

# BIOLOGICAL SULPHATE REMOVAL IN ACID MINE DRAINAGE USING ANAEROBIC FIXED BED REACTORS WITH CHEESE WHEY AS A CARBON SOURCE

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**Abstract --** The effect of the hydraulic retention time and the sulphate-total COD ratio on the performance of an anaerobic biological process applied to acid mine drainage water (AMD) was evaluated using cheese whey as a carbon source. Laboratory-scale fixed bed bioreactors operating at  $25 \pm 2$  °C were used in the experiments. Maximum sulphate reduction and hydrogen sulphide production were achieved at a hydraulic retention time of 8 days and at a sulphate-total COD ratio of 0.5. Under these conditions, the sulphate removal was found to be 68.1% while the hydrogen sulphide production was 0.27 L/day. At the same time, the total COD removal obtained was 55.4%.

**Keywords—** Acid mine drainage (AMD); Cheese whey; Sulphate removal; Carbon source; Sulphate-total COD ratio.

## I. INTRODUCTION

Acid mine drainage (AMD) is a big problem in the mining industries causing the pollution of surface and underground waters. In many cases this pollution provokes the disappearance of all aquatic life (Sheoran and Sheoran, 2006). The River Tinto, located in the county of Huelva (Southwestern Spain) is a typical case of extreme environment; its average pH is 2.3 and it has a high concentration of heavy metals (Fe, Zn and Cu). In this river extremophile microorganisms are the only ones that make up the biological community, which is composed of filamentous algae, fungi and bacteria. Among the eukaryotes, heterotrophic protist represents the main consumer group in this ecosystem (López-Archilla *et al.*, 2001). The pollution caused by the Rivers Tinto and Odiel is the equivalent of around 40,000 tons of sulphuric acid per year. The main source of pollution is originated in a deposit of 34 Km<sup>2</sup>, which produces 3 million m<sup>3</sup> AMD per year. This deposit is 3.2 % of the total area and 15.5 % of the hydraulic resources of the watershed. There are several technologies for AMD purification at laboratory, pilot and full-scales. One of the technologies developed has been the utilization of sulphate reducing bacteria (SRB) which can use

sulphate as an electron acceptor and form hydrogen sulphide leading to an increase in the pH of the water and the precipitation of heavy metal sulphides (Wakao *et al.*, 1979; Maree *et al.*, 1991; Dvorak *et al.*, 1992; Canty, 1994; Battaglia *et al.*, 2000; Chang *et al.*, 2000; Jong and Parry, 2003; Bhagat *et al.*, 2004; Boshoff *et al.*, 2004; Cohen, 2006; Sheoran and Sheoran, 2006; Van Roy *et al.*, 2006; Azabou *et al.*, 2007; Chang *et al.*, 2007; Hien-Hoa *et al.*, 2007). SRB require a source of carbon to use sulphate in anaerobic conditions as a terminal electron acceptor, which is reduced to H<sub>2</sub>S and HCO<sub>3</sub><sup>-</sup>. The process for heavy metals removal involves two stages: the production of H<sub>2</sub>S by SRB, as previously explained, and the precipitation of metals by the biologically produced H<sub>2</sub>S. This second step allows the selective recovery of some metal sulphides (Hammack and Edenborn, 1992; Kuyucak and Saint-Germain, 1994; Marchal *et al.*, 2001; Jong and Parry, 2003; Bhagat *et al.*, 2004, Cohen, 2006; Sheoran and Sheoran, 2006; Van Roy *et al.*, 2006; Azabou *et al.*, 2007; Chang *et al.*, 2007; Hien-Hoa *et al.*, 2007). In addition, metallic hydroxides and carbonates are also settled due to the alkalinity generated during the anaerobic process. It is known that carbonate/bicarbonate buffering is produced by the generation of CO<sub>2</sub> in the anaerobic process, which is partially dissolved in the anaerobic liquor. Therefore, pH values in a well-operated process remain in the range of 6.5-8.5 (Kaksonen and Puhakka, 2007; Neculita *et al.*, 2007). Different organic compounds have been used as sources of carbon: lactate (Dvorak *et al.*, 1992; Jong and Parry, 2003; Bhagat *et al.*, 2004; Azabou *et al.*, 2007), ethanol (Hien-Hoa *et al.*, 2007), sucrose (Zakaria *et al.*, 2007), phenols (Eccles, 1999), starch making process (Chang *et al.*, 2007), tannery effluent (Boshoff *et al.*, 2004), beet or sugar cane molasses (Maree, 1991; Kuyucak and Saint-Germain, 1994). The optimum sulphate-total COD ratio for SRB growth may be in the range of 1:0.5 to 1:4, while the recommended C:N:P ratio should be 110:7:1 (Dvorak *et al.*, 1992; Kuyucak and Sant-Germain, 1994). In general, the authors agree that the sulphate-total COD ratio

