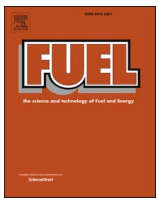
Fuel 323 (2022) 124367

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel



Full Length Article

Optimization of biogenic sulfur flocculation from an anoxic desulfurization bioreactor using response surface methodology

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ARTICLE INFO

Keywords:
Biogenic sulfur
Flocculation
Optimization
Response surface methodology
Turbidity

ABSTRACT

Elemental sulfur is an interesting byproduct obtained in the anoxic biodesulfurization of biogas. However, the colloidal properties of this biogenic elemental sulfur (S^0) make its efficient recovery difficult. The present study investigated the flocculation of S^0 produced in an anoxic bioreactor for biogas desulfurization using cationic (Sedifloc 40L4 C, Lizaflock 853 M), anionic (Innoflock 201) and non-ionic flocculants (NI-1009) and a coagulant (polyaluminum chloride). Cationic-type flocculants showed the highest flocculation efficiency, which led to its selection for further evaluation using the response surface methodology of the effects and interactions of its dose, stirring speed and pH on the biogenic sulfur flocculation rate. Optimum S^0 flocculation conditions were observed using Lizaflock 853 M at a dose of 0.82 mg L^{-1}, a stirring speed of 30 rpm and a pH of 8.0. A S^0 flocculation rate of 97.05% was achieved for an initial concentration of 1730 mg S^0 L^{-1}. The Zeta potential of the settled sulfur particles increased after the flocculation process.

1. Introduction

Hydrogen sulfide (H_2S) is one of the most undesirable compounds present in biogas because its presence precludes the application of the latter for most purposes [1]. Anoxic desulfurization has been demonstrated to be a robust technology that efficiently converts the hydrogen sulfide in biogas into elemental sulfur in suspended biomass bioreactors [2,3]. This biologically produced (biogenic) sulfur can be used as a fertilizer or raw material for chemical industries. However, as biogenic sulfur is generated as a stable colloidal suspension, the application of an isolation step to efficiently separate it from the liquid phase is essential for its valorization and to avoid other problems such as pipe blockage and secondary pollution [4].

The properties of biogenic sulfur differ markedly from those of chemically produced sulfur [5]. Thus, while chemically produced sulfur has extremely low solubility in water (5 μg L^{-1} at 20 °C), biogenic sulfur particles are hydrophilic and their structure and surface properties depend on the microorganism used in their production [6]. For example, while the elemental sulfur produced is granular in most cases, and can be deposited inside or outside the bacterial cells [7], some authors have found filamentous biogenic sulfur in bioreactors enriched with chemoautotrophic sulfur-oxidizing bacteria from coastal seawater [8]. Sun et al. [9] demonstrated how sulfur varied according to the reactor where it was produced. Whereas in abiotic reactors the predominant form of sulfur was S_6 with an average particle size of 34–40 μm, the predominant form of sulfur found in bioreactors was S_8 and with an average particle size of 10–29 μm.

Different physical and/or physicochemical methods can be used to separate the sulfur produced. The most popular such methods include gravity sedimentation [10], centrifugation [7], flotation [11], membrane separation [12], extraction [13] and coagulation-flocculation [14,15]. The method used usually depends on the characteristic properties of the biogenic sulfur. Despite gravity sedimentation being the most attractive method from a technical/economical point of view, the formation of easily settleable biogenic sulfur is rare. As such, flocculants and/or coagulants are typically added to increase the particle size and sedimentation rate [16].

The most effective electrolytes for net negatively charged particles

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https://doi.org/10.1016/j.fuel.2022.124367
Received 6 December 2021; Received in revised form 21 April 2022; Accepted 23 April 2022
Available online 2 May 2022

include the multivalent ions Al^{3+} and Fe^{3+} [17]. For example, polyaluminum chloride (PAC), which is formed by the prehydrolysis of aluminum, is one of the most widely used and preferred coagulants in wastewater treatment [18]. Flocculation agents, also known as flocculants, are divided into three main classes, cationic, anionic and non-anionic. Most of them are polyacrylamide-based and are widely used in wastewater treatment plants [19]; laboratory tests are usually the best way to select the best flocculant type.

Evaluation of the optimal conditions for the coagulation-flocculation-sedimentation process is usually carried out using the JarTest method. The results obtained in these tests help to determine the coagulant-flocculant type and its optimal dose. To optimize the coagulation-flocculation process, several factors, such as the flocculant type and dose, stirring speed, mixing time, pH, and temperature, must be considered [16,20]. Changing these factors will significantly affect the speed and size of floc formation, therefore correct optimization of these values becomes essential [21].

An efficient way to achieve this optimization is using the response surface methodology (RSM). Indeed, RSM has been extensively used to optimize the main parameters of the coagulation-flocculation process for many effluents [22].

