Study of immobilized biomass reactors for sulfate reducing activity characterization and improvement

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ABSTRACT
Immobilization of non-granular sludge is an auspicious option for sulfate reducing activity improvement. In this study, PVA-biomass granules and alginate-biomass granules were tested for mechanical stability, adsorption capacity and sulfate reduction. Moreover, two configurations of reactors, a Continuous Stirred Tank Reactor (CSRT) and a Column Reactor (CR) were operated, evaluating sulfate and glycerol consumption H_2S production in order to improve sulfate-reduction process within SONOVA process. The CR presented a stable sulfate reducing activity, higher production of H_2S and low wash out comparing to CSTR.

Keywords: sulfidogenic reactor; sulfate reducing sludge; polyvinylalcohol; alginate; immobilization.

1. INTRODUCTION
Combustion of sulfur fuels, mainly in the energy and the industrial sectors leads to the emissions of flue gases containing sulfur oxides (SO_x). Sulfur emission is responsible of air pollution and can be deposed in the environment. To this aim, SONOVA process has been developed to remove SO_x from combustion gases and their valorization as elemental sulfur. This process consists of a first step where SO_x is absorbed using a slightly alkaline solution, a biological step where absorbed SO_x (as a mixture of sulfate and sulfite) is anaerobically reduced to sulfide using glycerol as electron donor, and an aerobic sulfide oxidation step to obtain elemental sulfur (Mora et al., 2020).

Process development has highlighted that anaerobic sulfate-reduction is the process bottleneck. Several challenges have been reported for the improvement of sulfate reduction process, mainly related to maintaining a long-term efficiency and avoiding process failure caused by the inhibition of the biomass by Volatile Fatty Acids (VFAs) accumulation, sulfide toxicity, and oxygen presence (Fernandez-Palacios et al., 2019). Moreover, mass transfer resistance can be increased throughout the reactors operation, depending on their configuration. These operational limitations reduce H_2S production and thus process efficiency (Lens et al., 2003).

On the other hand, immobilization of biomass using synthetic polymers has been positioned as a promising approach for biomass immobilization. Synthetic granules improve reactors performance due to the generation of a protecting matrix that allows a decrease in starting up period and a fast adaptation to new conditions (Zhang et al. 2007). These methods can be an alternative for natural immobilization through Exopolysaccharides EPS production in cultures that have a slow production of these compounds. In addition, it has been described that polymer-biomass granules increase the mechanical strength of synthetic granules, preventing bacteria wash out (Zhang et al., 2016). These methods have been successfully used in sulfate-reduction process for acid mine draining treatment, avoiding toxicity of heavy metals and achieving high sulfidogenic activity (Selvaraj et al., 1997; Zhang et al., 2016).

Polymers used for biomass immobilization can be divided in two categories depending on the source where they have been obtained: natural or artificial polymers. Natural polymers such as alginate, chitin and carrageenan have high diffusion coefficients and low toxicity. On the other hand, synthetic polymers such as polyacrylamide, polypropylene and polyvinyl alcohol (PVA) have high mechanical strength. However, their preparation can be toxic for microorganism (Bouabidi et al., 2019).

In the present study, a new approach for sulfate reduction within SONOVA process is presented. For this purpose, two different polymers were tested for biomass immobilization, assessing sulfate adsorption, mechanical strength and removal efficiency of sulfate and chemical oxygen demand (COD). Besides, the long-term performance of both reactors operated under the same conditions (hydraulic residence time, sulfate load (HRT), C/S and biomass concentration) was evaluated using immobilized sludge as inoculum.

2. MATERIALS AND METHODS

2.1. Inoculum and cultivation

Inoculum was obtained from an anaerobic UASB reactor from Aigües de Manresa wastewater treatment plant. Sulfate-reducing activity was enhanced through sealed flask culture using anoxic mineral medium described by Mora et al. (2020).

2.2. Immobilization of sulfate reducing sludge

PVA-biomass granules were prepared using a PVA solution (13.6% w/w of PVA) that was blended (1:1) with concentrated sludge (3.78 g VSS/L). Mixture was frozen at -15 ˚C and cut as 5 mm granules.

Alginate-biomass granules were prepared using an alginate solution (3 %w/w of alginate) that was mixed (1:1) with concentrated sludge (also at 3.78 g VSS/L) and dropped into a $CaCl_2$ solution (4 %w/w) forming a 5 mm beads.

