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Biofilm formation for organic matter and sulphate removal in gas-lift reactors

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Abstract A start-up strategy was presented and evaluated to obtain a well-established biofilm in a gas lift-reactor capable both for the removal of organic matter and sulphate. Pumice stone was used as material support. The influence of shear forces, given by the biogas recirculation, the effect of the COD/SO_4^{-2} ratio and the OLRs increase were evaluated on the reactor performance. From the first stages, cell colonization was observed along with the presence of extracellular polymeric substances. The COD and sulphate removal was over 70%, for all conditions. The increase of gas flow did not have an adverse effect on biofilm development even though there was some detachment. Specific methanogenic activity of the biofilm increased along the experiments. Operational parameters as alkalinity and alkalinity ratio were within the recommended values for the operation with sulphate-rich wastewater. For gas-lift reactors operation it becomes fundamental to have a suitable start-up strategy that takes into account the initial biofilm development from a non-acclimatized biomass.

Keywords: anaerobic biofilm, gas-lift reactors, sulphate, start-up

INTRODUCTION

Nowadays, anaerobic digestion can be considered as a consolidated technology with more than 2200 high-rate reactors implemented worldwide, treating wastewater coming from industrial activities such as agro-food and beverage industries, alcohol distilleries, and pulp and paper production (van Lier, 2008).

Anaerobic treatment of sulphate-rich wastewater has an important inconvenience: the generation of hydrogen sulphide, a product of the sulphate-reducing bacteria (SRB) metabolism; and the competition for substrates that occurs between the SRB, methanogenic archaea (MA) and acetogenic bacteria (Chou et al. 2008; Dar et al. 2008; Muyzer and Stams, 2008). SRB are able to use sulphate as a terminal electron acceptor, which leads to the hydrogen sulphide reduction, an important methanogenesis inhibitor (Chen et al. 2008; Sarti et al. 2009). Wastewater containing high concentrations of sulphate are generated in the fermentation industry (especially in yeast production); in the food processing of marine products, in the Kraft pulping and bleaching for paper manufacturing, among others (Lens and Hulshoff Pol, 2000; Kaczala et al. 2010). Several anaerobic reactors have been used to treat this type of effluent, studies have been conducted in up-flow anaerobic sludge blanket (UASB) (Vallero et al. 2003; Boshoff et al. 2004a; Boshoff et al. 2004b; Kaparaju et al. 2010), expanded granular sludge blanket (EGSB) (Weijma et al. 2000), anaerobic filters (Khelifi et al. 2009; Kosińska and Miśkiewicz, 2009), packed and fluidized-bed reactors (Silva et al. 2002; Jong and Parry, 2006; Sahinkaya and Gungor, 2010), AnSBBR (anaerobic sequencing batch biofilm reactor) (Mohan et al. 2005; Sarti et al. 2008) and horizontal-flow anaerobic immobilized biomass reactor (Damianovic and Foresti, 2009). In UASB systems, mixing is mostly provided by biogas generation (Nicolella et al. 2000). During anaerobic treatment of sulphate-rich wastewater, biogas production is lower due to the production of H_2S, which can also leave the reactor dissolved in the liquid phase. Another important consideration is the possible precipitation of inorganic sulphides that causes problems in the UASB reactor and anaerobic filter (Lens et al. 1998). In addition, the presence of H_2S can adversely interfere in the formation of biofilms.

Gas-lift reactors offer the possibility of recirculating the biogas, enabling high liquid-gas mass transfer rates. This allows an internal H_2S stripping, reducing the sulphide concentration in the liquid phase, therefore decreasing the risk of microbial activity inhibition, providing conditions suitable for an effective treatment of this kind of wastewater. However, for the successful operation of these reactors, it is fundamental to consider one important aspect: an adequate start-up period that leads to a good formation and control of an active biofilm. The initial formation of the biofilm over carrier particles may be a time-consuming process, and mainly depends on the type of microorganism, the carrier characteristics and the hydrodynamic conditions (van Loosdrecht et al. 1995; Picanço et al. 2001; Liu and Tay, 2002; Esposito et al. 2003).