The main objective of this work was to optimize the flocculation of sulfur from an anoxic suspended biomass bioreactor used for biogas desulfurization. To that end, optimization of the main affecting parameters, such as flocculant dose, initial pH and stirring rate, was carried out using RSM.

2. Material and methods

2.1. Biological samples

Biogenic sulfur was produced in a continuous stirred tank bioreactor, performing desulfurization of biogas under anoxic conditions using nitrite as the final electron acceptor [23]. The bioreactor working volume was 5.5 L and it was operated under steady-state conditions at a hydraulic residence time of 1.73 days, a gas residence time of 119 s, H_2S inlet load (IL) of 77.4 gS-H_2S m^{-3} h^{-1}, and stirring speed of 60 rpm. The pH was controlled at between 7.8 and 8.0 by the addition of H_3PO_4 (2 N) or NaOH (2 N) and the temperature was controlled at 30 °C using a thermostatic bath (RM6 Lauda, Germany). At an N/S molar ratio of 0.75, the sulfur concentration in the reactor was between 1,700 and 1,800 mg S^0 L^{-1}.

The composition of the mineral medium was adapted from ATCC-1255 *Thiomicrospira denitrificans* [24]. The medium mineral composition was: KH_2PO_4 (2 g L^{-1}); NH_4Cl (1 g L^{-1}); $MgSO_4{\cdot}7H_2O$ (0.8 g L^{-1}); trace element solution SL-4 (2 mL L^{-1}). The SL-4 composition was: EDTA (0.5 g L^{-1}); $FeSO_4{\cdot}7H_2O$ (0.2 g L^{-1}); trace element solution SL-6 (100 mL L^{-1}). The SL-6 composition was: $ZnSO_4{\cdot}7H_2O$ (0.1 g L^{-1}); $MnCl_2{\cdot}4H_2O$ (0.03 g L^{-1}); H_3BO_3 (0.3 g L^{-1}); $CoCl_2{\cdot}6H_2O$ (0.2 g L^{-1}); $CuCl_2{\cdot}2H_2O$ (0.01 g L^{-1}); $NiCl_2{\cdot}6H_2O$ (0.02 g L^{-1}); $Na_2MoO_4{\cdot}2H_2O$ (0.03 g L^{-1}). The mineral medium was enriched with $NaNO_2$ (1.05 g of $N{-}NO_2^-$ L^{-1}) and $NaHCO_3$ (1.89 g L^{-1}) as a carbon source.

2.2. Coagulant-flocculants

Flocculants, prepared at a concentration of 0.1%, were used for the flocculation-sedimentation experiments. Flocculants in the solid state were left stirring at 400 rpm for 1 h to dissolve completely. It was decided to carry out preliminary tests with one coagulant (polyaluminum chloride, PAC) and cationic (Sedifloc 40L4 C, Lizaflock 853 M), anionic (Innoflock 201) and non-ionic flocculants (NI-1009). Both cationic flocculants are based on polyacrylamide and the main difference between them is the higher molecular weight (MW) and charge of Lizaflock 853 M compared to Sedifloc 40L4 C (Table S1). Flocculants based on aluminum salts were selected because higher flocculation rates were observed in previous studies [14,16,25,26]. The use of iron salts was discarded due to removal efficiencies below 40% of biogenic sulfur [27].

2.3. Batch flocculant studies

The Jar Test methodology was used to optimize the flocculation-coagulation process. Thus, a sample was taken from the bioreactor and diluted to a sulfur concentration of 475 mg S^0 L^{-1} and turbidity of 3,000 NTU. This solution was divided into 600 mL beakers with a final volume of 300 mL. The flocculation experiments were performed in a six-position flocculator type FC6S (Velp Scientifica, Italy), where the mixing speed (10–300 rpm) was controlled. After flocculant addition, the solution was mixed for 3 min at a high stirring speed (300 rpm) and then 10 min at medium stirring speed (120 rpm). At that point, the solution was allowed to settle for 30 min. Finally, a sample was taken at 2 cm below the surface of the solution for the turbidity measurement [16]. The percentage of biogenic sulfur flocculation without the addition of flocculants was also determined as control. In this case, the solution was mixed at high revolutions and then allowed to settle for 30 min. The pH was adjusted to that of the initial solution using H_2SO_4 (3 M) and NaOH (6 M).

The following equation was used to calculate the biogenic sulfur flocculation rate (θ, %) [16]:

$$\theta = \frac{A_i - A_f}{A_i} \text{ x } 100 \quad (1)$$

where A_i (mg S^0 L^{-1}) is the initial concentration of sulfur before the flocculation process, and A_f (mg S^0 L^{-1}) is the sulfur concentration at the final time.

