Removing organic matter from sulfate-rich wastewater via sulfidogenic and methanogenic pathways

Rogerio Silveira Vilela, Márcia Helena Rissato Zamariolli Damianovic and Eugenio Foresti

ABSTRACT

The simultaneous organic matter removal and sulfate reduction in synthetic sulfate-rich wastewater was evaluated for various chemical oxygen demand (COD)/sulfate ratios applied in a horizontal-flow anaerobic immobilized sludge (HAIS) reactor. At higher COD/sulfate ratios (12.5 and 7.5), the removal of organic matter was stable, likely due to methanogenesis. A combination of sulfate reduction and methanogenesis was clearly established at COD/sulfate ratios of 3.0 and 1.9. At a COD/sulfate ratio of 1.0, the organic matter removal was likely influenced by methanogenesis inhibition. The quantity of sulfate removed at a COD/sulfate ratio of 1.0 was identical to that obtained at a ratio of 1.9, indicating a lack of available electron donors for sulfidogenesis. The sulfate reduction and organic matter removal were not maximized at the same COD/sulfate ratio; therefore, competitive inhibition must be the predominant mechanism in establishing an electron flow.

Key words | anaerobic reactor, biological treatment, COD/sulfate ratio, methanogenesis, sulfidogenesis

Rogerio Silveira Vilela
Márcia Helena Rissato Zamariolli Damianovic
Eugenio Foresti (corresponding author)
Department of Hydraulic and Sanitation
    Engineering,
Escola de Engenharia de São Carlos,
Universidade de São Paulo,
Av. Trabalhador São-Carlense,
400, São Carlos – São Paulo,
Brazil
E-mail: eforesti@sc.usp.br

INTRODUCTION

The presence of sulfate in wastewater that has been subjected to anaerobic treatment processes can interfere with methanogenic processes in different ways, including competing for electron donors and producing sulfide and CO_2 through complete oxidation via sulfate-reducing bacteria (SRB) or acetate and sulfide via SRB that are incomplete oxidizers (Colleran et al. 1995; Omil et al. 1998; Damianovic & Foresti 2009). Other effects include stimulation (Zehnder & Wuhrmann 1977; Speece 1983) due to sulfur's role as a macronutrient for MA (methanogenic archaea); toxicity (Muyzer & Stams 2008) caused by a high concentration of sulfide in the liquid phase; and syntrophism (Bryant et al. 1967) resulting from incomplete SRB-mediated oxidation, which produces acetate, the main substrate for acetoclastic methanogens.

In the presence of sulfate without other electron acceptors, the electron flux in organic matter degradation is directed toward sulfidogenesis, methanogenesis or a combination of the two (Lens et al. 1998). The chemical oxygen demand (COD)/sulfate ratio is deemed the most important parameter driving the electron flux, as recently studied by several authors (Mulopo et al. 2011; Subtil et al. 2011; Cao et al. 2012; Li et al. 2012). For COD/sulfate ratios equal to or below 0.67, the organic matter is completely removed via sulfidogenesis. The reactor configuration, the wastewater composition, the operating time and the sulfate concentration are also important factors affecting the ability of SRB to outcompete MA. At higher COD/sulfate ratios, the complete removal of organic matter is only possible through methanogenesis (Lens et al. 1998).

However, the long-term performance of immobilized biomass reactors that treat wastewater subjected to different COD/sulfate ratios remains uncertain. The literature contains controversial information regarding the extent of competition for electron donors, the factors affecting the acclimatization of the MA to sulfide and the influence of the reactor configuration on the processes.

This study evaluates the performance of a horizontal-flow anaerobic immobilized sludge (HAIS) reactor for the treatment of sulfate-rich wastewater subjected to different COD/sulfate ratios. Special attention is given to evaluating the electron flux, which supports methanogenesis and sulfidogenesis at varying COD/sulfate ratios.