Biofilm formation is related to the balance between the growth of the adhered biomass and its detachment (Nicolella et al. 2000). van Houten demonstrated that SRB are able to form stable biofilms in gas-lift reactors (van Houten et al. 1997). However, when shear forces are considerably high, as those provided by the upward-flow velocity reached in gas-lift reactors, cell adhesion to the surface carrier for initial microcolonies formation may be prevented. Furthermore, several studies have demonstrated how shear stress has an important influence in the detachment of biomass (Michaud et al. 2003; Lewandowski and Beyenal, 2007; Andersson et al. 2008).

The chemical oxygen demand (COD)/SO_4^{-2} ratio is a key operational condition during the treatment of sulphate-rich wastewater, since it determines the competition between different microorganisms (Vela et al. 2002; Mockaitis et al. 2010; Lopes et al. 2010). At low COD/SO_4^{-2} ratios, sulfidogenesis is widely favoured, which may cause inhibition of the MA. A theoretical COD demand for SRB is 0.67 milligram of COD per milligram of SO_4^{-2} condition at which methane production is close to zero. Generally, to avoid the decrease methane production, these treatments are carried out with COD/SO_4^{-2} ratios values above 10 (Lens et al. 1998).

The aim of this study was to assess the performance of an adequate start-up strategy for gas lift reactors treating sulphate-rich wastewater. Despite the importance of the start-up process in these biofilm reactors, there is no information specifically describing the best procedure for this. For this reason, it is important to generate start-up recommendations for guiding gas-lift reactors implementation, achieving the formation and growth of an active biofilm capable both, the removal of organic matter and sulphates.

MATERIALS AND METHODS

Experimental set-up

A 2.5 L methacrylate gas-lift reactor was used to conduct this study (Figure 1). Reactor dimensions were 69.0 cm in height, 7.0 cm in diameter and 4.2 cm for the riser diameter. The reactor was operated with biogas recirculation, at different rates. Temperature was maintained at 35ºC ± 2.

Carrier selection

Several support materials for the immobilization of both SRB and methanogenic archaea has been evaluated (Silva et al. 2006). Previous experiments (data not shown) were carried out to compare the adherence ability of the biofilm on two carriers, pumice stone and sand particles, under the same conditions described for the start-up of this study. Pumice stone (Table 1) was selected since higher biofilm formation was achieved. The latter was most likely the result of the greater roughness of pumice stone surfaces, which could have provided a protected environment from shear stress, allowing the initial adhesion of cells.