2.4. Initial screening for flocculants and dose

In order to perform an initial selection of the best flocculants, primary tests were carried out using those mentioned in Section 2.2. Different final concentrations at the point of application were used for each flocculant. The range used was estimated based on the technical data sheets provided by the manufacturer and the literature. For the cationic and non-ionic flocculants (Sedifloc 40L4 C, Lizaflock 853 M and NI 1009) a range of between 0.05 and 3 mg L^{-1} was used, as suggested by Chen et al. [16]. The range provided by the manufacturer (0.2–10 mg L^{-1}) was added using the anionic flocculant Innoflock 201. A PAC dose range between the dose recommended by the manufacturer and that used by Üstün et al. [28] was used (0.2–8 mg L^{-1}).

Within these ranges, six different flocculant concentrations were used in the test. The sulfur was recovered directly from the total volume of the bioreactor and diluted. The test was performed at room temperature, the initial sulfur concentration was 479.1 ± 2.4 mg S^0 L^{-1}, and the pH was maintained between 7.8 and 8.2, the range in which the desulfurization reactor was working at that time.

2.5. Optimization of sulfur flocculation

The two best flocculants were selected to optimize the sulfur flocculation process using RSM. The 17 experiments were based on a composite central design (CCD) of centered faces: 2^3 (factorial design), three repetitions of central points and six axial points (α = 1). The

Table 1
Factors and levels for the Composite Central Design.

Factors	Levels		
	−1	0	+1
Stirring speed (rpm)	30	90	150
pH	7.4	7.8	8.2
Flocculant dose (mg L^{-1})	0.1	0.55	1

experimental factors and levels are shown in Table 1. The selected response variable was the biogenic sulfur flocculation rate.

Once the flocculant dose, pH and stirring speed had been optimized, the reproducibility of the sulfur removal methodology was determined. To that end, a test was carried out on four different days with three replicates per day. The initial sulfur concentration was 532.1 ± 16.4 mg S^0 L^{-1}. A determination of the particle size and Zeta potential of three sulfur samples was also performed. These samples corresponded to the initial biogenic sulfur suspended in the bioreactor (Sample A) and the biogenic sulfur remaining in the clarified (Sample B) and settled (Sample C) effluents after the flocculation-sedimentation process was performed.

2.6. Effect of concentration on the flocculation method

The efficacy of the optimum flocculation conditions was verified for solutions with different concentrations of biogenic sulfur. The biogenic sulfur flocculation rate was determined at the following initial sulfur concentrations: 221.9, 545.0, 907.3, 1,200.7 and 1,729.8 mg S^0 L^{-1}.

2.7. Analytical methods

The turbidity (Nephelometric Turbidity Unit, NTU) was measured using a 2100AN turbidimeter (Hach, USA) with round borosilicate glass cuvettes.

The biogenic sulfur concentration of the samples was measured using gas chromatography coupled with a pulsed flame photometric detector (GC-PFPD) [29]. A linear relationship was established between simple turbidity (NTU) and sulfur content (mg S^0 L^{-1}) (Fig. S1). The linear regression obtained was considered valid for turbidity values in the range 1,000–11,000 NTU, with a correlation coefficient of $R^2 = 0.9963$. Equation (2) was used to calculate the sulfur concentration under the initial operating conditions:

$$[S^0] = \frac{T}{6.3347} \quad (2)$$

where T is the turbidity in NTU, and [S^0] is the sulfur content in mg S^0 L^{-1}.

Dynamic light scattering (DLS) was utilized in order to measure suspended particle hydrodynamic size. Measurements were carried out at 25 °C, using a Malvern Zetasizer NanoZS ("Malvern Instruments",