METHODS

The bench-scale HAIS reactor, with a total volume of 1,991 mL, consisted of a 1-m-long tube with a 5-cm diameter and four ports equally spaced (20 cm) along its length for sampling purposes. The HAIS reactor was filled with polyurethane foam matrices for biomass immobilization. These characteristics defined the fluid dynamics as a non-ideal plug-flow system. The reactor was installed inside a 30 °C temperature-controlled chamber (Figure 1).

The inoculum was the sludge taken from an up-flow anaerobic sludge blanket (UASB) reactor treating poultry slaughterhouse. The granular sludge was macerated before immobilization in polyurethane foam matrices, and inoculation was according to procedures adopted by Foresti *et al.* (1995). The reactor was subjected to synthetic wastewater containing 30 mg L^{-1} of sulfate (COD/sulfate = 50 mg L^{-1}) for 2 weeks. At the end of this startup period, the reactor presented high COD removal efficiency and the operation was performed through six stages.

The hydraulic retention time was set at 12 ± 1 h with a constant flow maintained by a peristaltic pump at a flow rate of 60 mL h^{-1}. Anaerobic sludge (total volatile solids of 500 mg L^{-1}) from the UASB reactor for the treatment of poultry slaughterhouse wastewater was used as the inoculum. The HAIS reactor was operated for 216 days.

The HAIS reactor was supplied with synthetic sulfate-rich wastewater and ethanol as the carbon source and electron donor (Table 1). The operating conditions comprised six distinct stages in which the organic matter concentration was fixed while the sulfate concentration gradually increased (through the addition of Na$_2$SO$_4$), thus decreasing the COD/sulfate ratio (Table 2).

Ethanol (CH$_3$CH$_2$OH) was chosen as an organic electron donor because it is easy to manipulate and its performance in combined sulfidogenesis and methanogenesis is well understood. Sodium hydroxide (NaOH) was added to maintain a pH between 7.0 and 8.0 to minimize the toxic effects of the sulfide (Hulshoff Pol *et al.* 1998).

The reactor performance was evaluated based on the influent and effluent analyses of sulfate, COD, volatile fatty acids (VFA) and sulfide according to APHA (2005). The concentration gradients of COD, sulfate and sulfide along the reactor's length were determined in duplicate samples taken twice weekly at the end of every experimental stage except stage 1.

The sulfate was determined via ion chromatography using a Dionex® chromatograph, model ICS-5000 (Thermo Scientific™, Sunnyvale, CA, USA), and the results were obtained using Dionex® Chromeleon Instrumental Control 6.8 software. The sulfide in the liquid phase was determined using the colorimetric method according to APHA (2005). For COD determination, sulfide was previously removed by precipitation with zinc acetate.

The electron fluxes in the organic matter removal via sulfidogenesis and methanogenesis were calculated by assuming that the electron donor was completely oxidized by SRB (Equations (1)–(3)). The fraction of organic matter
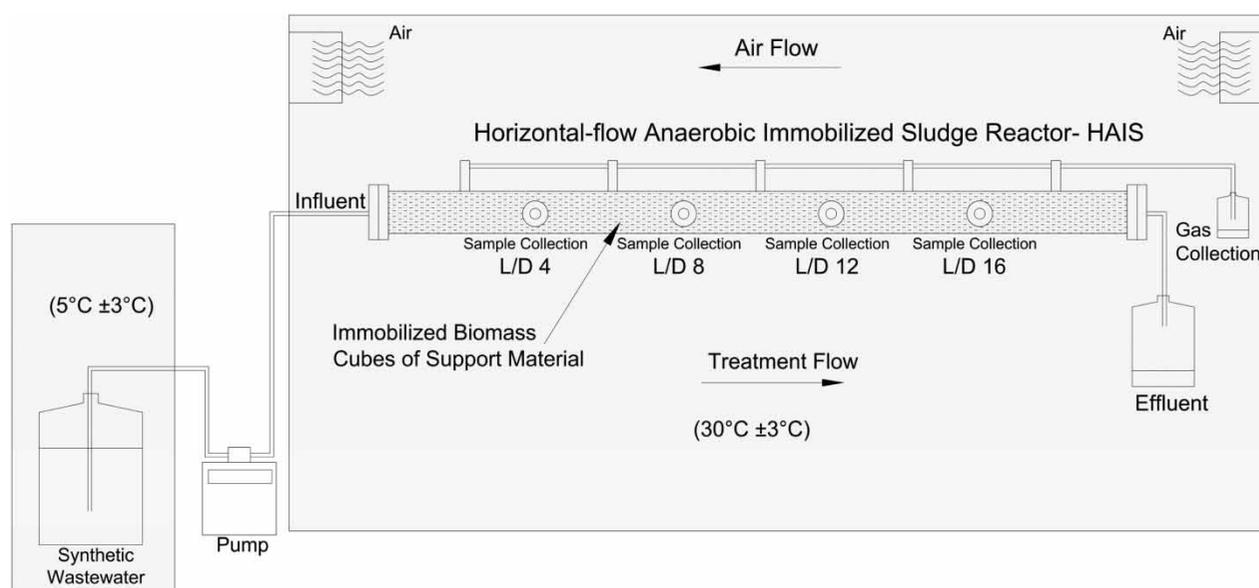

