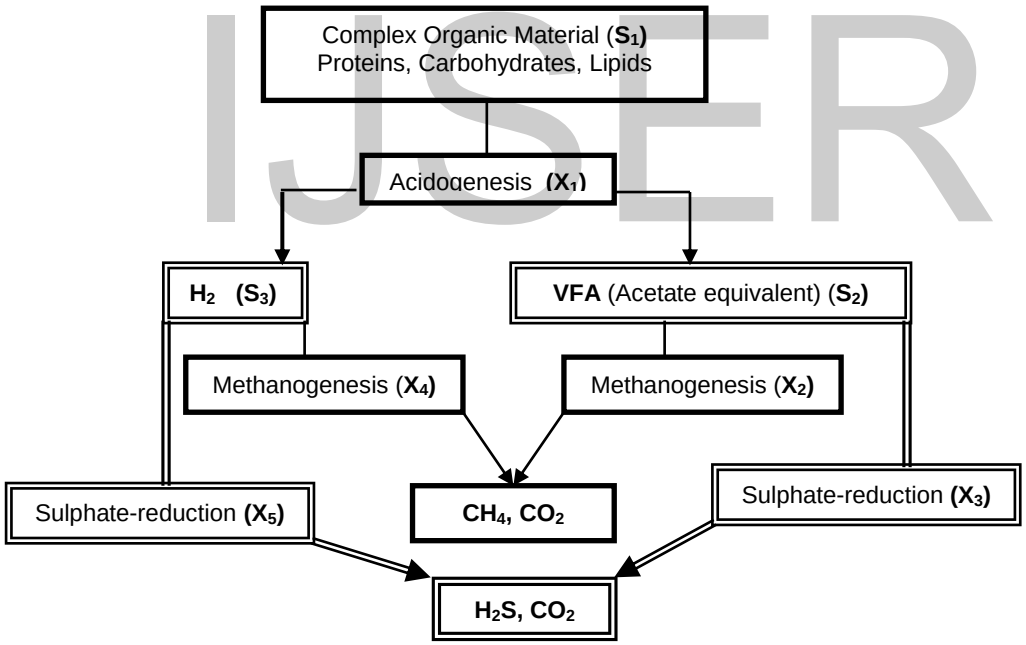

Fig. 1. Flow chart of APM with sulphate reduction processes

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 (kgCOD m^{-3})	Hydrogenotrophic SRB (kgCOD m^{-3})	

TABLE 3: KINETICS 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_4^{2-}

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: METABOLISM 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

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

2.2 Kinetic of sulphate reducing 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 ($gSO4_{reduced}.gVSS^{-1}.d^{-1}$);

KS: saturation constant for S (g/l); XSRB: sulphate-reducing bacteria (SRB) ($gVSS.l^{-1}$); VSS: volatile suspended solids (biomass); μmax: Maximum specific growth rate of SRB, KSO4: saturation constant for SO_4^{2-}.

Important for application of one of these equations is the estimation of typical model parameters like υ_{max}, μ_{max} and Y. Those model parameters are specific and dependent on COD sources.

3 Materials and Methods

3.1 Microorganisms 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 litters of anaerobic ponds sewage were centrifuged at 3500 rpm for 10 minutes. The tests were conducted on a synthetic wastewater [14]. For all experiments, a basal medium was used in such a way that C/N/P ratios did not constitute limitation in nutrients for bacterial growth (Table 6).

TABLE 6: COMPOSITION OF THE SYNTHETIC WASTEWATER USED FOR GROWTH OF SRB

Components	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	

3.2 Experimental procedure

The study was performed in Phase 1 at 20°C and Phase 2 at 30°C. 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 , 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; 700 mg.l^{-1}, for reactor D. 125 ml serum vials were used and filled with 100ml of sample, leaving a headspace of 25 ml. The pH of all media was set to value above 8. Nitrogen was bubbled thought the sample for at least 5 minutes after the addition of sodium acetate and potassium sulphate. This is to ensure that wastewater sample and the headspace are free from oxygen that would otherwise inhibit the sulphate reduction processes. Water samples in the reactors were continuously mixed by magnetic stirrer. Syringes were used to withdraw 4ml of liquid samples. The samples were immediately filtered through a 0,45 µm membrane filter. Sulphate was analysed by using ion chromatography. VSS, chemical oxygen demand (COD) were determined according to standard methods [15].