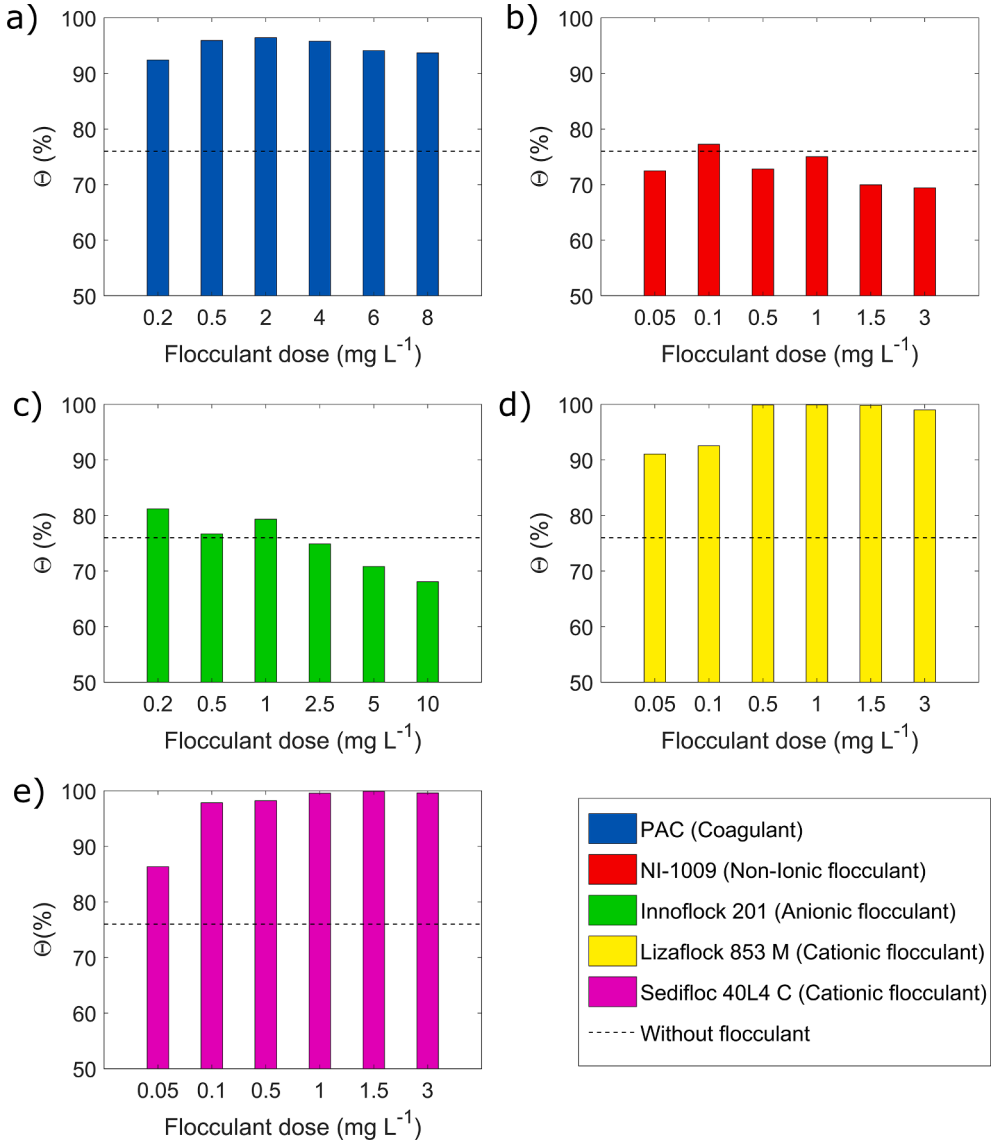

Fig. 1. Initial screening of biogenic sulfur flocculation rate at different doses and flocculant types: (a) PAC, (b) NI-1009, (c) Innoflock 201, (d) Lizaflock 853 M and (e) Sedifloc 40L4 C. Sulfur removal by gravity sedimentation without flocculant (dotted line).

Worcestershire, UK), with a 1 cm path cell. This equipment also allows for the Z-potential to be measured which evaluates colloidal stability in solution.

3. Results and discussion

3.1. Initial screening

An initial screening of the proposed flocculants was performed to identify the best-performing flocculants. The results obtained in this preliminary test are shown in Fig. 1. In the absence of flocculant, sulfur removal remained at 75.79 ± 3.96% after 30 min of gravity sedimentation. In the absence of polyelectrolyte, the biogenic sulfur flocculation rate for the biogenic sulfur studied was remarkably high compared to the removal rate obtained by other authors such as Janssen et al. [30], who found similar removal rates but after gravity sedimentation for 24 h. This settleability increase may be caused by the large size of the sulfur particles studied in the present research. This aggregation of sulfur particles may be caused by the low shear stress forces (0.012 Pa) of the bioreactor in which the sulfur was produced [23] and the high H_2S inlet load applied to the bioreactor, which results in an increase in the particle size [9,30]. Indeed, it is likely that the high H_2S inlet load led to an increase in the particle size and, hence, its settleability. The biogenic sulfur obtained in this study also showed a better removal efficiency than the biosulfur used by Mora et al. [14], which did not show any settling capacity after 1 h of the Imhoff test.

The results obtained using the non-ionic (NI-1009) and anionic (Innoflock 201-MA) flocculants were not satisfactory, since these compounds did not improve sulfur recovery compared to the control (Fig. 1b and 1c). In both cases, an increase in the concentration of the flocculant led to a stabilization of the colloidal sulfur particles, which may be due to their charge density. Other authors have reported that non-ionic flocculants are the best for sedimenting sulfur at pH values of between 4.7 and 6.5 [31]. However, the sulfur in these studies was not produced biologically and the pH values are more acidic, which means that the charge on the sulfur particles is completely different from that of the particles used in the present study.

Sulfur recovery was improved when using PAC as coagulant, exceeding 95% at doses higher than 0.5 mg L^{-1} (Fig. 1a). This good performance may be attributed to the destabilization of the suspension caused by its mechanism of action, which involves surface-charge neutralization and bridging [32]. Chen et al. [16] obtained similar successful results when using PAC as coagulant to improve the sedimentation of biogenic sulfur. In their study, a maximum sulfur removal rate of 97.53% was obtained under specific conditions of pH 4.73, a stirring speed of 129 rpm and a PAC dose of 2.42 mg PAC L^{-1}. Such low doses of PAC are in contrast with other results obtained in the literature like Yuan et al. [26] and Lohwacharin et al. [33] who achieved flocculation rates higher than 90% with PAC flocculant doses higher than 300 mg L^{-1}. The settling percentage of the biogenic sulfur is highly dependent on the source from which it comes [34].