2.2.1. Adsorption and mechanical stability of immobilized biomass

Adsorption tests were performed by incubating both polymer-granules without cells for 24 h, in a sealed flask with anoxic mineral medium. Percentage of sulfate removal was calculated.

Regarding to mechanical stability, polymer-biomass granules were incubated in a sealed flask with anoxic mineral medium for 24 h, stirred at 300 rpm. Granules were counted and weighted (wet weight) at 0 and 24 h of incubation. Percentages of granules and weight loss were calculated.

2.2.2. Sulfate and COD consumption of synthetic biomass-granules and free cells

Alginate-biomass granules, PVA-biomass granules and free cells were incubated with anoxic mineral medium at 32 ˚C. Consumption rates were calculated measuring sulfate and COD concentration of the media every 6 hours, during 72 h.

2.3. Reactors set up and operation

A column reactor (CR) and continuous stirred tank reactor (CSTR), both of 500 mL of volume, were inoculated with 100 mL of artificial PVA-biomass granules (Figure 1). Both reactors were fed using mineral medium described by Mora et al. (2020) with an HRT of 5 h. Sulfate load was increased from 0.2 to 4 kg/m^{3}d, keeping a C/S ratio of 6.25 mg O_2/mg SO_4^{2-}. Reactors were operated during 101 days. pH, sulfate, COD, H_2S, volatile suspended solids (VSS) concentration and VFAs concentration were measured at the outlet of both reactors.

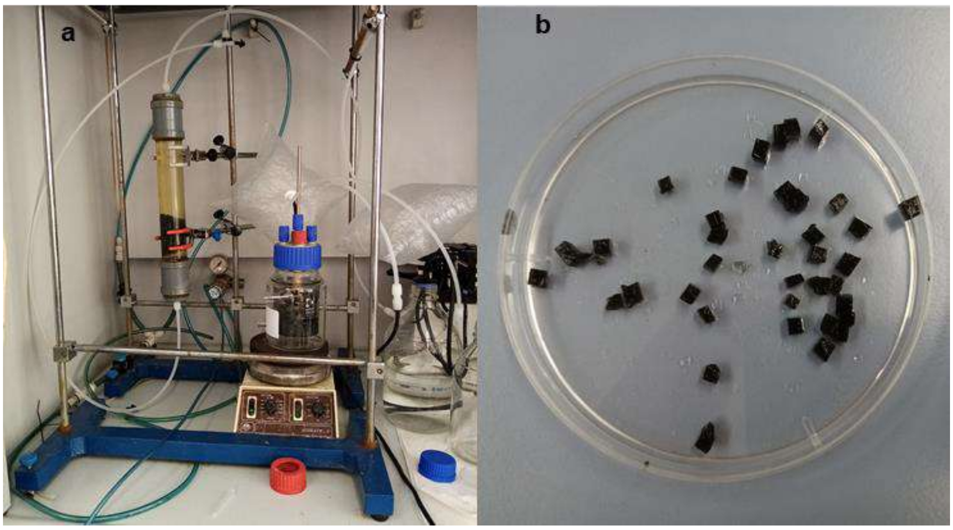

***Figure 1:** a) CR and CSRT reactors set-up. b) Inoculum of PVA biomass-granules.*

2.4. Analytical methods

Sulfate was measured using spectrophotometry (HACH, Spain), VSS and COD were analyzed using Standard methods (APHA, 1998). VFAs concentration were obtained by HPLC with an ICSep ICE-CPREGEL 87H3 column and a at 210 nm wavelength detector. H_2S was measured using an electrochemical sensor (SULF-NP, UNISENSE, Aarhus, Denmark).

3. RESULTS

3.1. Characterization of immobilized biomass using PVA and Alginate

3.1.1. Adsorption test and mechanical strength evaluation

Alginate and PVA were used as synthetic polymer for biomass immobilization. Sulfate adsorption and mechanical stability of granules were quantified (Table 1). Adsorption tests highlighted that sulfate was not absorbed into either polymer. According to these results changes in sulfate concentration are only caused by microbial sulfate reducing activity.

Mechanical stability test presented differences between polymers (Table 2). A low degradation of PVA-biomass granules was observed at 24 h of stirred incubation. However, alginate biomass-granules were degraded up to 77%.