4 Results

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

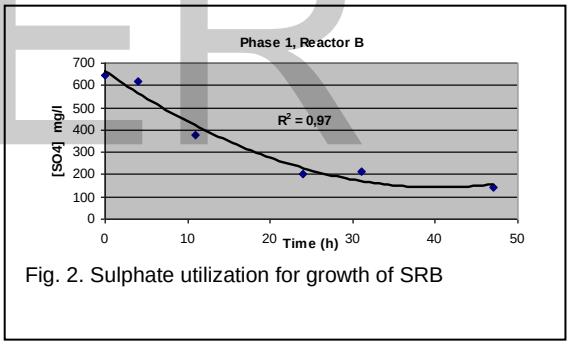

Fig. 2. Sulphate utilization for growth of SRB

Sulphate depletion data have been used to estimate kinetics parameters. Assuming the biomass concentration XSRB is constant and Si>>Ki, equation (1) can be transformed into (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 (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}

constant, the data for sulphate reduction activity (υ_{SO4}) as function of initial sulphate concentration (SO_4^{2-}) are obtained, as can be seen from fig. 3.

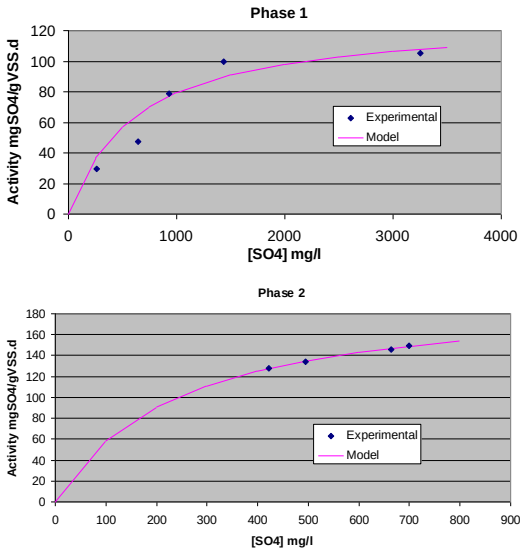

Fig. 3. Sulphate-reducing activity for various sulphate concentrations in Phase 1 and 2 (COD acetate = 2133mgCOD.l^{-1}; T° = 20 and 30°C)

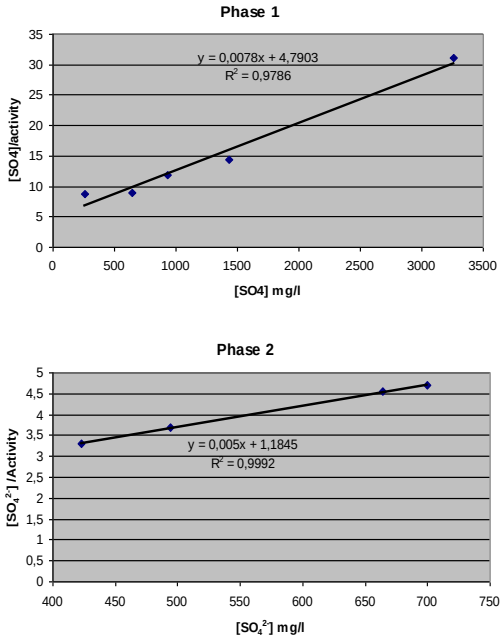

Fig. 4. Estimation of K_{SO4} and υ_{max} for sulphate in Phase 1 and 2

Linearization

Inverting (3) gives:

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

Multiplying (4) by $S_{SO_4^{2-}}$ produces (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} (fig. 4).

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

Based of these graphs, the results of the tests have shown in table 7 (X is here the total VSS):

TABLE 7: υ_{max} AND K_{SO4} VALUES FOR SRB IN PHASE 1 (20°C) AND PHASE 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

5 DISCUSSION

The values of K_{SO4} calculated for Phase 1 and Phase 2 are 614 mg/l and 240 mg/l respectively. Values of saturation constant decrease as temperature increased. Using data from a batch system, Characklis and Marshall [16] reported also a K_{SO4} increase 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 [17]. This means that the sulphate reduction

processes in stabilisation pond is slower in reaching the maximum processes rate compared to the processes in other anaerobic reactors. In our case the biomass was not pure strains of SRB species.
When the temperature was increased in the range 20°C to 30°C, the maximum specific rate of sulphate utilization increased from 128 to 200 $mgSO4_{reduced}.gVSS^{-1}.d^{-1}$. This increase in maximum specific rate with the temperature increase has also been reported [18]. The υ_{max} values obtained for this work compare well with those reported in the literature. υ_{max} Values reported by [19] for bioreduction of sulphate vary between 40 and 190 mg $SO4_{reduced}.gVSS^{-1}.d^{-1}$.

6 CONCLUSION

A structured mathematical model of sulphate reduction in anaerobic ponds was developed and the true yield values are estimated from thermodynamic method.

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

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