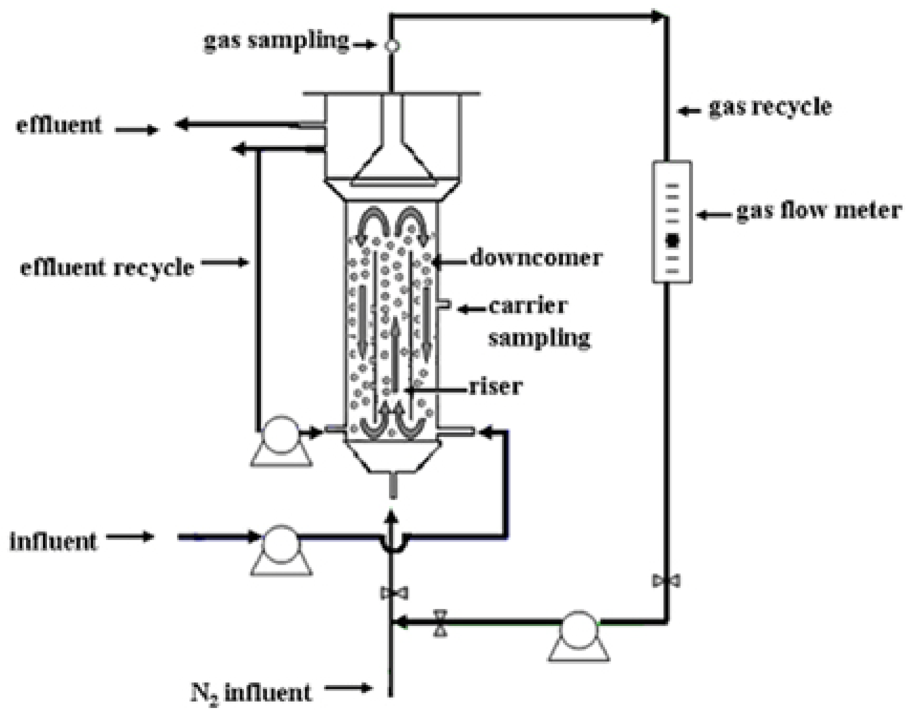

Fig. 1 Gas-lift reactor.

Determination of fluid velocities

In a gas lift reactor, gas is injected through the bottom of the reactor in the riser zone. This induces a density difference for the fluids contained in the downcomer and the riser sections, producing the fluid circulation (Nicolella et al. 2000). Determination of the liquid velocities at different zones in the reactor is necessary since this parameter is directly related to the shear forces applied in the system. Velocities inside the reactor, at different gas flows (Table 2), were determined by measuring conductivity changes after the addition of a pulse of a sodium chloride saturated solution.

It was observed that the riser velocity necessary to achieve the complete circulation of the carrier particles was 75.24 m/h, value 10-fold greater than the upward velocity used to operate the EGSB reactors, and about 100-fold greater than that used in UASB reactors.

Wastewater and reactor inoculation

For the start-up evaluation, the reactor was fed with a semi-synthetic effluent composed by diluted wine in concentrations as describes Table 3, sodium bicarbonate, yeast extract, macro and micro nutrient solutions, and sodium sulphate when the COD/SO_4^{-2} ratio decrease was done. This semi-synthetic effluent was chosen because it is an easily degradable substrate with appropriate sulphate content for the developing biomass.

The reactor was filled in 1/3 of its reaction volume with pumice stone and inoculated with 0.8 L of granular sludge from a UASB reactor treating tobacco industry effluent (methanogenic activity of 0.77 $gCOD_{CH4}/gVSS \cdot d$). Prior to inoculation, the granules were disintegrated by agitation with glass small balls, under a N_2 atmosphere. Under these conditions, the experimental runs (E0 to E7) started as described above.

Table 1. Physical characteristics of pumice particles.

Characteristic	Value
diameter	0.8-3.0 mm
density	1.0 g/cm^3
bulk density	0.4 g/cm^3
superficial area	1.11 m^2/g
total pore superficial area	0.55 m^2/g
total pore volume	1.03 µl/g
pore diameter	28.1 Å

Start-up operation strategy for the Gas-lift reactor

The influence of shear forces on the biofilm formation (or detachment), given by the biogas recirculation, was evaluated in eight experimental stages (E0 to E7) as a start-up strategy. A gradual increase of gas flow was done from the recirculating gas phase using a pump and rotameter regulation as it show in Figure 1. At the beginning of the operation, due to the minimal biogas generation, nitrogen injection was necessary to fill the system's gas piping and achieve the particle fluidization, thus during the whole operation, the gas flow was fixed by the initial amount of injected nitrogen gas plus the biogas produced. The increase of organic loading rates (OLR), necessary for the anaerobic biomass adaptation, was done deferred from the gas flow increase to avoid overlapping effects (possible detachment caused by the gas flow increase; and biofilm growth caused by the organic matter consumption). Finally, the COD/SO_4^{-2} ratio was decreased by increasing the amount of SO_4^{-2} in order to develop the sulphate reduction ability in the biomass. All stages were ended when constant COD or sulphate removals were reached (steady state conditions) and the standard deviation of measurements at these conditions was calculated (represented as error bars in the figures). Table 3 shows the experimental design used in this study.