Figure 1 | Scheme of the bench-scale HAIS reactor.

Table 1 │ Synthetic wastewater composition

Constituent	Stock solution (g L^{-1})	Volume added (mL L^{-1})
Macronutrient solution 1 (Visser et al. 1993)		
NH$_4$Cl	15.00	1.00
KH$_2$PO$_4$	17.50	
Macronutrient solution 2 (Visser et al. 1993)		
KCl	27.00	0.03
MgCl$_2$·6H$_2$O	15.00	
Solution for pH control		
NaOH	80.00	1.50
Micronutrient/metal solution		
C$_6$H$_9$NO$_6$	12.80	1.00
FeCl$_3$·6H$_2$O	01.35	
MnCl$_2$·4H$_2$O	0.100	
CoCl$_2$·6H$_2$O	0.024	
CaCl$_2$·2H$_2$O	0.100	
ZnCl$_2$	0.100	
CuCl$_2$·2H$_2$O	0.025	
H$_3$BO$_3$	0.010	
NaMoO$_4$·H$_2$O	0.024	
NaCl	01.00	
Na$_2$SeO$_3$·5H$_2$O	0.026	
NiCl$_2$·6H$_2$O	0.120	
Vitamin solution (Touzel & Albagnac 1983)		
C$_{10}$H$_{16}$N$_2$O$_3$S	0.009	0.5
C$_{10}$H$_{16}$N$_2$O$_3$S	0.009	
C$_{12}$H$_{17}$N$_4$OS	0.023	
C$_{17}$H$_{20}$N$_4$O$_6$	0.023	
C$_6$H$_5$NO$_2$	0.023	
C$_{18}$H$_{32}$CaN$_2$O$_{10}$	0.023	
C$_8$H$_{11}$NO$_3$	0.046	
C$_{63}$H$_{88}$CoN$_{14}$O$_{14}$P	0.0001	
C$_8$H$_{14}$O$_2$S$_2$	0.023	
NH$_2$C$_6$H$_4$COOH	0.023	

removed by methanogenesis was calculated as the difference between total COD removed and (COD – S^{2-}), corresponding to the quantity of COD removed via sulfidogenesis. These routes are the only way to direct the electron flux from the ethanol present in the synthetic wastewater.

$$\text{Total COD removal}(\text{COD}_{\text{Total}})(\%)$$
$$= \left(\frac{(\text{COD influent} - \text{COD effluent})}{\text{COD influent}} \right) \times 100 \qquad (1)$$

$$\text{COD removal via sulfidogenesis} \left(\text{COD} - \text{S}^{2-} \right)(\%)$$
$$= \left(\frac{0.67(\text{sulfate influent} - \text{sulfate effluent})}{(\text{COD influent} - \text{COD effluent})} \right) \times 100 \qquad (2)$$

$$\text{COD removal via methanogenesis}(\text{COD} - \text{CH}_4)(\%)$$
$$= \left[(\text{COD}_{\text{Total}}) - \left(\text{COD} - \text{S}^{2-} \right) \right](\%) \qquad (3)$$

RESULTS AND DISCUSSION

The organic matter was efficiently removed (>88%) through sulfate reduction via combined methanogenic and sulfidogenic pathways at a COD/sulfate ratio between 12.5 and 1.9 (Figures 2(a) and 2(b); Table 3). At higher COD/sulfate ratios, nearly 100% of the organic matter was removed. Under these higher COD/sulfate ratios, methanogenesis should prevail over sulfidogenesis (Dar et al. 2008).

