

# Influence of the COD to sulphate ratio on the anaerobic organic matter degradation kinetics

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## Abstract

The influence of the COD to sulphate ratio on the substrate consumption kinetic parameters was evaluated using a differential reactor filled with polyurethane foam matrices, taken from a horizontal-flow anaerobic immobilised biomass (HAIB) reactor. Experiments were carried out with synthetic substrate containing glucose, ammonium acetate and methanol, besides sodium bicarbonate. Micronutrients were provided by adding salts and trace metals from a stock solution. The influent chemical oxygen demand (COD) was maintained as constant as possible around 2 000 mg·l<sup>-1</sup> in all the experiments. The reactors were subjected to increasing sulphate concentrations from 100 to 2 800 mg·l<sup>-1</sup>. First-order apparent kinetic parameters ( $K_1^{app}$ ) decreased from  $1.96 \times 10^{-4}$  to  $1.55 \times 10^{-4}$  l·mg VSS<sup>-1</sup>·h<sup>-1</sup> as the COD to sulphate ratio decreased from 22.6 to 0.8. The progressive but moderate inhibition of the organic matter conversion observed indicates that high sulphate concentrations can be accommodated in HAIB reactors even at very low COD to sulphate ratios.

## Introduction

The presence of sulphate in wastewaters can sometimes represent a serious problem when the anaerobic treatment process is used. Sulphide generation may cause problems such as odour and corrosion, besides toxicity in an aquatic medium. In addition, the anaerobic treatment of sulphate-rich wastewaters deserves special attention, since several interactions between methane-producing archaea (MPA) and sulfate-reducing bacteria (SRB) take place in the anaerobic reactor. The result of these syntrophic or competitive interactions can compromise the successful application of anaerobic biotechnology.

Sulphate emissions are not a direct threat to the environment, but high sulphate concentrations can cause an imbalance in the natural sulphur cycle. Sulphide production can present serious operational problems in anaerobic reactors used for the treatment of wastewaters containing high sulphate concentrations (Lens et al., 1998).

Hydrogen sulphide (H<sub>2</sub>S) in aqueous and gaseous solution causes chemical (corrosion, odour, increase of the effluent COD) and biological (toxicity, inhibition) problems that can affect the wastewater treatment process. These factors have been a barrier to the wider application of anaerobic processes for the treatment of wastewaters containing high sulphate concentrations generated in several types of industries. Therefore, it is necessary to elucidate the factors governing the interactions among the different types of micro-organisms involved in the process, and to develop technologies and strategies to guarantee the successful application of the anaerobic process for the treatment of sulphate-containing wastewaters (Colleran et al., 1995).

The problems of odour and corrosion have been conveniently solved by the collection and treatment of the biogas and the use of materials resistant to corrosion. However, maintaining methano-

genic stability is only possible under favourable conditions for process self-control. Among other conditions, an effective management of the factors that interfere in the equilibrium of the microbial community in anaerobic reactors is required. Such factors include pH, temperature, hydraulic retention time (HRT), organic and sulphate loading rates and the relationship between chemical oxygen demand (COD) and sulphate.

The main current problems related to the presence of high sulphate concentrations in the influent of anaerobic reactors are recognised as: competition between sulphate-reducing bacteria (SRB) and methane-producing archaea (MPA) for the same substrates (H<sub>2</sub>, acetate); sensitivity of MPA to sulphide, leading to methanogenesis inhibition when the sulphide concentration surpasses certain limits; precipitation of trace metals, causing nutritional deficiencies in the reactor. Competition between MPA and SRB in an anaerobic consortium is based on subtle inter-relationships still not clarified (Speece, 1996).