This poor correlation between flocculant dose and flocculation rate in biogenic sulfur from different sources is probably caused by the effluent characteristics (organic matter, salts, and other sulfur compounds) [14]. The sulfur studied in the present study was obtained from a bioreactor fed with a synthetic mineral medium, which lacks organic matter. Organic matter represents a challenge in the flocculation process because its presence requires the use of higher doses of coagulants or flocculants [35–37].

Finally, the best results in terms of biogenic sulfur flocculation rate were obtained using the polyacrylamide-based cationic flocculants (Sedifloc 40L4 C and Lizaflock 853 M). Indeed, the biogenic sulfur flocculation efficiency was over 98% at flocculant doses higher than 0.5 mg L^{-1} (Fig. 1d and 1e). These exceptional results can be explained by considering that the biogenic sulfur particles are negatively charged at the pH values used [30]. The mechanism of action for this type of polyacrylamide flocculant involves polymer bridging to the different negatively charged particles, which leads to faster settling [38]. The negative charge of the biogenic sulfur at high pHs has been widely reported by other authors, which mainly relates the origin of this charge to the presence of carboxylic acid and amino groups from proteins that cover these sulfur particles [6,7]. Many authors have obtained good results using cationic coagulants and/or flocculants to improve the sedimentation of biogenic sulfur. Mora et al. [14], for example, improved the settling capacity of biogenic elemental sulfur by adding two different cationic coagulants with linear and branched molecular structures. Similarly, Yuan et al. [26] reported that the addition of cationic coagulants led to a very high removal efficiency for colloidal biosulfur. However, Chen et al [16] obtained worse results when using cationic polyacrylamide in comparison with PAC. These different results may be related to the low charge of their colloidal biosulfur particles, which made the adsorption capacity of polyacrylamide insufficient to destabilize the colloid suspension.

3.2. Optimization of sulfur flocculation

Taking into consideration the results obtained in the initial screening, the main parameters affecting the flocculation process were optimized using the best performing flocculants, i.e. Sedifloc 40L4 C and Lizaflock 853 M.

The variables stirring speed (rpm), initial pH and flocculant dose (mg L^{-1}) were optimized by applying RSM. Table 2 shows the values used and the percentage biogenic sulfur flocculation rate for both polyacrylamide-based cationic flocculants. The flocculation rate ranged from 71.56% to 99.88%, showing excellent results under all the conditions tested.

Lizaflock 853 M was subsequently selected due to the overall better results obtained in terms of sulfur removal percentages. An analysis of variance (ANOVA) was performed with the results obtained using this flocculant (Table 3).

As shown in Table 3, when the p-value is lower than 0.05 the effect terms are considered significant. As such, it can be considered that the most important parameter is the flocculant dose, which has a positive effect. On the other hand, the effect of the other two parameters (stirring speed and pH) was still significant but lower than that for the flocculant dose. The effect of these two parameters was negative in the case of stirring speed and positive in the case of pH.

The optimization results were fitted to a quadratic model that predicts a curve function as a response, the model equation being as follows:

Table 2
Sulfur removal rates obtained using the best-performing flocculants in the flocculation optimization experimental design.

Stirring speed (rpm)	pH	Flocculant dose (mg L^{-1})	θ (%) Lizaflock 853 M	θ (%) Sedifloc 40L4 C
30 (−1)	7.4 (−1)	0.1 (−1)	91.69	77.56
150 (+1)	7.4 (−1)	0.1 (−1)	91.32	71.99
30 (−1)	8.2 (+1)	0.1 (−1)	96.26	89.58
150 (+1)	8.2 (+1)	0.1 (−1)	91.33	86.75
30 (−1)	7.4 (−1)	1 (+1)	99.76	94.37
150 (+1)	7.4 (−1)	1 (+1)	99.72	88.57
30 (−1)	8.2 (+1)	1 (+1)	99.66	99.67
150 (+1)	8.2 (+1)	1 (+1)	99.69	98.13
30 (−1)	7.8 (0)	1 (+1)	99.65	93.94
150 (+1)	7.8 (0)	1 (+1)	98.19	89.77
90 (0)	7.4 (−1)	0.55 (0)	97.29	84.05
90 (0)	8.2 (+1)	0.55 (0)	99.25	95.8
90 (0)	7.8 (0)	0.1 (−1)	94.22	92.48
90 (0)	7.8 (0)	1 (+1)	99.88	99.35
90 (0)	7.8 (0)	0.55 (0)	99.00	93.64
90 (0)	7.8 (0)	0.55 (0)	99.23	93.08
90 (0)	7.8 (0)	0.55 (0)	99.42	93.21

Table 3
Analysis of variance (ANOVA) for the CCD model*.