According to Jeris & McCarty (1965), 70% of the methane produced by organic matter degradation derives from acetoclastic methanogenic fermentation, and 30% derives from H$_2$ respiration with CO$_2$ as the only electron acceptor. Therefore, up to 30% of the removed COD was derived from SRB incomplete oxidizers, as observed in stages 1, 2 and 3 (Table 3). In stages 4, 5 and 6, the sulfate reduction exceeded the maximum H$_2$ available, thus

Table 2 │ HAIS operating conditions

Compounds	Sulfidogenic and methanogenic evaluation stages					
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
COD (mg L^{-1})	1,500	1,500	1,500	1,500	1,500	1,500
Sulfate (mg L^{-1})	120	200	500	800	1,500	2,000
COD/sulfate ratio	12.5	7.5	3	1.9	1	0.75

Table 3 │ Statistical analysis of organic matter removal via sulfidogenesis and methanogenesis[a]

	Stages					
	1	2	3	4	5	6
COD/sulfate	12.5	7.5	3	1.9	1.0	0.75
Total COD removal (%)	97.1 ± 2.4	95.0 ± 1.6	93.8 ± 3.1	87.9 ± 3.7	81.2 ± 3.3	86.5 ± 6.9
COD removal via sulfidogenesis (%)	1.8 ± 2.0	4.7 ± 2.6	20.3 ± 5.0	32.8 ± 2.3	44.7 ± 6.3	42.7 ± 5.2
COD removal via methanogenesis (%)	94.6 ± 4.2	91.4 ± 4.0	74.7 ± 6.9	54.1 ± 6.0	36.6 ± 6.3	44.1 ± 5.4
N (number of data)	08	07	14	07	07	09

[a]According to Equations (1)–(3).

confirming the consumption of acetate by SRB complete oxidizers. Figure 4 corroborates this hypothesis. The removal of organic matter was greater than that predicted by the sulfate reduction stoichiometric equation (Figure 2), indicating the coexistence of sulfidogenic and methanogenic pathways.

In stage 5, the biological sulfate reduction efficiency decreased from that of earlier stages, with nearly 40% of the influent sulfate concentration remaining (Figure 2(b)). However, the quantity of sulfate removed increased (COD/sulfate ratio of 1), but this removal was not proportional to the quantity of sulfate loaded (Figure 2(b)), which indicated limitations on the electron availability for sulfidogenesis. According to Damianovic *et al.* (2006), incomplete oxidation

of ethanol by SRB requires a COD/sulfate ratio of approximately 1.92 for efficient sulfidogenesis.

The same result occurred in stage 6 (Figure 2(b)), with a similar quantity of sulfate reduced as in the previous stage, confirming the hypothesized limited electron availability. Regarding the COD, both the removal efficiency and the quantity removed decreased from stage 3 onward. At stage 4, the COD removal efficiency was approximately 90% and decreased to 80% in stage 5 (Figure 2(a)) when the sulfide concentration reached 250 mg L^{-1}. A slight increase in the COD removal efficiency in stage 6 (Figure 2(a)) coincided with the reduction of sulfide (Figure 2(b)) to 150 mg L^{-1}. The data obtained do not provide a clear explanation for the decreased sulfide concentration in the liquid. Furthermore, the sulfide concentrations were below the expected stoichiometric values, suggesting the formation of compounds of intermediate oxidation state such as sulfite and/or thiosulfate (Dannenberg *et al.* 1992). However, the acclimation of methanogenic organisms to sulfide (Muyzer & Stams 2008; Silva *et al.* 2011) can explain the increased organic matter removal observed in stage 6 (Figure 2(a)).