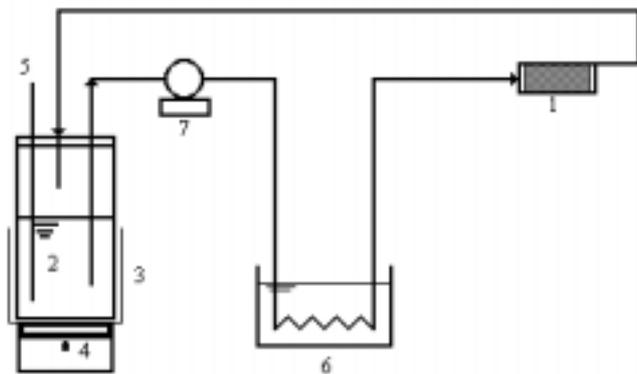
Verstraete & Vandevivere (1999) and Silva et al. (2002) reported on the use of anaerobic processes for sulphate removal. Such technology has been optimised in order to recover sulphur, thus avoiding its emission as pollutant and recycling it as raw material.

The COD to sulphate ratio is a parameter widely used to control biological sulphate reduction, as well as the methanogenic process leading to organic matter consumption. Wastewaters with a COD/sulphate ratio of 0.67 contain enough sulphate available to completely remove the organic matter via sulphate reduction. This is a theoretical ratio based on stoichiometry and assumes that all the COD is in a form that can be utilised by SRB. However, several factors can influence the microbial competition between MPA and SRB and an oversimplified analysis can lead to contradictory results (Speece, 1996).

The kinetic approach can be useful to elucidate some aspects of sulphate reduction and methanogenesis in anaerobic reactors. Thus, biochemical reaction rates and kinetic parameters can be used to investigate the effect of sulphate in an anaerobic reactor. Evaluation of organic substrate consumption rates in a reactor

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**Figure 1**

Experimental apparatus to estimate kinetic parameters. (1) Differential reactor; (2) Agitated tank; (3) Ice batch; (4) Magnetic stirrer; (5) Sampler; (6) Heat exchange; (7) Peristaltic pump.

subjected to various sulphate concentration levels can assist in elucidating the extension of the effects of the sulphate on the overall process of organic matter decomposition.

In this work, kinetic studies were carried out aimed at investigating organic matter consumption rates under different COD to sulphate ratios. Apparent kinetic parameters were estimated and used to evaluate the influence of the progressive increase of sulphate concentration on the organic matter degradation rates.

## Material and methods

The kinetic studies here performed evaluated the organic material degradation rates under different sulphate contents levels. Experiments were carried out using a differential reactor with a volume of 10 ml and 15 mm-length, as presented in Fig. 1. This type of apparatus was first used in kinetic assays by Zaiat et al. (1997).

The differential reactor was filled with polyurethane foam particles containing immobilised anaerobic biomass. A synthetic sulphate-rich wastewater used in the kinetic assays contained glucose ( $1\,750\text{ mg}\cdot\text{l}^{-1}$ ), ammonium acetate ( $400\text{ mg}\cdot\text{l}^{-1}$ ) and methanol ( $0.5\text{ mg}\cdot\text{l}^{-1}$ ) besides sodium bicarbonate. Micronutrients were added from a stock solution of salts and trace metals (nickel sulphate –  $0.5\text{ mg}\cdot\text{l}^{-1}$ , ferrous sulphate –  $5.0\text{ mg}\cdot\text{l}^{-1}$ , ferric chloride –  $0.5\text{ mg}\cdot\text{l}^{-1}$ , calcium chloride –  $44.5\text{ mg}\cdot\text{l}^{-1}$ , cobalt chloride –  $0.08\text{ mg}\cdot\text{l}^{-1}$ , magnesium sulphate –  $22.5\text{ mg}\cdot\text{l}^{-1}$ , monobasic potassium phosphate –  $8.5\text{ mg}\cdot\text{l}^{-1}$ , dibasic potassium phosphate –  $21.8\text{ mg}\cdot\text{l}^{-1}$ , dibasic sodium phosphate –  $33.4\text{ mg}\cdot\text{l}^{-1}$ ). Initial COD was maintained as constant as possible throughout all the experiments, achieving  $2,272 \pm 74\text{ mg}\cdot\text{l}^{-1}$ . Sulphate was added progressively as  $\text{Na}_2\text{SO}_4$ , in the concentrations of 100, 500, 1 000, 1 750 and 2 800  $\text{mg SO}_4^{2-}\cdot\text{l}^{-1}$ , resulting in COD/sulphate ratios ranging from 22.6 to 0.8.