Analytical methods

Volatile suspended solids (VSS), volatile attached solids (VAS), chemical oxygen demand (COD), sulphate concentration and total alkalinity (TA) were determined according to Standard Methods for the Examination of Water and Wastewater (Eaton et al. 2005). VSS measurement of a sample previously centrifuged, was determined by evaporating the moisture content in a drying oven at 105ºC and then subjected to temperatures of 550ºC where the volatile solids are volatilized. The total organic matter fixed on the support corresponding to VAS were measured, on washed samples of bioparticles, by weight loss between drying at 105ºC and burning at 550ºC.

Both methods are adapted from the 2540-E method. COD was measured by the dichromate closed reflux method 5220-C. The increase of the COD given by the H_2S interference in the COD determination due to its oxidation at the analysis conditions, were theoretically corrected from stoichiometry calculation of H_2S oxidation. Sulphate concentration was determined using the turbidimetric method 4500-SO_4^{-2}-E. TA was measured according to method 2320-B. TA expressed as $CaCO_3$ concentration, can be considered approximately by the sum of the alkalinity due to bicarbonate and volatile fatty acids (VFA), and it is a measure of the acid neutralizing capacity due to the presence of buffering substances, which behaviour can vary markedly with the pH of the solution. Recommended alkalinity values correspond to $CaCO_3$ concentration above 1500 mg/L (Lema et al. 1991).

The alkalinity ratio (IA/TA), the ratio between the intermediate alkalinity and total alkalinity, was determined according to the procedure proposed by Jenkins. Partial alkalinity was measured by titration at pH 5.75, corresponding to the bicarbonate alkalinity, while the intermediate alkalinity (IA), is the difference between the total alkalinity (pH 4.30) and partial alkalinity (pH 5.75), which approaches to the Volatile fatty acids (VFA) concentration (Jenkins et al. 1991). A fine performance of an anaerobic system depends on an adequate buffering capacity and a non-excessive concentration of VFA, so the alkalinity ratio IA/TA can be used as a control parameter, recommending not exceeding a value of 0.3 to avoid the acidification of the reactor (Wetzel et al. 1994).

Table 2. Velocities inside the reactor at different gas flows.

Gas flow [L/min]	Riser velocity [m/h]
1	64.8
2	75.3
3	92.6
4	120.0
5	170.5

The specific methanogenic activity (SMA) test aims to determine the maximum degradation capacity of the methanogenic population in optimal conditions. Tests were performed according to the procedure previously proposed (Soto et al. 1993), were the pressure generated by the biogas was monitored through time. The methanogenic activity is defined as the maximum slope of the curve generated by volume of methane produced per gram of VAS (or VSS, whichever is applicable) versus time. Several samples, which correspond to the bioparticles where biofilm grows, were taken from the reactor. The analyzed consortium is placed in a mini reactor under anaerobic conditions at 37ºC with a solution of volatile fatty acids composed of butyric acid, propionic acid and acetic acid, in addition to micro and macro nutrients. Since the determination of the SMA was performed without agitation, it is assumed that no biomass detachment occurs and therefore only its measures the activity of the biofilm formed. The methane production is measured by the displacement of a solution of NaOH 30 g/L, which solubilises the CO_2 content in the biogas, thus quantifying only methane.

Total sulphur concentration in the liquid phase was determined by a hydrogen sulphide ion selective electrode Orion model 9616 (Orion Research, Beverly) The sample preparation included the addition of antioxidant solutions that prevent the sulphur compounds oxidation and buffer solutions that shift the acid-base balance of the species towards the ionized form, in order to avoid the H_2S volatilization. Non-ionized hydrogen sulphide was calculated from the acid-base balance of H_2S, considering the pH at the time of measurement.

Scanning electron microscopy (SEM) was used to register the development of the anaerobic biofilm. Samples for SEM observation were fixed with 3% (v/v) glutaraldehyde in a 0.1 M cacodylate buffer (pH 7.2) and then dehydrated through a graded series of ethanol solutions (30%, 50%, 80%, 96% and 100% ethanol). The samples were then at critical point dried and coated with gold. SEM micrographs were taken with an ETEC Autoscan U-1 scanning microscope (Etec Corporation of Hayward, California, USA).