Figure 3 presents a statistical analysis of the COD, sulfate and sulfide data throughout the experiment.

The sulfate removal was unstable during stages 1 and 2 (~40%) at COD/sulfate ratios of 12.5 and 7.5, respectively (Figure 3). Sulfur is an essential nutrient for methanogens (Speece 1983). Therefore, at low sulfide concentrations, such as those in stages 1 and 2 (Figure 3), the sulfide likely stimulated methane formation (Table 2), as observed in the UASB reactor experiments by Paula & Foresti (2009). As expected, the increased sulfide concentration caused a gradual increase in the effluent COD, likely due to competition for electron donors.

Despite the slight increase in the average COD removal efficiency during stage 6, the overall organic matter removal was quite unstable, unlike the stable sulfidogenesis
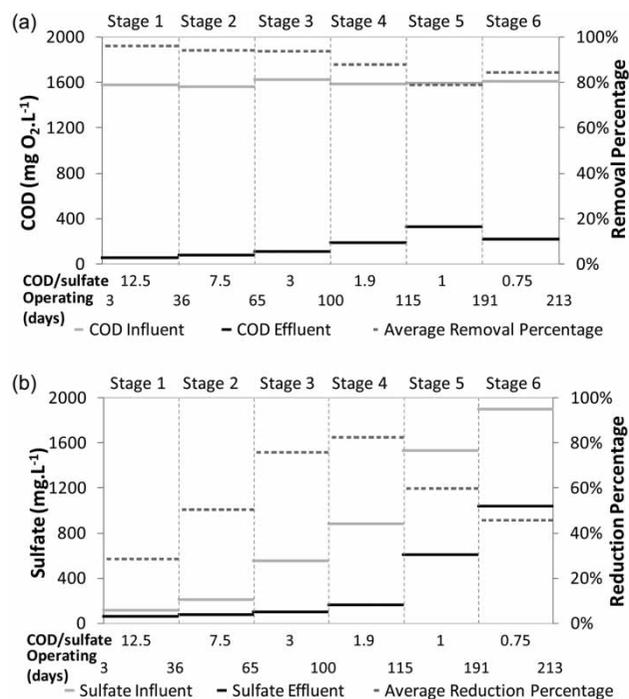
Figure 2 │ (a) Average quantity of COD removed; (b) average quantity of sulfate reduction.
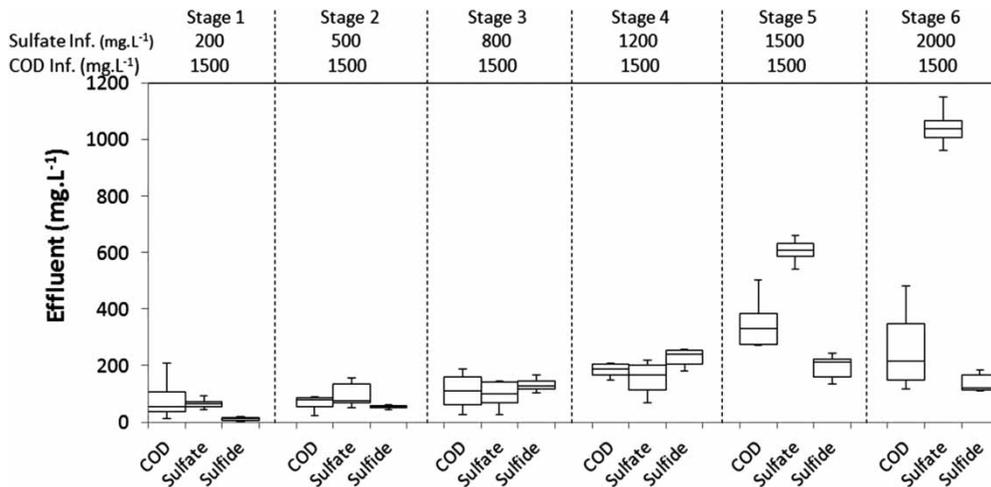
Figure 3 │ Boxplot analysis of organic matter removal (COD), sulfate reduction and sulfide concentration.
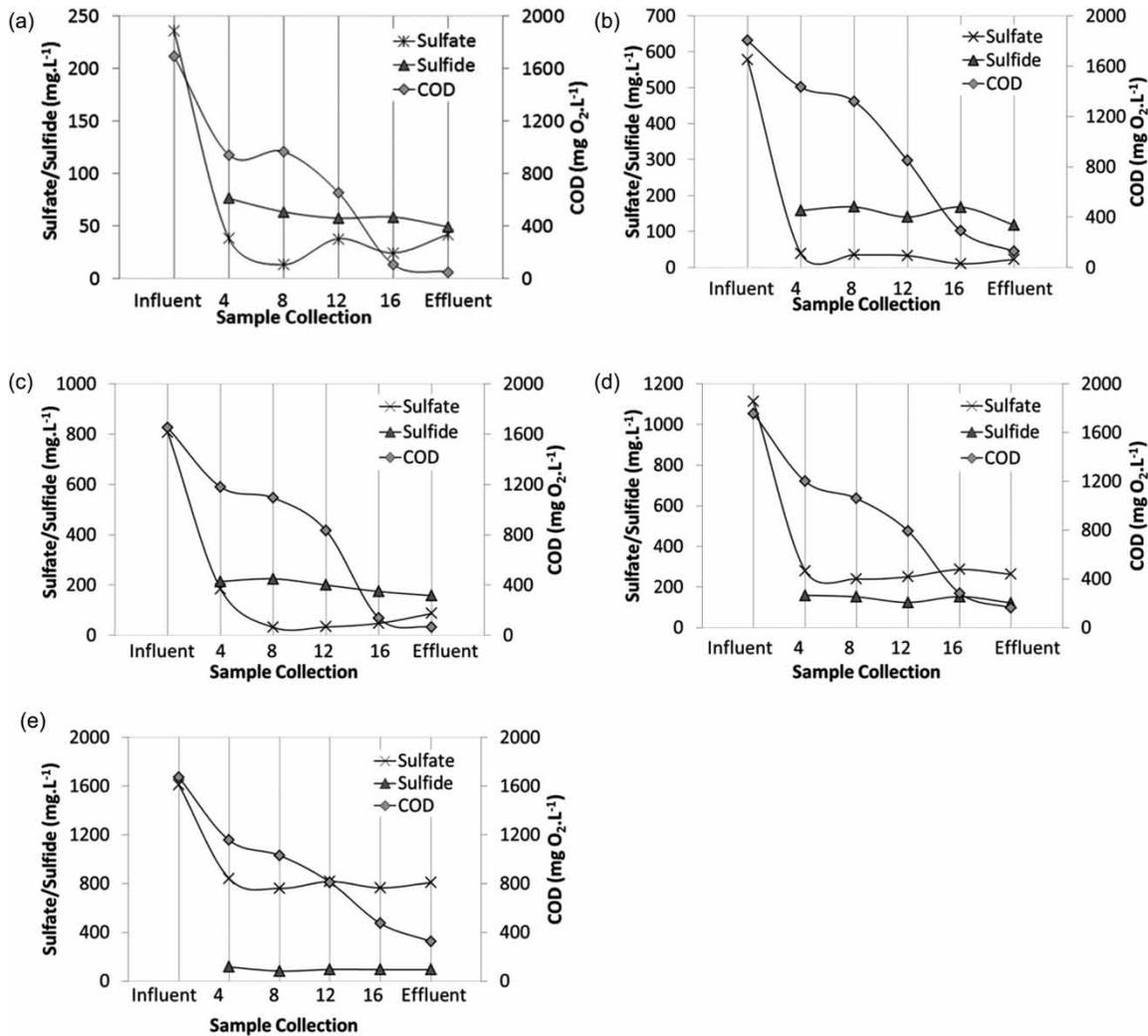
Figure 4 │ Reactor concentration gradients: (a) stage 2 – sulfate concentration = 200 mg L^{-1}; (b) stage 3 – sulfate concentration = 500 mg L^{-1}; (c) stage 4 – sulfate concentration = 800 mg L^{-1}; (d) stage 5 – sulfate concentration = 1,500 mg L^{-1}; (e) stage 6 – sulfate concentration = 2,000 mg L^{-1}.