(or its inverse: total COD-sulphate ratio) is the key to favouring the metabolism of SRB against the methane-forming bacteria (McCartney and Oleszkiewicz, 1993; Vavilin *et al.*, 1994; Li *et al.*, 1996; Annachatre and Suktrakoolvait, 2001; Mussati *et al.*, 2005). Several researchers (Tyagi *et al.*, 1988; Van Hille *et al.*, 1999; Ghigliazza *et al.*, 2000; and Bhagat *et al.*, 2004) developed a procedure for AMD treatment based on two circuits: a biological circuit where a mixture of AMD and the source of carbon are mixed and subjected to the anaerobic process with hydrogen sulphide production and a chemical circuit where the raw wastewater is fed to a reactor where the precipitation of heavy metals takes place when the wastewater is in contact with the hydrogen sulphide obtained in the anaerobic process. Similar procedures have been successfully applied by other authors for heavy and radioactive metals in surface and underground-polluted waters (Kuyucak and Sant-Germain, 1994; Boshoff *et al.*, 2004; Azabou *et al.*, 2007; Chang *et al.*, 2007; Hien Hoa *et al.*, 2007; Yi *et al.*, 2007; Zakaria *et al.*, 2007; Hintermeyer *et al.*, 2008). Hammack and Edenborn (1992) developed a multistage chemical circuit and obtained a selective separation of metallic sulphides by varying the pH at each stage. The anaerobic biological process produced the alkalinity required for the pH variation.

A previous work reported the treatment of AMD by SRB using polyacrylate cylinders of 31 litres of effective volume with a sand bed and a layer of crushed stones at the bottom. These reactors were filled with AMD and diluted whey (5.7% vol./vol.) to support bacterial growth and incubated in batch mode for 203 days (Christensen *et al.*, 1996). Rough calculations indicated that less than 27% of the sulphate present at the start was reduced to sulphide over the course of the study, and it was therefore not surprising that a temporal increase in dissolved sulphate concentrations was seen.

Based on the literature review, the aim of the current work was to study the effect of the hydraulic retention time (HRT) and sulphate-total COD ratio on the performance of an anaerobic fixed bed bioreactor operating in continuous mode, as a biological process for hydrogen sulphide production, treating AMD, using undiluted cheese whey as a carbon source.

## II. MATERIALS AND METHODS

### A. Experimental Set-Up

Three transparent acrylic cylinders with a total volume of 7.8-L and an effective volume of 7.0-L were used as laboratory-scale anaerobic bioreactors. The cylinders had a total height of 43 cm and a diameter of 18 cm. The cylinders were divided into four sections. An upper section with a dome at the top, 2 cm in height and a cylindrical section of 5 cm in height, which was used for biogas storage and was connected to the gasholder, raw wastewater inlet and effluent recycling. The second section, 20 cm in height, was the main component of the anaerobic fixed bed bioreactor (AFBB) with a total volume of 5 L. The AFBB was packed with a support for