Polyurethane foam bioparticles were taken from a bench-scale packed-bed reactor treating the same sulphate-rich wastewater at the same environmental conditions of the differential reactor used for kinetic assays. The bench-scale packed-bed bioreactor was operated at a constant hydraulic retention time of 12 h. Each specific experimental condition was maintained in the packed-bed reactor for at least two months before the corresponding kinetic experiment was performed.

Substrate (600 ml) was recirculated in a closed circuit from the 1 l agitated flask to the differential reactor. Before entering the reactor, the substrate was heated to  $30^\circ\text{C}$  and returned to the tank, which was maintained at  $5^\circ\text{C}$  to minimise the occurrence of

biochemical reactions outside the reactor.

The differential reactor was subjected to different liquid flow rates, allowing for the application of varying liquid superficial velocities ( $v_s$ ) for each COD to sulphate ratio applied in order to verify the influence of the liquid-phase mass transfer on the overall degradation rate. Each experiment lasted 10 h and 12 ml samples were taken every 2.5 h for analysis of chemical oxygen demand (COD) according to *Standard Methods* (1995). Therefore, it was possible to obtain the temporal profile of substrate concentrations in the liquid phase ( $S_b$ ) for each COD to sulphate condition and for each applied  $v_s$ . The sampling procedure adopted allowed for only 8% of the total liquid volume to be altered during the experimental period.

At the end of each experiment, the total mass of microorganisms immobilised in the matrices was determined as volatile suspended solids (VSS) after removing the solids from the foam with 100 ml distilled water.

Equations were adjusted to the experimental profiles of  $S_b$  using the software Excel 97 (Microsoft®), thus permitting the estimation of the apparent kinetic parameters for organic matter degradation.

## Results and discussion

Profiles of organic matter concentration (expressed as COD) as a function of time at different liquid superficial velocities ( $v_s$ ) were obtained for each applied COD to sulphate ratio.

A first-order kinetic model was found to represent the kinetic behaviour in all cases studied. So, exponential decay expressions were adjusted to all the profiles with good correlation coefficient, as:

$$S_b = S_{bo} \cdot e^{-k_1^{app} \cdot t} \quad (1)$$

Equation (1) correlates the concentration of organic matter in the liquid phase ( $S_b$ ) with time ( $t$ ) and with the initial concentration of the substrate in the liquid phase ( $S_{bo}$ ).  $K_1^{app}$  is the first-order apparent kinetic constant. Such a parameter is apparent, because it includes the phenomenon of mass transfer in the liquid and solid phases, besides the organic matter conversion rate.

The adjusted curves, presented in Tables 1 to 5, provide values of first-order apparent specific kinetic parameters ( $K_1^{app}$ ) for each liquid superficial velocity applied to the differential reactor. No tendency for an increase in  $K_1^{app}$  values as  $v_s$  was increased was observed in any experimental condition assayed, thus indicating that the liquid-phase mass transfer resistance did not affect the overall reaction rate. Therefore, the overall conversion rates were mainly influenced by the biochemical reactions rates and by the solid-phase mass transfer flux in the experiments here described.

The first-order kinetic model represented the observed organic matter consumption rate well and the kinetic parameter was found to be independent of the liquid superficial velocity for all the COD to sulphate ratios assayed. In that way, a mean value of  $K_1^{app}$  could be obtained for each COD to sulphate ratio (Table 6).

It can be observed that the value of  $K_1^{app}$  decreased with the COD to sulphate ratio. Thus, the organic matter consumption rate decreased with the increase of sulphate content in the wastewater. Such a decrease was probably related to the interactions between sulphidogenic and methanogenic activities. The pH in all the experiments ranged between 7.5 and 8.0.

The  $K_1^{app}$  behaviour in respect of the COD to sulphate ratio could be represented adequately by a hyperbolic expression as:

**TABLE 1**  
Expressions adjusted to the experimental COD profiles for each liquid superficial velocity and respective correlation coefficients ( $r^2$ ). COD/sulphate = 22.6.