Table 3. Conditions for OLR, gas flow, Hydraulic RetentionTime (HRT), COD concentration and COD/SO_4^{-2} in each start-up stage.

Stage	Gas flow [L/min]	OLR [gCOD/L*d]	HRT [d]	COD [gCOD/L]	COD/SO_4^{-2}
E0	0	0.5	0.9	0.5	10
E1	1	0.5	0.9	0.5	10
E2	1	1	0.5	0.5	10
E3	2	1	0.4	0.4	10
E4	2	2	0.4	0.8	6
E5	2	2	0.3	0.7	4
E6	2	3	0.3	1.1	4
E7	2	3	0.3	1.1	3

RESULTS AND DISCUSSION

Reactor performance

Figure 2 and Figure 3 show COD removal and the IA/TA ratio for each stage of the start-up operation. COD removals were over 70%, and the IA/TA ratio remained below 0.4. The increase of gas flow from E0 to E1 did not have an adverse effect on biofilm development, despite certain detachment occurred (Table 4). Instead, it was favourable in terms of COD removal and alkalinity ratio. This higher performance could be attributed to better mixing conditions generated by the higher gas injection, reducing mass transfer limitations (Lazarova and Manem, 1996). In stage E2, a slight decrease of COD removal was detected since the first increase of OLR, as well as in stage E4, probably because the biofilm was not developed yet. The same criterion can be applied to explain the alkalinity ratio increase in stage E2, indicating an accumulation of volatile fatty acid.

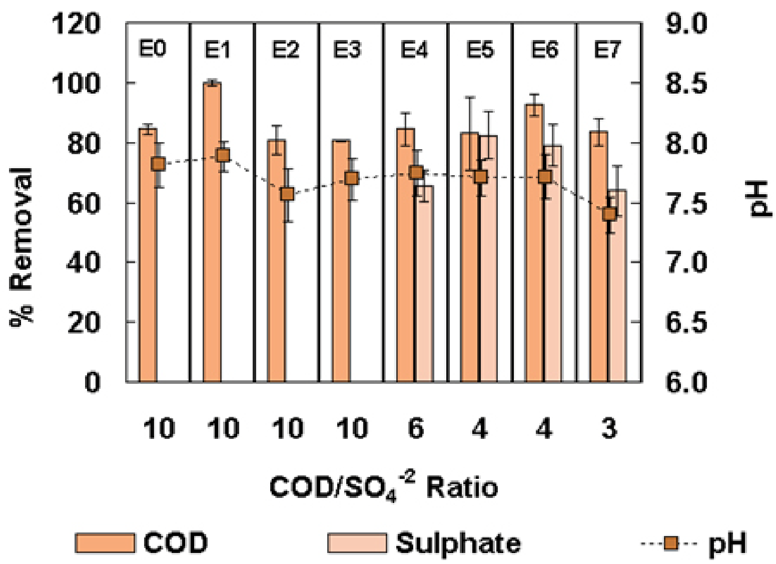

Fig. **2 Reactor behaviour on % COD removal, % sulphate removal and pH.** From E0 to E3, sulphate removal was not measured.

Biofilm activity

SMA assays were carried out at the end of each start-up stages to evaluate the biofilm development. In Figure 4 it is possible to appreciate how SMA increases along the experiments. It can be commented that no null SMA was obtained at the first stage (E0); indicating that cell colonization was achieved due to the low shear stress that allows the establishment of the first group of the cells in the carrier. In previous experiments, in which the gas-lift reactor was operated from the beginning with a constant recirculation biogas flow at its maximum value (2 L/min), it was determined that after 140 days, there was no adhesion of active cells on the carrier, where it only reached 16 mg per gram of carrier VAS, with a zero methanogenic activity per gram of VAS. This was also evident in the performance of the reactor (data not shown), where the organic matter removal, alkalinity and alkalinity ratio values, were far from those that indicate a good achievement in anaerobic systems.