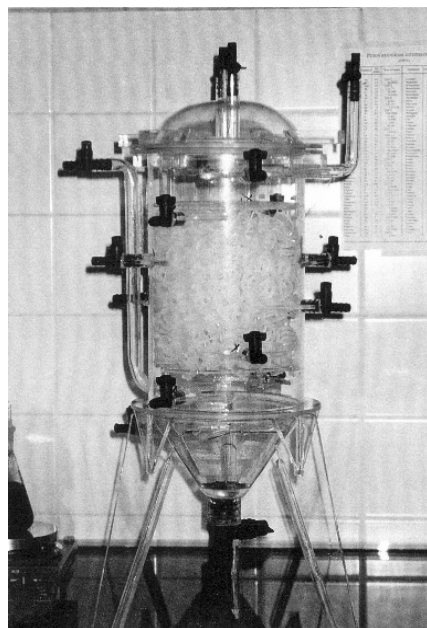


Figure 1. Photograph of one of the laboratory-scale anaerobic fixed bed reactors used in the experiments.

microorganisms immobilization, which consisted of propylene cylinders of 2.5 cm in diameter with internal baffles to increase the specific surface area. The medium had a porosity of 90 % and a specific surface area of 205 m<sup>2</sup>/m<sup>3</sup>. A perforated plate with 5-mm holes limited the top while a screen limited the lower part of the bed. The third section consisted of a cylindrical zone, 5 cm in height, where a connection was situated for the effluent outlet. The fourth section consisted of an inverted cone with a 90° angle and 11 cm in height at the bottom where the sloughed sludge accumulates. In the upper part of this section a connection for nitrogen gas bubbling was placed to enhance the effluent outlet. The bottom of this section was provided with a connection for sludge outlet. A part of the sludge was used for recycling to the top of the bioreactors. A peristaltic pump working at 30 rpm and at a flow-rate of 2.25 L/h operated for 15 minutes every hour to allow for sludge recycling. Figure 1 shows a photo of one of the bioreactors used.

### B. Inoculum and Bioreactors Acclimatization

The sludge used as inoculum in the AFBBs was obtained from an anaerobic pond treating piggery waste from a farm in Los Alcores (Seville, Spain). The inoculum had a total solid concentration (TS) of 14.1 %, while 47.0 % corresponded to volatile solids (VS). The sludge was collected in an isotonic medium at pH 7.0 (Durán-Barrantes *et al.*, 2001) to obtain a biomass concentration of 10 g/L as volatile suspended solids (VSS) in the reactors. The isotonic medium had an initial sulphate concentration of 2.0 g/L to enhance the development of SRB and a total COD of 14.1 g/L. The anaerobic biomass contained in the AFBBs were acclimated progressively by increasing the concentration of sulphate at the inlet from a concentration of 2.0 g/L at the start of this stage up to a concentration of 10.0 g/L at the

end. Therefore, the sulphate-total COD ratio ranged from 1:7 (0.1) to 1:1.4 (0.7). The acclimatization period lasted 38 days. The main objective of this start-up period was to acclimatize the anaerobic microorganisms inoculated to high sulphate concentrations, similar to those used during the experiments by using real AMD, as well as to favour the predominance of SRB against the methanogens. After this preliminary step, the liquor in the bioreactors had the following characteristics: pH, 7.1; alkalinity, 7.5 g CaCO<sub>3</sub>/L; total COD, 0.15 g/L and sulphate, 1.6 g/L.