(Figure 3). With limited availability of electron donors, the competing roles of the incomplete and complete oxidizing SRB remain uncertain.

The highest average sulfate reduction efficiency and stability occurred during stage 4 (Table 3; Figure 3). A previous study using an HAIS reactor detected acetogenesis associated with sulfate reduction followed by methanogenesis (Damianovic & Foresti 2009). Sulfate reduction and organic matter removal occurred as sequential reactions along the HAIS reactor. The same phenomenon was observed in this study (Figure 3).

The concentration gradients of the COD, sulfate and sulfide indicated that the sulfidogenic activity occurred at a higher rate in the first section of the reacto, from the influent inlet to the first sampling port, than in the other sections – for all stages. Organic matter was removed along the entire length of the reactor, as indicated by the COD profiles.

The high sulfate reduction efficiencies obtained at the first sampling port (Figure 4) can be attributed to the intense SRB activity, whereas the COD removal via methanogenesis prevails from port 8 onward. From ports 4 to 8, the COD was level, indicating a transition zone, possibly responsible for the acclimation of MA to sulfide.

CONCLUSIONS

The operation of the HAIS reactor at decreasing COD/sulfate ratios provided a better understanding of the sulfidogenesis and methanogenesis processes that occur along a non-ideal plug-flow unit containing immobilized biomass.

At a high COD/sulfate ratio (12.5 and 7.5), the organic matter removal was stable and efficient, primarily due to methanogenesis. The highest sulfate reduction efficiency was obtained at a COD/sulfate ratio of 3 to 1.9 for influent sulfate concentrations up to 800 mg L^{-1}. The increase in the influent sulfate concentration for COD/sulfate ratios of 1 and 0.75 did not increase the quantity of sulfate reduced owing to limited electron donor availability. However, the overall COD removal efficiency decreased. The quantities of sulfate reduced at ratios of 1 and 0.75 were quite similar. The highest COD and sulfate removal rates were not achieved at the same COD/sulfate ratio; thus, competition was the primary mechanism in establishing the electron flux.

The high sulfate reduction in the first reactor segment led to a gradual COD removal via methanogenesis along the subsequent segments. The increase in sulfide concentration with decreasing COD/sulfate ratio resulted in a decrease in the COD removal rate along the reactor. At a COD/sulfate ratio of 0.75, sulfidogenesis was likely inhibited by the competition for electron sources.

REFERENCES

APHA 2005 *Standard Methods for Examination of Water and Wastewater* 19th edn, American Public Health Association/ American Water Works Association/Water Environment Federation, Washington, DC, USA.

Bryant, M. P., Wolin, E. A., Wolin, M. J. & Wolfe, R. S. 1967 *Methanobacillus omelianskii* a symbiotic association of two species of bacteria. *Archiv fur Microbiology* **59**, 20–31.

Cao, J., Zhang, G., Mao, Z., Li, Y., Fang, F. & Chao, Y. 2012 Influence of electron donors on the growth and activity of sulphate-reducing bacteria. *International Journal of Mineral Processing* **106–109**, 58–64.

Colleran, E., Finnegan, S. & Lens, P. 1995 Anaerobic treatment of sulphate-containing waste streams. *Antonie van Leeuwenhoek* **67**, 29–46.

Damianovic, M. H. R. Z. & Foresti, E. 2009 Dynamics of sulfetogenesis associated to methanogenesis in horizontal-flow anaerobic immobilized biomass reactor. *Process Biochemistry* **44**, 1050–1054.

Damianovic, M. H. R. Z., Sakamoto, I. K. & Foresti, E. 2006 Biofilm adaptation to sulfate reduction in anaerobic immobilized biomass reactors subjected to different COD/sulphate ratios. *Water Science and Technology* **54** (2), 119–126.