Figure 4 also shows which values of SMA differed significantly from the maximum value of SMA reached at E6. It was established that there is a significant difference in the SMA value of E6 with those obtained at the initial stages, while not significantly different was obtained for the final stages. Thus, from stages E4, E5, and E7, is possible to establish that the initial adhesion of microorganisms to the carrier was done and maintained, leading to the biofilm stabilization.

In stages where equal OLR were applied (*i.e*. E2 and E3, or E4 and E5), the value of methanogenic activity was almost the same. This fact indicates that once the biofilm is established, the applied OLR is the main factor responsible for the system response, rather than the gas flow.

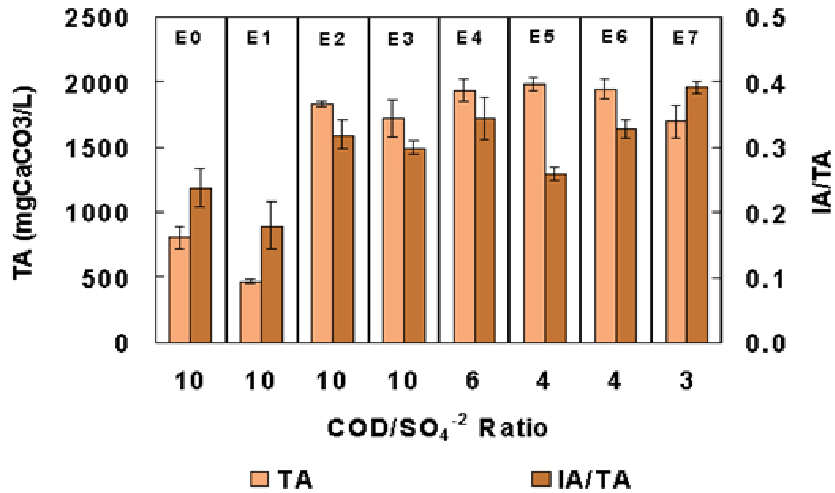

Fig. **3 Operational parameters, alkalinity ratio (IA/TA) and total alkalinity (TA) at each start-up stage.**

The gradual increase of methanogenic activity during the operation can be due to two not necessarily excluding factors: the increase of specific mass content in the biofilm and/or the increase in the proportion of methanogenic population. More research could be performed in areas such as molecular biology to corroborate this last proposal. The results suggest that the increase in the methanogenic activity in E1 might be mainly due to the increase in the adhered biomass, since also was observed a reduction of VSS measured in the reactor effluent, considered as a measure of the detachment or not adhered cells. Whereas, in the following stages this increase can be attributed to the increase in the proportion of methanogenic microorganisms, since the Volatile attached solids (VAS) and VSS concentration stays relatively constant (Table 4).

Despite the increase in methanogenic activity of biofilm during the study, the inoculums value (0.77 $gCOD_{CH4}$/gVSS·d) was not reached. Nevertheless, the values obtained for methanogenic activity are according to the values reported for biofilm reactors (Visser et al. 1993). These samples corresponded to a methanogenic/sulfidogenic system, which is comparable to the conditions of this investigation. In an anaerobic fluidized bed reactor, activity values of 0.2 $gCOD_{CH4}$/gVAS·d were reported in those particles with 331mm biofilm thickness (Buffière et al. 1998); similar thicknesses were reached in the present investigation.

Biofilm development

In Figure 5, Scanning electron microscopy (SEM) photographs of biofilm formation on pumice stone particles are presented. At initial stages it can be seen how cells colonize the cavities or pores. In Figure 4b, it is possible to distinguish the presence of extracellular polymeric substances, which are an indicator of biofilm maturation (Lens et al. 2003). Also, at final stages (E5 and E6) it is possible to distinguish more biodiversity, a favourable index for the microbial biofilm formation (Bramucci and Nagarajan, 2006).