### C. Analytical Determinations

All the analytical determinations were based on the standard methods (APHA, 1989). Total alkalinity, total and soluble COD, total solids (TS), mineral solids (MS), volatile solids (VS), total suspended solids (TSS), mineral suspended solids (MSS), volatile suspended solids (VSS), sulphate, dissolved sulphide and pH were determined using standard analytical techniques. Heavy metals concentrations were analysed by atomic absorption. Free hydrogen sulphide was indirectly determined from the pH value and the concentration of dissolved sulphide of the samples (Isa *et al.*, 1986). The biogas production was measured by displacing water saturated in H<sub>2</sub>S and CO<sub>2</sub> in Boyle-Mariotte vessels. They were connected to the top of the reactors as was previously described. The concentration of CO<sub>2</sub>, H<sub>2</sub>S and CH<sub>4</sub> in the biogas was determined by using an ORSAT apparatus. The H<sub>2</sub>S concentration was measured by passing 100 ml of biogas through a PbSO<sub>4</sub> saturated solution, while the CO<sub>2</sub> was measured passing the remaining gas through a half saturated KOH solution. The differences in volumes are equivalent to the amounts of H<sub>2</sub>S and CO<sub>2</sub> respectively and the final remaining volume is equivalent to the amount of methane in the biogas. The experiments were carried out at a room temperature of 25 ± 2°C according to the average temperature in the county of Huelva. The volumes of biogas produced were corrected to standard temperature and pressure conditions.

### D. Characteristics of the AMD

The acid mine drainage water used in the experiments was obtained from the River Tinto. Table 1 shows the average characteristics and features of the AMD based on triplicate samples.

Table 1. Average characteristics and features of the AMD collected from the River Tinto and used in the experiments.\*

Component	Units	Average value
Fe	mg/L	2830.0
Cu	mg/L	168.0
Zn	mg/L	120.0
Mn	mg/L	56.0
Ni	mg/L	2.2
Cd	mg/L	1.9
Cr	mg/L	0.2
SO <sub>4</sub> <sup>-2</sup>	g/L	8.5
pH	-	2.3

\* Values are averages of three determinations. There was virtually no variation (less than 3%) between analyses.

Table 2. Characteristics and features of the cheese whey used as a source of carbon in the experiments.\*

Parameter	Units	Average value
Total COD	g/L	82.6
Soluble COD	g/L	65.7
TS	g/L	53.1
MS	g/L	5.3
VS	g/L	47.7
TSS	g/L	9.6
MSS	g/L	0.0
VSS	g/L	9.6
Alkalinity	g/L	0.7
N <sub>T</sub>	g/L	1.8
P <sub>T</sub>	g/L	0.4
Fats	g/L	2.0
Lactose	g/L	50.0
pH	-	6.3

\* Values are averages of three determinations. There was virtually no variation (less than 5%) between analyses.

### E. Characteristics of the Carbon Source

Cheese whey was used as a carbon source in the experiments. It was collected from a factory located in "San José de la Rinconada", Seville, Spain. The average characteristics and features of this wastewater based on triplicate samples are summarized in Table 2. This wastewater shows a high concentration of soluble organic matter, mainly as lactose, a pH value near neutrality and a good C:N:P balance for an anaerobic biological process.

### F. Experimental Procedure

Three AFBBs working in parallel at semi-continuous and down-flow modes were used in the experiments. Therefore, all experiments were carried out in triplicate and the final results expressed as means. The reactors operated at hydraulic retention times of 16, 11, 8 and 4 days with intermittent feedings every 2.5 hours. Steady-state conditions were assumed to be achieved after a period equivalent to three times the nominal hydraulic retention time (HRT) selected. Effluent samples were collected and analysed for at least six consecutive days. The steady-state value of a given parameter was taken as the average of these consecutive measurements for that parameter when the deviations between the observed values were less than 3% in all cases (Isa *et al.*, 1986). The effect of sulphate-total COD concentrations was evaluated at ratios of 0.50, 0.66 and 1 using volumetric ratios of cheese whey and AMD of 1:5, 1:7.7 and 1:10, respectively.