Table 1: *Sulfate adsorption test for PVA and alginate matrix.*

	PVA		Alginate	
Time (h)	0	24	0	24
Adsorbed Sulfate (mg/L)	94.7	94.7	95.1	95

Table 2: *Mechanical stability test for mixtures of PVA-sludge and Alginate-sludge.*

	PVA biomass-granules		Alginate biomass-granules	
Time (h)	0	24	0	24
Initial granule amount	40	38	40	2
Final granule amount	100	95	100	15
Wet weight (g)	1.6077	1.4022	1.2910	0.0309
% degraded	100	12.9	100	77.6

3.1.2. Kinetic study

Kinetic study was performed with PVA-biomass granules, Alginate-biomass granules and suspended cells (Figure 2). Regarding sulfate consumption, PVA-biomass granules presented a high consumption rate compared to alginate-biomass granules and suspended cells, removing 98% of sulfate in 15 h. These results can be explained by the diffusion capacity and the finale structure of each matrix. It has been reported that immobilization allows high concentration of substrates in the matrix. Thus, free cells have a poor performance compared to synthetic biomass granules (Chen et al., 2015). However, cell density and diffusion rates of subtracts and products can affect the performance of the synthetic biomass-granules (Plieva et al. 2008). Diffusion limitations have been described in alginate beads due to the high volume of the structure, generating larger diffusion distances and lower kinetic rates (Seifert & Phillips, 1997). Since PVA biomass-granules have a compact structure and a higher cell density than alginate granules, higher kinetic rates were obtained.

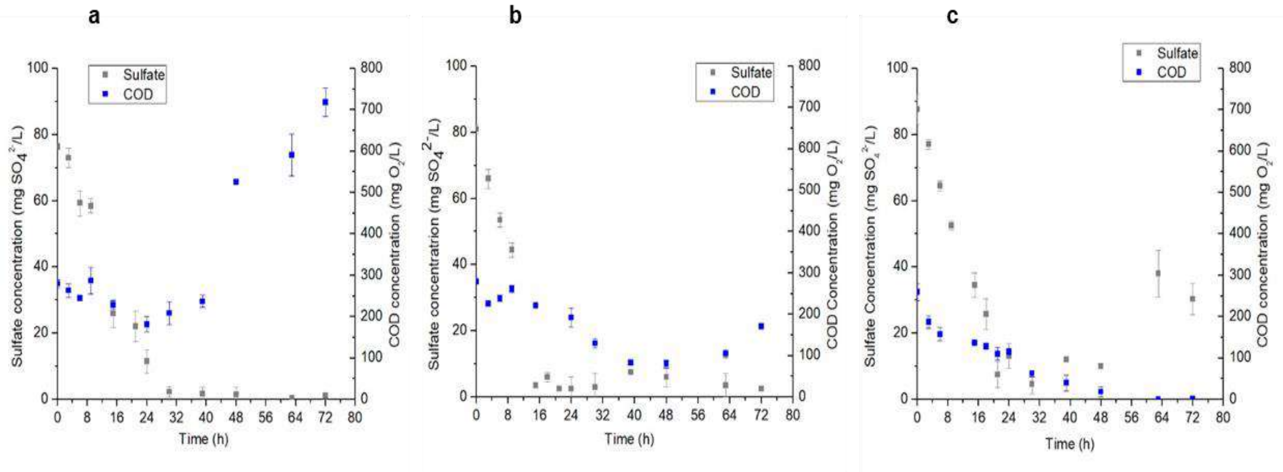

Figure 2: *Kinetic study of a) Alginate-biomass granules b) PVA-biomass granules c) Free cells.*

COD concentration was measured to quantify glycerol intake. Only in the assay of suspended cells, glycerol was completely depleted. In PVA-biomass-granules and alginate-biomass granules assays, an increase of COD concentration at 48 h of the experiment was observed due to degradation of polymer materials and dissolution in mineral medium. Since PVA-biomass granules showed a higher sulfate consumption rate and mechanical stability, it was selected as inoculum for reactors operation.

3.2. Long-term performance of CR and CSTR

3.2.2. Sulfate and glycerol removal

CR and CSTR were simultaneously operated under the same operational conditions (inlet sulfate concentration, C/S and HRT). Results obtained during reactors operation and monitoring are presented in Figure 3. Sulfate reduction is more than 95% when reactors were fed with sulfate loads from 0.2 to 3 kg/m^{3}d. When sulfate load was set at 4 kg/m^{3}d, CR and CSTR sulfate RE decreased a 50%. Nevertheless, CR regained a 97% removal efficiency in 10 days, in contrast with CSTR. This difference can be explained by accumulation of H_2S and VFAs that can be toxic for the biomass. In CSTR, lower mass transfer resistance exposed granules to inhibitors (Lens et al., 2003). Moreover, COD was not completely removed in any of the reactors. However, in CR COD RE varied between 60% and 80% and in CSTR COD RE had a decreasing trend, ranging from 90% in day 1, to 58% at the end of the experiment. It is possible to observe that sulfate reduction is not limited by glycerol concentration.