Organic matter removal and sulphate reduction

In Figure 2 it is possible to see the reactor behaviour on COD and sulphate removal after an increase of the sulphate load at E4. Sulphate and COD removals were always over 70%. A fast response for sulphate removal indicates the natural presence of SRB in the system. The operation of initial stages at the no inhibitory COD/SO_4^{-2} ratio of 10, seems to be an appropriate acclimatization procedure for the biomass to sulphate-rich wastewater. Decrease in COD and sulphate removals in stage E7, corresponding to a COD/SO_4^{-2} ratio of 3, cannot be attributed to the inhibiting effect of sulphide, because the H_2S concentrations did not reach inhibitory levels (Figure 6), which should be around 250 mg/L (Kosińska and Miśkiewicz, 2009).

Table 4. Volatile suspended solids in the effluent and total content of volatile attached solids in the reactor, at each stage of start-up operation.

Stage	Duration [days]	VSS in reactor effluent [g/L]	VAS in the reactor [mg/g carrier]
E0	75	2.28	29
E1	12	0.74	36
E2	86	0.34	48
E3	15	0.34	52
E4	48	0.20	51
E5	11	0.22	54
E6	8	0.12	58
E7	10	0.12	58

The system was always operated at pH higher than 7.0 (Figure 2), where most of the available sulphide is present in its ionized form, which is considered less toxic (Chen et al. 2008). The latter indicates that the system did not present any inhibition by sulphide, as commented previously. On the other hand, since most of the sulphur produced is in ionized form, the H_2S fraction in equilibrium with the gaseous phase was lower, and it's desorption will always be limited to acid-base conditions. Hence, to promote desorption of H_2S, increasing the amount of H_2S which leaves the reactor in the gas phase, is recommended to operate the system with slightly acidic pH value, around 6.5.

Figure 3 shows that TA is within the recommended values for the operation with sulphate-rich wastewater (E4 to E7). However, the sulfidogenesis produces an increase in the proton concentration given by species in acid-base equilibrium. Indeed, the increase in the proton concentration diminishes the pH and alkalinity, especially in the E7 stage, where is possible to distinguish the hydrogen sulphide production effect, although not to an inhibiting level for the system.

Increases in the IA/TA ratio values at E4, E6 and E7 can also be attributed to sulfidogenesis. SRB compete for substrates in the sulphate reduction, potentially leading to an accumulation of intermediate products such as volatile fatty acids, which, as consequence, precisely cause the increase of the IA/TA ratio value.

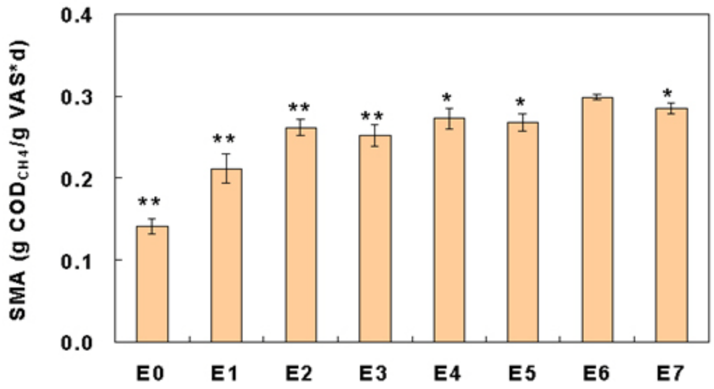

Fig. 4 Specific methanogenic activity of biofilm at each stage of start-up operation. ** means significant difference with SMA value of E6 stage; * means no significant difference with SMA value of E6 stage. Statistical analysis using two-sided test Student's distribution and confidence level of 0.1.