## III. RESULTS AND DISCUSSION

### A. Effect of the Hydraulic Retention Time (HRT) on the Process Performance

Figure 2 shows the effect of the HRT on the sulphate and total COD removals and on the methane and hydrogen sulphide gas production. An increase in the HRT determined an increase in the sulphate removal. However, when the HRT increased from 4 to 8 days, the maximum increase of sulphate removal was obtained (63.5%). A similar trend was appreciated for total COD

removal in the range of 4-8 days. However, when the HRT increased to values higher than 8 days, the total COD removal decreased and sulphate removal was decelerated. Methane and sulphide gas production increased when the HRT increased from 4 to 8 days, while at higher values the production of both methane and hydrogen sulphide decreased. The maximum production of methane and hydrogen sulphide and maximum removals for sulphate and total COD were obtained at a HRT of 8 days. It was found that at HRTs higher than 8 days, total COD removal and methane gas production decreased faster than sulphate removal. This behaviour may be determined by the existing competition between methanogenic and sulphate reducing bacteria as is well known. When environmental conditions favour the development of SRB the development of methanogenic bacteria is affected and, likewise, if methanogenic metabolism is favoured, the SRB is inhibited and sulphate reduction reaction affected (Rabus *et al.*, 1996). Effects of HRT on efficiency of this type of bioreactors have been widely studied (Rockhold *et al.*, 2002; Kaksonen and Puhakka, 2007; Neculita *et al.*, 2007). The variability of hydraulic properties of porous media used in reactive mixtures may result in HRTs specific to each bioreactor. It is usually accepted that hydrogen sulphide production occurs in at least 3 to 8 d (Neculita *et al.*, 2007). A longer HRT may imply depletion of either the available organic matter source or the sulphate source for SRB. In a semicontinuous anaerobic laboratory reactor, more sulphates were reduced to sulphides with a 3-5 d HRT compared to a 1-d HRT, regardless of the organic carbon/sulphate ratio (Neculita *et al.*, 2007). Sulphate removal greater than 80% could be achieved in biological sulphate reduction assays using molasses as a carbon source at total COD-sulphate ratios greater than 10 when methane producing bacteria (MPB) predominated (Annachhatre and Suktrakoolvait, 2001). Competition between SRB and MPB was more intense as the total COD-Sulphate ratio was reduced from 5 to 2. The COD removal decreased to approximately 30% at a total COD-Sulphate ratio less than 2 because of the accumulation of sulphurous precipitates and the non-biodegradable portion of molasses in the sludge (Annachhatre and Suktrakoolvait, 2001). In addition, sulphate removal yields higher than 89% were achieved in the anaerobic treatment of industrial effluents with a HRT of 2 days using propionate as the carbon source (Ghigliazza *et al.*, 2000).

Figure 3 shows the effect of the HRT on the pH and alkalinity in the effluent of the process. An increase in the pH and the alkalinity values was observed with the increase in the HRT. A slightly acid pH and an alkalinity value of around 3 g/L appeared to be favourable for the maximum conversion of sulphate to sulphides. pH and alkalinity increased considerably at a HRT of 8 days, coinciding with the increase in the sulphate removal and with the maximum production of hydrogen sulphide. The results obtained show that the

maximum sulphate removal was achieved at a HRT of 8 days with a maximum  $H_2S$  production and a considerable increase in pH and alkalinity. In order for SRB to thrive, they require a pH in the range of 5 to 8 (Neculita *et al.*, 2007). Outside this range, the rate of microbial sulphate reduction is reduced. Low pH (< 5) normally inhibits sulphate reduction. It was also reported that when the pH in the reactor is much higher than 7.0, only a small amount of  $H_2S$  is generated, as occurred in the biological sulphate reduction process using tannery effluents as a carbon