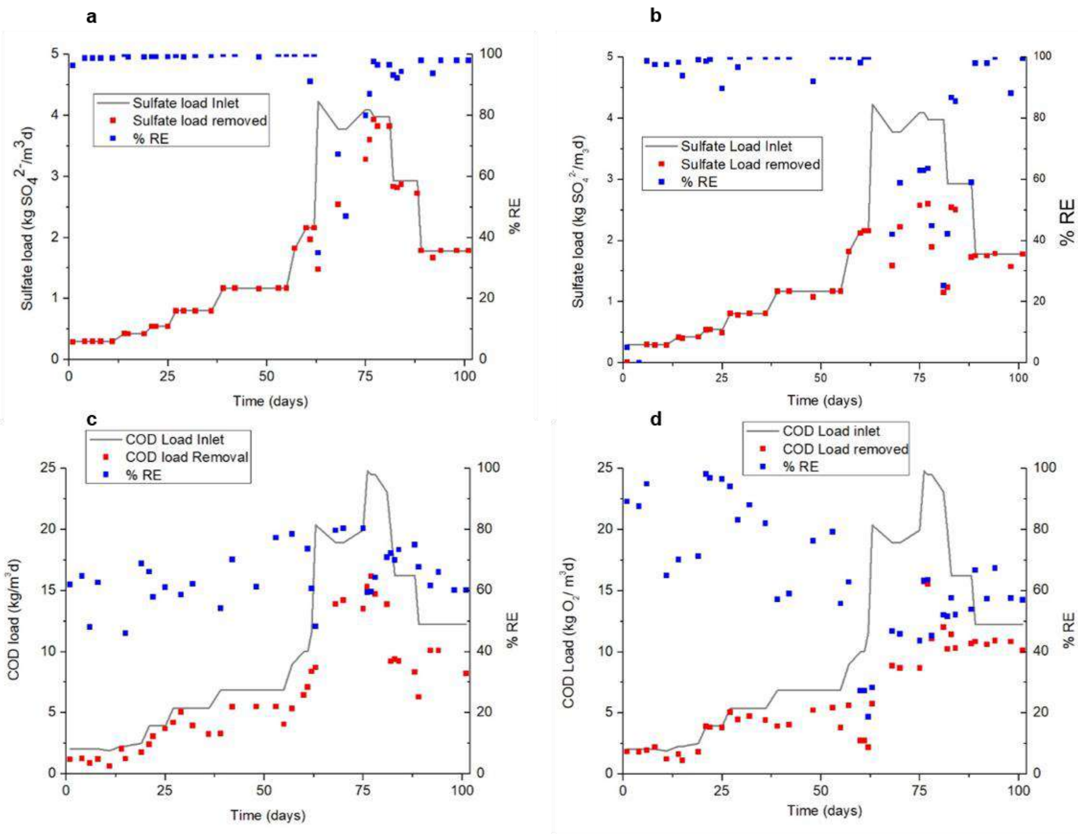

Figure 3: *Long-term performance of CR and CSTR reactors. a) Sulfate removal in CR, b) sulfate removal in CSTR c) COD removal in CR, d) COD removal in CSTR.*

3.3.2. H_2S production

H_2S concentration in the outlet of CR and CSTR was measured (Figure 4). It was possible to determine that H_2S concentration was higher in CR than CSTR. This is explained because H_2S stripping takes place in stirred reactor. Furthermore, H_2S concentration in both reactors increase at sulfate load of 4 kg/m^{3}d as a consequence of a raised sulfate reducing activity.