$v_s$ (cm·s <sup>-1</sup> )	First-order expression	$r^2$
0.058	$S_b = 2271.7.e^{-0.0570.(t)}$	0.9939
0.083	$S_b = 2279.1.e^{-0.0568.(t)}$	0.9937
0.107	$S_b = 2170.4.e^{-0.0641.(t)}$	0.9696
0.138	$S_b = 2187.9.e^{-0.0588.(t)}$	0.9925
0.162	$S_b = 2342.5.e^{-0.0626.(t)}$	0.9926

Number of observations (n): 5

**TABLE 4**  
Expressions adjusted to the experimental COD profiles for each liquid superficial velocity and respective correlation coefficients ( $r^2$ ). COD/sulphate = 1.3.

$v_s$ (cm·s <sup>-1</sup> )	First-order expression	$r^2$
0.048	$S_b = 2244.4.e^{-0.0468.(t)}$	0.9978
0.071	$S_b = 2188.2.e^{-0.0587.(t)}$	0.9983
0.098	$S_b = 2169.8.e^{-0.0679.(t)}$	0.9850
0.119	$S_b = 2197.8.e^{-0.0703.(t)}$	0.9901
0.159	$S_b = 2249.4.e^{-0.0646.(t)}$	0.9931

Number of observations (n): 5

**TABLE 2**  
Expressions adjusted to the experimental COD profiles for each liquid superficial velocity and respective correlation coefficients ( $r^2$ ). COD/sulphate = 4.8.

$v_s$ (cm·s <sup>-1</sup> )	First-order expression	$r^2$
0.046	$S_b = 2356.1.e^{-0.0568.(t)}$	0.9949
0.070	$S_b = 2361.1.e^{-0.0503.(t)}$	0.9827
0.094	$S_b = 2358.5.e^{-0.0664.(t)}$	0.9894
0.119	$S_b = 2328.7.e^{-0.0594.(t)}$	0.9810
0.159	$S_b = 2292.5.e^{-0.0612.(t)}$	0.9601
0.182	$S_b = 2291.9.e^{-0.0627.(t)}$	0.9623

Number of observations (n): 5

**TABLE 5**  
Expressions adjusted to the experimental COD profiles for each liquid superficial velocity and respective correlation coefficients ( $r^2$ ). COD/sulphate = 0.8.

$v_s$ (cm·s <sup>-1</sup> )	First-order expression	$r^2$
0.033	$S_b = 2332.8.e^{-0.0531.(t)}$	0.9916
0.042	$S_b = 2293.5.e^{-0.0639.(t)}$	0.9955
0.068	$S_b = 2132.3.e^{-0.0602.(t)}$	0.9649
0.094	$S_b = 2143.6.e^{-0.0714.(t)}$	0.9961
0.120	$S_b = 2129.8.e^{-0.0624.(t)}$	0.9798

Number of observations (n): 5

**TABLE 3**  
Expressions adjusted to the experimental COD profiles for each liquid superficial velocity and respective correlation coefficients ( $r^2$ ). COD/sulphate = 2.3.

$v_s$ (cm·s <sup>-1</sup> )	First-order expression	$r^2$
0.042	$S_b = 2258.4.e^{-0.063.(t)}$	0.9947
0.065	$S_b = 2240.4.e^{-0.0686.(t)}$	0.9962
0.086	$S_b = 2151.0.e^{-0.0593.(t)}$	0.9724
0.107	$S_b = 2193.0.e^{-0.0524.(t)}$	0.9672
0.144	$S_b = 2257.9.e^{-0.0599.(t)}$	0.9898
0.166	$S_b = 2215.0.e^{-0.0505.(t)}$	0.9780

Number of observations (n): 5

**TABLE 6**  
Mean values of estimated as a function of COD to sulphate ratio and the mean biomass concentration (X) for each experiment