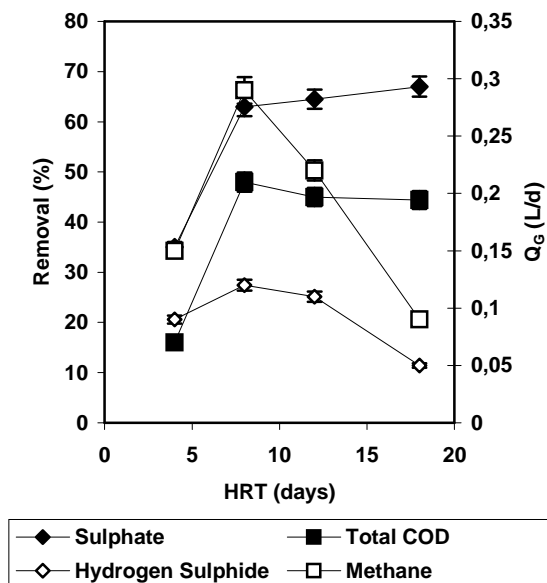


Figure 2. Variation of the sulphate and total COD removals and hydrogen sulphide and methane productions with the hydraulic retention time.

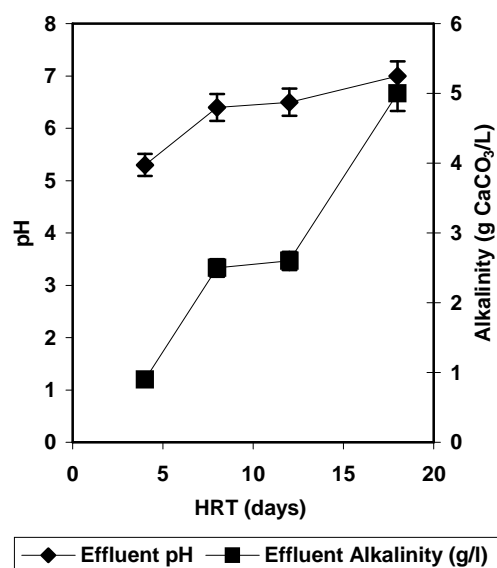


Figure 3. Variation of the pH and alkalinity with the hydraulic retention time.

source (Boshoff *et al.*, 2004). Effluent pH from anaerobic solid-substrate reactors treating AMD, with cow manure and sawdust (2:1 in weight) as the carbon source, dropped over time from 6.7 to approximately 5.5. This fact brought about a decrease in the sulphate removal rate from 250 to 40 mmol/m<sup>3</sup>·d (Drury, 1999).

### B. Effect of the Sulphate-Total COD ratio

Figure 4 shows the effect of the sulphate-total COD ratio on the sulphate and total COD removals, methane and hydrogen sulphide productions for a hydraulic retention time of 8 days, a value that was considered as optimum according to the previous results obtained. As can be seen, the decrease in the sulphate-total COD ratio caused an increase in sulphate and total COD removals and consequently in the production of methane and hydrogen sulphide. Specifically, total COD and sulphate removals of 56% and 69% respectively were found at a sulphate-total COD ratio of 0.5. Several studies have been conducted to find the best sulphate-total COD ratios for AMD treatment under sulphate-reducing conditions but the results were not consistent (Neculita *et al.*, 2007). With sludge as the carbon organic source (Al-Ani, 1994) the best performance was found for a sulphate-total COD ratio of 0.2 (total COD-sulphate ratio of 5.0), whereas other studies using natural or synthetic substrates found that SRB were predominant for a sulphate/total COD ratio higher than 0.59 (total COD/sulphate ratio below 1.7) (Neculita *et al.*, 2007). Moreover, a sulphate removal of 50% was achieved in UASB reactors used for lead removal through a biological sulphate reduction process with ethanol as an electron donor at a sulphate-total COD ratio of 0.5 (Hien-Hoa *et al.*, 2007). Lower sulphate removals were observed at a sulphate-total COD ratio of 0.27 when lactate was used as a carbon source as a result of the competition between methanogens and SRB (McCartney and Oleszkiewicz, 1993).