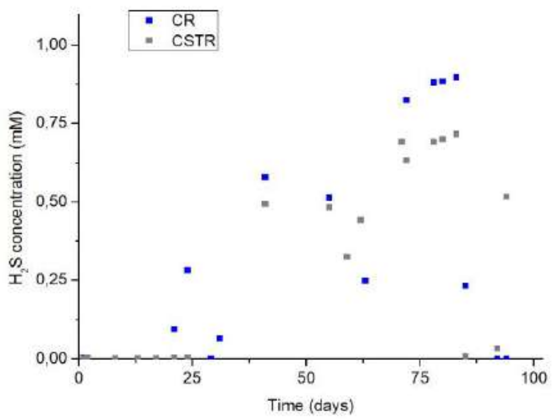

Figure 4: *H_2S concentration in CR and CSTR outlets.*

3.2.3. VFAs Analysis

VFAs accumulation were lower in CSTR (0.8 mg/L) than in CR (1.4 mg/L) due to gaseous compounds stripping. Besides at high loads of sulfate there is more production and accumulation of VFAs due to C/S value remained constant (Figure 5). Acetic and propionic acid accumulation demonstrated the absence of microorganism that could use these compounds as an electron donor. This trend is reported in studies of sulfidogenic reactor operation and can trigger a process failure (Jing et al., 2013; Mohan et al., 2005). Despite VFAs accumulation, synthetic biomass granules prevent inhibition by toxic compounds and do not affect sulfate reducing activity.

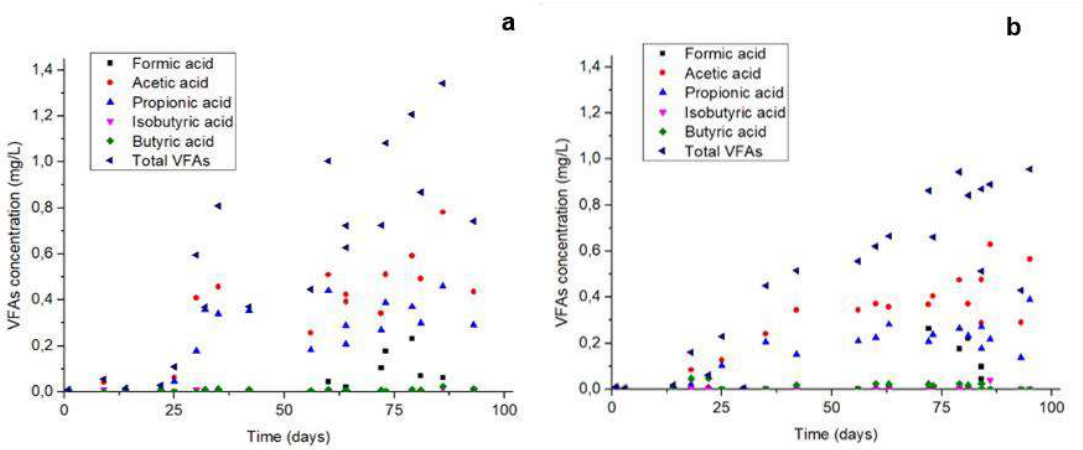

Figure 5: *VFAs concentration in outlet of a) CR b) CSTR.*

3.3. Granules degradation during CR and CSTR operation

VSS was determined in the outlet of CR and CSTR during al operation time (Figure 6). CSTR presented a final degradation of granules of 0.078 g VSS/L due to mechanical stirring and CR 0.023 g VSS/L. Despite of it is possible to improve PVA mechanical strength increasing the PVA concentration and the density of polymer matrix, this can be detrimental for mass transfer capacity (El-Naas et al., 2013).

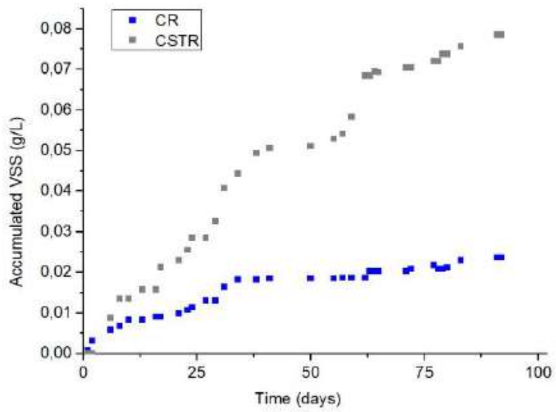

Figure 6: *Biomass accumulation in the outlet of CR and CSTR.*

4. CONCLUSIONS

The aim of this study was to analyze polymer-biomass granules performance for sulfate reduction process and to select a reactor configuration that allows high sulfate removal and H_2S production. PVA-biomass granules do not limit the activity of biomass and present high mechanical resistance and no adsorption of sulfate, thus can be used for scaling-up applications. Besides, CR reactor filled with PVA biomass granules presented a high stability in presence of toxic compounds such as H_2S and VFAs, therefore it could be an interesting option for future studies.

5. ACKNOWLEDGEMENTS

Authors acknowledge Ministerio de Economía y Competitividad (Spain), through the project RTI2018-099362-B-C22 MINECO/FEDER, EU, for the financial support provided to perform this research.

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