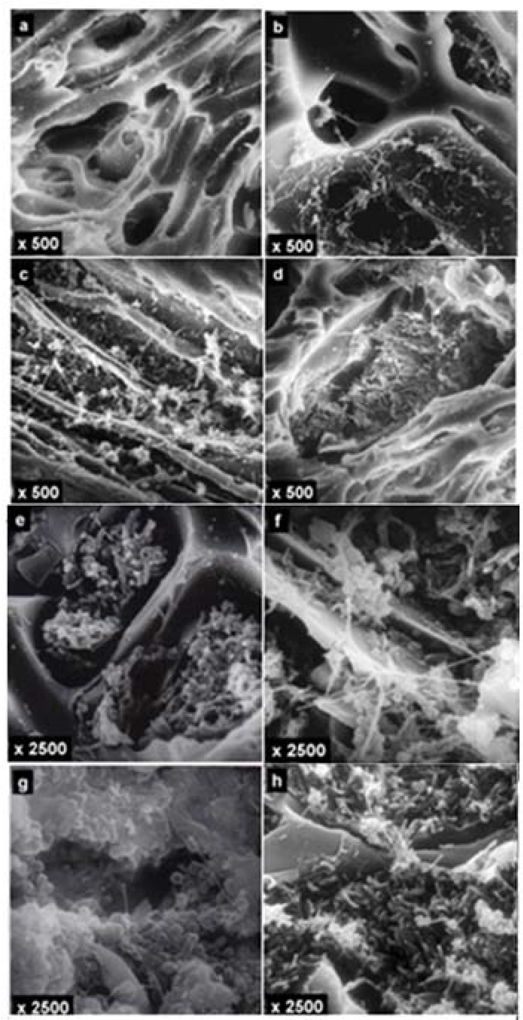

Fig. 5 SEM photography at two different magnification values for biofilm formation on pumice particles at the end of each stage: a) carrier surface before biofilm formation; b) stage E1, c) stage E3, d) stage E4, e) stage E1, f) stage E2, g) stage E5, h) stage E6.

CONCLUDING REMARKS

A start-up strategy of increase gradually the gas flow and the Organic loading rate (OLR), allowed the establishment of an active biofilm. It is not trivial to conclude that for the adequate performance of an anaerobic biofilm gas-lift reactor, it is essential to have a proper start-up procedure that includes the formation of the biofilm; even more if this system is destined to treat sulphate-rich wastewater, which implies an additional acclimatization to this substrate.

In a system with high shear stress, such as a gas-lift reactor, the morphology of particle carriers becomes very important in the biofilm development, particularly in the initial adhesion of the cells' surface cavities. Particles with higher porosity have better carrier features, such as pumice stone.

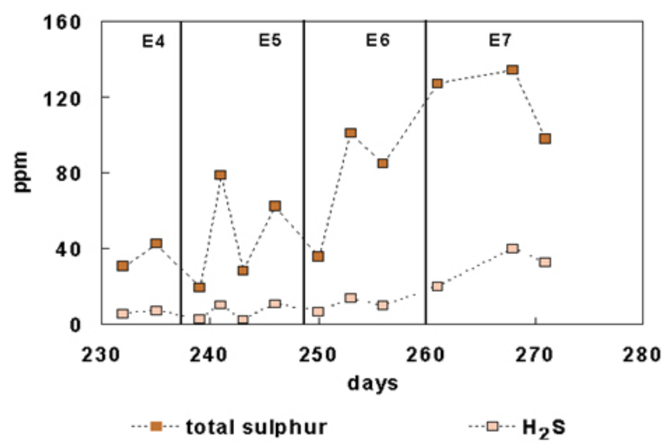

Fig. 6 Concentration of non-ionized hydrogen sulphide and total sulphur measured in reactor effluent.

Once the initial adherence of the biofilm is achieved at low shear stress (early stages of start-up), carrier colonization occurs. At the final stages of start-up, where shear stresses are increased, the concentration of biomass per gram of carrier stays virtually constant; probably due to a balance between growth and detachment of biofilm, which defines its morphology.

The high rates of sulphates and organic matter removal reached during the start-up, also indicate that establishment of both methanogenic and sulphate-reducer bacteria populations occurred during the biofilm development.

Furthermore, no inhibition was observed in the system despite the fact that at final stages of start-up the COD/SO_4^{-2} ratio was 4 and 3, values that are less than the critical value (10) for sulfidogenesis prevalence. This was due to the early reactor acclimatization, where from the first stages of start-up the COD/SO_4^{-2} ratio was 10. Measures of H_2S concentrations in the reactor effluent, reached levels that were reported as non inhibitory, which also possibly predicts that the use of this type of reactor is favourable to assess the stripping of this sulphur species into the gas phase.

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