Figure 5 shows the effect of sulphate-total COD ratio on the pH and alkalinity in the effluent. As can be observed, an increase in pH value and alkalinity concentration occurred when the sulphate-total COD ratio decreased. This meant that a better performance and hydrogen sulphide production were achieved. Within the range of ratios tested, the best results were obtained at a sulphate-total COD ratio of 0.5. It was found that maximum values of total COD and sulphate removals were no higher than 56% and 69%, respectively. These results coincided with those obtained by other authors (Dvorak *et al.*, 1992, Kuyucak and Sant-Germain, 1994). In addition, Annachatre and Suktrakoolvait (2001) found that total COD removal decreased to 30% when the sulphate-total COD ratio was higher than 0.5.

Finally, under the optimal conditions obtained in the present work, the average value of sulphate reduction rate was found to be 0.34 g/L·d and the percentage of hydrogen sulphide in the biogas achieved 30%. Lower sulphate removal rates (0.25 g/L·d) were achieved in

stirred tank reactors (STR) using tannery effluents as a carbon source for biological sulphate reduction, while higher values (0.6 g/L·d) were obtained when UASB reactors were used to treat the same type of substrate (Boshoff *et al.*, 2004).

### IV. CONCLUSIONS

The best results for biological sulphate removal in acid mine drainage water using anaerobic fixed bed reactors with undiluted cheese whey as a carbon source were achieved at a HRT of 8 days and at a sulphate-total

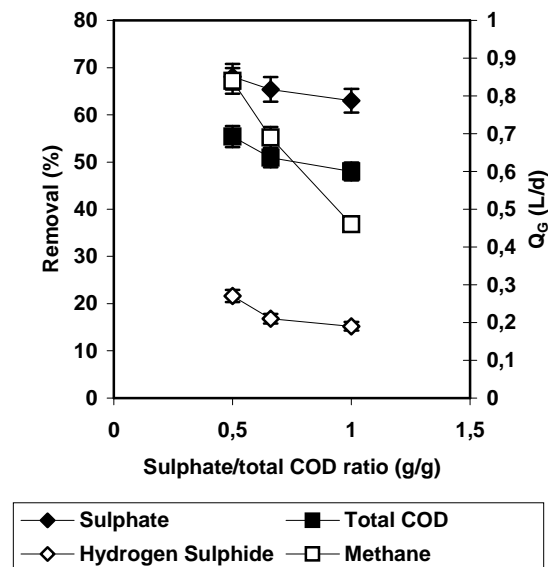


Figure 4. Variation of the sulphate and total COD removals and hydrogen sulphide and methane productions with the sulphate-total COD ratio for a HRT of 8 d.

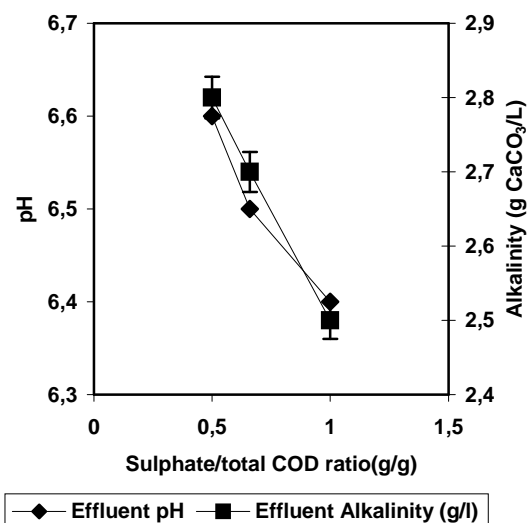


Figure 5. Variation of the pH and alkalinity with the sulphate-total COD ratio.

COD ratio of 0.5. Under these conditions, the maximum removal of sulphate was 68.1%, which was equivalent to a sulphate reduction rate of 0.34 g/L-d, while the hydrogen sulphide production was 0.27 L/d and the hydrogen sulphide concentration in the biogas was 30%. The decrease in the HRT to values lower than 8 days produced the decrease in the pH and alkalinity of the process due to the inhibition of the microorganisms responsible for carbonaceous compound decomposition and buffer production.

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