Dannenberg, S., Kirder, M., Dilling, W. & Cypionka, H. 1992 Oxidation of H_2, organic compounds and inorganic sulfur compounds coupled reduction of O_2 or nitrate by sulfate reducing bacteria. *Archives of Microbiology* **158**, 93–99.

Dar, S. A., Kleerebezem, R., Stams, A. J. M., Kuenen, J. G. & Muyzer, G. 2008 Competition and coexistence of sulphate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulphate ratio. *Applied Microbiology Biotechnology* **78**, 1045–1055.

Foresti, E., Zaiat, M., Cabral, A. K. A. & Del Nery, V. 1995 Horizontal-flow anaerobic immobilized sludge (HAIS) reactor for paper industry wastewater treatment. *Brazilian Journal of Chemical Engineering* **12** (4), 235–239.

Hulshoff Pol, L. W., Lens, P. N. L., Stams, A. J. M. & Lettinga, G. 1998 Anaerobic treatment of sulphate-rich wastewaters. *Biodegradation* **9** (3–4), 213–224.

Jeris, J. S. & McCarty, P. L. J. 1965 The biochemistry of methane fermentation using C^{14} tracers. *Water Pollution Control Federation* **57**, 178–192.

Lens, P. N. L., Visser, A., Janssen, A. J. H., Hulshoff Pol, L. W. & Lettinga, G. 1998 Biotechnological treatment of sulfate-rich wastewaters. *Critical Reviews in Environmental Science and Technology* **1** (28), 41–88.

Li, L., Wang, J., Luan, Z., Ji, Z. & Yu, L. 2012 Biological sulphate removal from acrylic fiber manufacturing wastewater using a two-stage UASB reactor. *Journal of Environmental Sciences* **24** (2), 343–350.

Mulopo, J., Greben, H., Sigama, J., Radebe, V., Mashego, M. & Burke, L. 2011 The relationships between sulphate reduction and COD/VFA utilization using grass cellulose as carbon and energy sources. *Applied Biochemistry Biotechnology* **163**, 393–403.

Muyzer, G. & Stams, A. J. M. 2008 The ecology and biotechnology of sulfate-reducing bacteria. *Nature Reviews: Microbiology* **6**, 441–454.

Omil, F., Lens, P. N. L., Visser, A., Hulshoff Pol, L. W. & Lettinga, G. 1998 Long-term competition between sulfate reducing and methanogenic bacteria in UASB reactors treating volatile fatty acids. *Biotechnology Bioengineering* **57**, 676–685.

Paula Jr, D. & Foresti, E. 2009 Sulfide toxicity kinetics of a UASB reactor. *Brazilian Journal of Chemical Engineering* **26** (4), 669–675.

Silva, A. J., Domingues, M. R., Hirasawa, J. S., Varesche, M. B. A., Foresti, E. & Zaiat, M. 2011 Kinetic modeling and microbial assessment by fluorescent in situ hybridization in anaerobic sequencing batch biofilm reactors treating sulphate-rich wastewater. *Brazilian Journal of Chemical Engineering* **28** (2), 209–219.

Speece, E. R. 1983 Anaerobic biotechnology for industrial wastewater treatment. *Environmental Science & Technology* **17** (9), 416A–427A.

Subtil, E. L., Cassini, S. T. A. & Gonçalves, R. F. 2011 Sulphate and dissolved sulfide variation under low COD/sulphate ratio in up-flow anaerobic sludge blanket (UASB) treating domestic wastewater. *Interdisciplinary Journal of Applied Science* **7** (1), 130–139.

Touzel, J. P. & Albagnac, G. 1983 Isolation and characterization of *Methanococcus mazei* strain MC_3. *FEMS Microbiology Letters* **16** (2–3), 241–245.

Visser, A., Gao, Y. & Lettinga, G. 1993 Effects of pH on methanogenesis and sulphate reduction in thermophilic (55°C) UASB reactors. *Bioresource Technology* **44**, 113–121.

Zehnder, A. J. B. & Wuhrmann, K. 1977 Physiology of a *Methanobacterium* strain AZ. *Archives of Microbiology* **111** (3), 199–205.