Parameter	Sum of squares	Degree of freedom	F-value	p-value
A (Stirring speed)	4.58329	1	8.25	0.0239
B (pH)	4.10881	1	7.39	0.0298
C (Flocculant dose)	114.853	1	206.66	0.0000
AB	2.52001	1	4.53	0.0707
AC	3.49801	1	6.29	0.0405
BC	2.77301	1	4.99	0.0606
A^2	0.06022	1	0.11	0.7516
B^2	1.71442	1	3.08	0.1225
C^2	10.9316	1	19.67	0.0606
Error	3.89041	7		
Total	164.389	16		

*$R^2 = 0.976$; adj. $R^2 = 0.946$.

$$\theta = -253.257 + 0.165149 \cdot A + 83.4993 \cdot B + 41.8119 \cdot C - 0.0000416471 \cdot A^2 - 0.023854 \cdot A \cdot B + 0.0244907 \cdot A \cdot C - 4.99956 \cdot B^2 - 3.27083 \cdot B \cdot C - 9.97496 \cdot C^2 \quad (3)$$

where A is the stirring speed (rpm), B is the initial pH of the solution and C is the flocculant dose (mg L^{-1}). The R^2 value for this model was 0.9763, thus indicating the good fitting of the model to the experimental data. This indicates that less than 2.4% of the total variation cannot be explained by the model [20,26].

The response surface graph for the optimal values reported by the model is shown in Fig. 2a for a flocculant dose of 0.82 mg L^{-1}, in Fig. 2b for a stirring intensity of 30 rpm and in Fig. 2c for a pH of 8.0.

Chen et al. [16] performed a similar study regarding sulfur recovery during the treatment of sulfate-containing wastewater with an initial NTU of 350. In that study, it was concluded that the best flocculation conditions were the use of PAC as flocculant at a dose of 2.42 mg L^{-1}, a stirring speed of 129 rpm and a pH of 4.73. Yuan et al. [26] obtained a 94.1% sulfur flocculation efficiency under similar conditions with the same flocculant and at a flocculant dose of 396 mg L^{-1}. Mora et al. [14] studied different coagulants and cationic flocculants to settle biogenic sulfur produced in a CSTR under microaerophilic conditions and a pH of 7.4. The optimum value reached was 64.4% of settled solids for the FL4820 coagulant (linear structure cationic) dose of coagulant of 0.5%. These results differ markedly from the optimized parameters obtained in the present study, thus leading us to conclude that it is important to determine the characteristics of the sulfur to be recovered since this will determine the optimum method for its recovery.

The Zeta potential is an indirect measurement that evaluates the charge on the particle surface. Values between −10 mV and +5 mV allow rapid removal of the particles in raw water while values close to 0 mV promote particle aggregation [39]. The initial biogenic sulfur sample (Sample A), showed a Zeta potential of -10.0 ± 1.59 mV, while the sample obtained from the settled effluent after the flocculation and sedimentation process (Sample C) showed a Zeta potential of +7.03 ±