COD/sulphate	X (mg VSS·l <sup>-1</sup> )	$K_1^{app}$ (l·mg VSS·h <sup>-1</sup> )
22.6	306	$1.96 (\pm 0.10) \times 10^{-4}$
4.8	323	$1.84 (\pm 0.17) \times 10^{-4}$
2.3	338	$1.74 (\pm 0.20) \times 10^{-4}$
1.3	361	$1.71 (\pm 0.26) \times 10^{-4}$
0.8	401	$1.55 (\pm 0.16) \times 10^{-4}$

$$k_1^{app} = 1.94 \times 10^{-4} \frac{(COD/SO_4^{2-})}{0.204 + (COD/SO_4^{2-})} \quad (2)$$

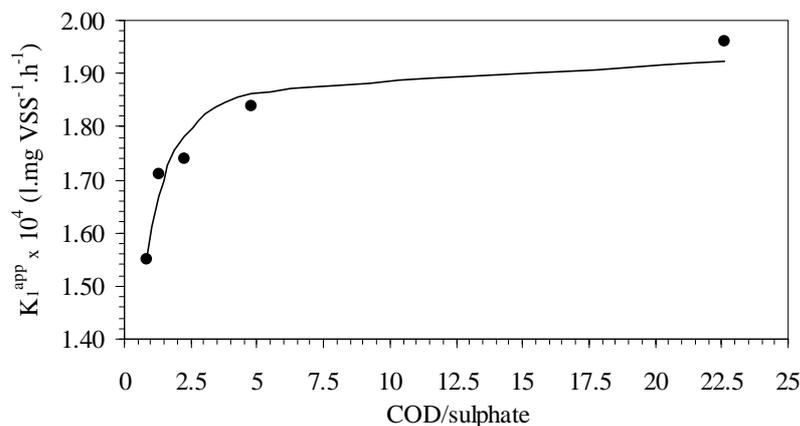
Equation (2) adjusted to the experimental data, with correlation coefficient of 0.9466, is presented in Fig. 2.

The maximum value of  $K_1^{app}$  predicted by Eq. (2) is  $1.94 \times 10^{-4}$  l·mg<sup>-1</sup>VSS·h<sup>-1</sup>. Such value is achieved under conditions of high

COD to sulphate ratio. The expression also indicates that the reaction rate would be half its maximum value at COD/SO<sub>4</sub><sup>2-</sup> of 0.20, approximately.

The influence of the COD to sulphate ratio on the overall reaction rate is more effective for ratios lower than 5.0, probably due to the higher sulphide concentrations in these conditions, inhibiting methanogenesis and causing an imbalance in the anaerobic conversion process.

Although simple, the experimental protocol proposed here was found to be useful for process analysis purposes. The inclusion of microbiological characterisation and determination of other constituents can sometimes improve the method.



**Figure 2**  
Apparent first-order kinetic parameters as a function of COD to sulphate ratio. Experimental values (•) and adjusted expression (—).

## Conclusions

Kinetic studies were found to be a useful tool to assess data of the influence of the COD to sulphate ratio on the anaerobic conversion process. Very simple experiments can be conducted leading to important information on organic matter consumption under different influent sulphate concentrations.

In this study, the first-order kinetic model adequately represented the organic matter degradation rates. It was verified that the apparent kinetic parameters ( $K_1^{app}$ ) were not affected by altering the liquid superficial velocity. Therefore, the liquid-phase mass transfer did not have an influence on the overall reaction rates.

The values of  $K_1^{app}$  decreased with the COD to sulphate ratios and a hyperbolic function was found suitable to represent such a variation. This behaviour indicates a possible moderated inhibition of the organic matter conversion process as the sulphate concentration increases. This inhibition provoked a decrease of approximately 21% in the observed reaction rate when the COD/sulphate decreased from 22.6 to 0.8.

The method proposed here was found to be useful to assess data of the overall organic matter degradation rate under different sulphate concentrations, thus permitting the evaluation of the COD to sulphate ratio influence on the anaerobic organic matter conversion process. However, the data obtained are limited to the experimental conditions imposed, such as temperature, condition of cell immobilisation, type and size of immobilisation matrix. It is well known that the nature of the organic matter compounds in the substrate can affect the overall reaction rate. For this reason, extrapolations to other systems treating different organic matter sources have to be carefully made.

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