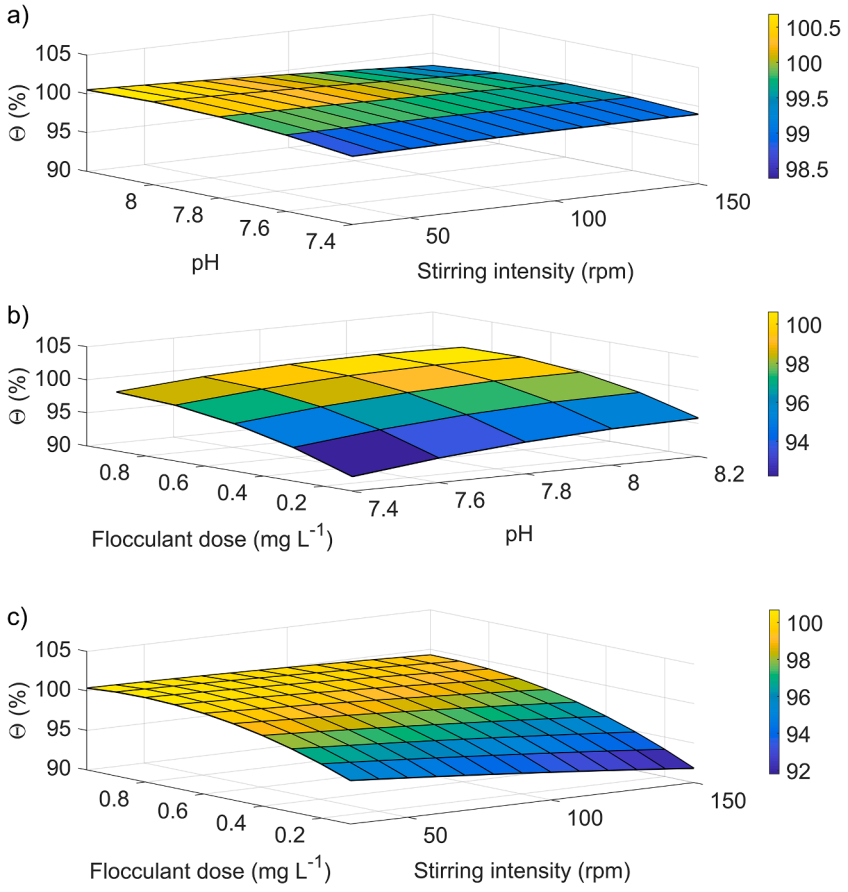

Fig. 2. Effect of the interaction between flocculant (Lizaflock 853 M) dose, stirring intensity and pH on the biogenic sulfur flocculation rate at values of (a) flocculant dose of 0.82 mg L^{-1}, (b) stirring intensity of 30 rpm and (c) pH of 8.0.

1.85 mV. Such low values of the initial sample indicate that a low dose of flocculant (0.82 mg L^{-1}) is needed to neutralize the negative charges. If the flocculant dose increases too much, it would re-stabilize the system causing a decrease in the particles' settlement [26]. A positive Zeta potential value is a result of absorption of flocculant into the particles giving them a positive charge. According to Razali et al. [40], the Zeta potential of wastewater from the paper mill at pH above +9 was −10 mV, indicating that 1.2 mg L^{-1} of the dose of flocculant added was not enough to neutralize the negative charges. Such a pH effect, however, becomes ineffective if flocculants with high organic charge are used, where charge neutralization has a major effect [41].

To evaluate the effect of the flocculant on the suspended particle size, the initial sample (Sample A) and a sample of the biogenic sulfur present clarified effluent after the flocculation and sedimentation process (Sample B) were measured. The particles in Sample A was ranged from 531 to 1,720 nm (mean particle size of 955 nm; 23.5% of the total particles), while the particles in Sample B was ranged from 79 to 122 nm (mean particle size of 91.3 nm; 39.7% of the total particles (Fig. 3). The size of the particles characterized in the present study was smaller than others reported in the bibliography. Some of the sulfur particle sizes reported were 2–5 μm in sulfur from biological desulfurization of paper mill gas streams [5]; 4.69 μm in sulfur from the Thiopaq® process [42], 0–29 μm in sulfur produced in a batch reactor to nitrate and sulfide removal [9], and 39.8 μm in sulfur produced CSTR under micro-aerophilic conditions [14]. According to Mol et al. [34], the optimal particle size for settling would be between 5 and 20 μm, while our values were much lower than this. Hence, the favorable results obtained in this study can be mainly attributed to the initial Zeta potential value.

The water quality parameters of the initial flocculation sample (Sample A) and clarified sample (Sample B) under the optimal operation conditions were measured. As might be expected, almost all physico-chemical parameters remained constant. There was a decrease in turbidity (NTU), and other parameters associated with particles in suspension such as chemical oxygen demand (COD), total suspended solids, total volatile solids and biomass (Table S2).

The production of biogenic sulfur is characterized by a high size and charge variability. Therefore, in order to test the reproducibility of the flocculation process, three replicates of the experiment were carried out on three different days of operation of the desulfurization reactor. Although the equation of the quadratic model establishes the optimum pH value for the flocculation process as 8.0, it was decided to use a pH of 7.8. This was due to the fact that 7.8 was demonstrated to be the optimum pH value to perform the anoxic biodesulfurization of biogas [43] and a change in this pH would require the addition of chemicals, thus resulting in higher operating costs. Moreover, the effect of pH was very low in this range of values (7.8–8.0). Hence, the experimental conditions tested were the use of the cationic flocculant Lizaflock 853 M at a pH of 7.8, a dose of 0.82 mg L^{-1} and a slow stirring speed of 30 rpm.

Table 4
Biogenic sulfur flocculation rate and the initial [S^0] obtained during the reproducibility tests performed on three different days.

Day	θ (%)	Initial [S^0] (mg S^0 L^{-1})
1	99.64	537.77 ± 1.97
	99.69	
	99.64	
2	99.84	513.46 ± 15.86
	99.85	
	99.84	
3	99.91	544.99 ± 2.18
	99.97	
	99.95	

As shown in Table 4, the percentages of biogenic sulfur flocculation were always higher than 99.5% on the three different days. These results highlight the reproducibility and excellent results for the flocculation conditions tested.

3.3. Effect of biogenic sulfur concentration on the flocculation method

The robustness of the optimum flocculation conditions (30 rpm, Lizaflock 853 M and 0.82 mg L^{-1}) was evaluated at pH 7.8 for different initial concentrations of biogenic sulfur.

The biogenic sulfur flocculation rate and the final sulfur concentration for each initial concentration are shown in Fig. 4. While the biogenic sulfur flocculation efficiency remained higher than 99% at sulfur concentrations up to 907.3 mg S^0 L^{-1}, the flocculation rate started to decrease at an initial sulfur concentration of 1,200.7 mg S^0 L^{-1}. However, it should be noted that the sulfur flocculation always remained above 97% and the final sulfur concentration below 122.9 mg S^0 L^{-1}. Consequently, it can be seen that, with a stirring intensity of 30 rpm, a Lizaflock 853 M flocculant dose of 0.82 mg L^{-1} and a pH of 7.8, we obtained a sulfur flocculation of more than 97% for S^0 concentrations below 1,729.8 mg S^0 L^{-1}.

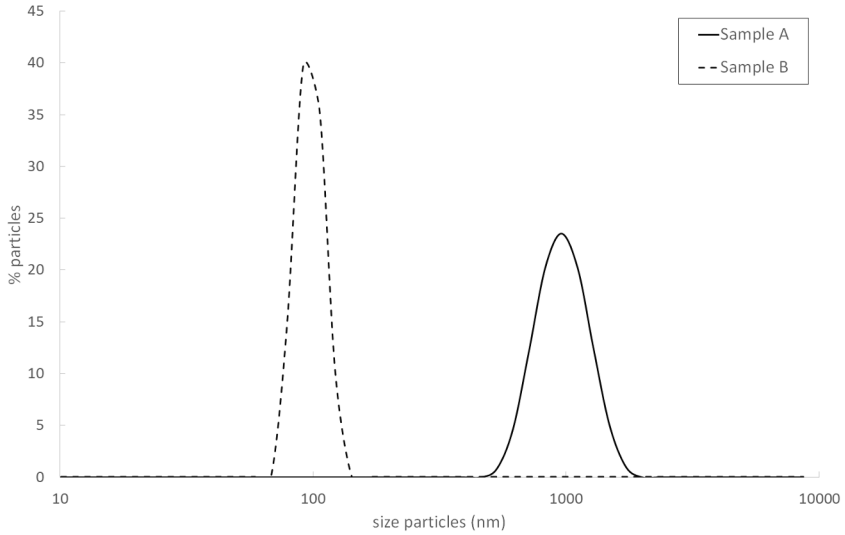

Fig. 3. Log-normal size distribution of sulfur particles in the bioreactor (Sample A) and the clarified effluent after the flocculation and sedimentation process (Sample B).

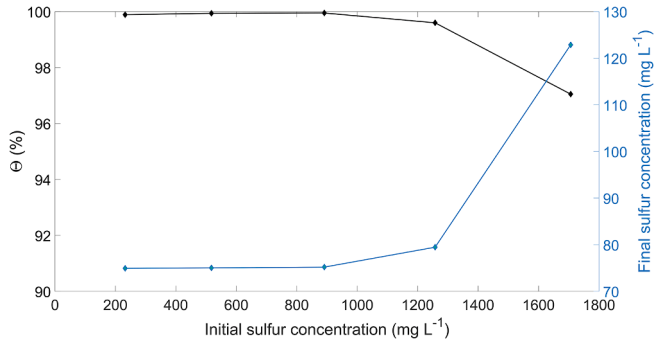

Fig. 4. Biogenic sulfur flocculation rate under the optimized conditions at different concentrations of biogenic sulfur.

4. Conclusions

This research successfully studied the flocculation of biogenic sulfur and established the optimum operating parameters for the flocculation of biogenic sulfur from an anoxic bioreactor. The cationic flocculants Sedifloc 40L4 C, Lizaflock 853 M and PAC18 showed flocculation rates over 95% for a dose of 0.5 mg L^{-1}. According to the RSM approach, which uses a CCD experimental design, the optimal conditions for Lizaflock 853 M were pH 8.0, a stirring speed of 30 rpm and a dose of 0.82 mg L^{-1}. The flocculant dose was the most significant variable. Under the optimum conditions, a flocculation rate of 97.05% was achieved for an initial sulfur concentration of 1,730 mg L^{-1}. The Zeta potential of the sulfur particles increased after the flocculation. It is worth highlighting the fact that- in view of these satisfactory results- there is significant progress from the application point of view in the scientific and engineering fields.

CRediT authorship contribution statement

Sandra Torres-Herrera: Investigation, Formal analysis, Writing – original draft. **J. Joaquín González-Cortés:** Formal analysis, Methodology, Writing – review & editing. **Fernando Almenglo:** Conceptualization, Methodology, Supervision, Writing – review & editing. **María Pilar Yeste:** Investigation, Formal analysis. **Martín Ramírez:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Domingo Cantero:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was co-financed by the 2014-2020 ERDF Operational Programme and by the Department of Economy, Knowledge, Business and University of the Regional Government of Andalusia. Project reference: FEDER-UCA18-106138.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fuel.2022.124367.

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