Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2023.29227

285 (2023) 83–92
February

Kinetic study and process optimization of simultaneous biological elemental sulfur (S^0) production and denitrification by using an oil-field consortium

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Received 2 July 2022; Accepted 12 December 2022

ABSTRACT

This study focused on the challenges of the maximum and fastest production of sulfate-free elemental sulfur through simultaneous reduction of sulfide by autotrophic denitrifying microbes. A microbial oil consortium was used to supply biomass which developed and synthetized by Research Institute of Petroleum Industry of Iran (RIPI). Having main feeds of concentrated nitrate and sulfide, this reaction was anaerobically conducted under batch conditions (8 h) and 3 different molar S/N ratios (1.75, 2.00, and 2.25). The results convinced us to look for a fourth S/N ratio (the optimal point) that our data did not show. Molar S/N ratio 1.9 was the point that the highest amount of elemental sulfur was achieved and sulfide removal rate reached the highest and fastest value (197 mg/L, 3 h) with no sulfate production. Bio-kinetics studies showed that a substrate limitation and a chemical/physical product inhibition mechanism controlled the reaction conditions. Three bio-kinetic mechanisms were investigated to predict the parameters of bio-kinetic models. Inhibition-free substrate limitation, substrate inhibition, and product inhibitions parameters were calculated and studied by MATLAB software version 17b.

Keywords: Simultaneous desulfurization and denitrification; Biological elemental sulfur; Molar S/N ratio; Oil-field microbial consortium; Bio-kinetic models

1. Introduction

High sulfide concentrations in wastewater streams cause various environmental issues that affect ecosystems, microorganisms, and human health even at low concentrations [1,2]. As an electron donor in anaerobic reactions, the presence of sulfide leads to the accumulation of reduced sulfur compounds such as HS^- (another form of sulfide) and light mercaptans [3,4]. Moreover, considerable amounts of H_2S production from HS^- could occur during the anaerobic sludge digestion in municipal wastewater treatment plants, causing many restrictions and much destruction to energy generation and environmental impact [5].

Although the conversion of sulfide to sulfate, at first glance, can solve HS^- problem simply, some chemical and practical realities avoid specialists following this reaction with blindfolded eyes. By controlling the range of oxidation–reduction potential (ORP), reduced sulfur can be converted into various transient forms of sulfur such as thiosulfate, elemental sulfur S^0, and sulfate [6–8]. In contrast to sulfur-oxidizing bacteria (SOB), sulfur-reducing bacteria (SRB) are not so active and inherently have weak performance due to their extremely negative ORP requirements; therefore, they played no role in this research [9]. Eventually, the unbalanced sulfate accumulation blocks the sulfur cycle, which is followed by many expected and unexpected environmental impacts [10].

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The intermediate product S^0 is used widely in various industries [11,12]; however, its thermodynamics is precarious, though it could not remain and be collected from within the bioreactor easily. The autotrophic denitrification, especially at low molar S/N ratios (less than one), leads the oxidation process toward the complete conversion of sulfide to sulfate [13,14]. Moreover, the energies read by Eqs. (1) and (2) showed that oxidation by aeration resulted in sulfate production. One can conclude simply that the overall energy for conversion of H_2S to sulfate is –796.5 kJ/gmol, which is an attractive and unavoidable reaction to consume the elemental sulfur. Even attempts such as micro-aeration could not prevent sulfate production reliably [15].

$$H_2S + 0.5O_2 \rightarrow S^0 + H_2O,$$
$$\Delta G^0 = -209.4 \text{ kJ/gmol } H_2S \quad (1)$$

$$S^0 + 1.5O_2 + H_2O \rightarrow SO_4^- + 2H^+,$$
$$\Delta G^0 = -587.1 \text{ kJ/gmol } S^0 \quad (2)$$

The autotrophic simultaneous denitrification and desulfurization process, shown by Eq. (3) (S/N is 2.5), is an environmentally friendly and cost-effective process that has four significant advantages: low energy consumption (no aeration), low investment, nitrate removal, and recoverable elemental sulfur as an intermediate product [16,17].

$$S^{2-} + 0.4NO_3^- + 2.4H^+ \rightarrow S^0 + 0.2N_2 + 1.2H_2O,$$
$$\Delta G^0 = -191.0 \text{ kJ/gmol } S^{2-} \quad (3)$$

Because thermodynamics are involved essentially with the beginning and end of a reaction (not the path), it is expected that the standard Gibb's energy of the reaction read by Eq. (3) will become more or less equal to that illustrated by Eq. (1). The importance of Eqs. (1) and (3) is not about the amount of their $\Delta G°$, but all about their product's stability [18].

Although nitrate is an electron acceptor, it cannot complete the reaction in the direction of sulfate production because of its weakness compared to dissolved oxygen [19]. Furthermore, the stoichiometry of nitrate reduction is in a form that does not allow the oxidation surplus to the way of sulfate formation [14]. In this regard, considering nitrate removal and pH effect, good attempts were made to determine the S/N ratio for the most effective autotrophic denitrification [20–23]. However, these studies could be more complete if attempts were made to investigate the condition resulting in elemental sulfur production and its stability.

For a practical approach to simultaneous desulfurization and denitrification, which could be counted as our main focus in this work, we investigated the critical factors, including determining the optimum S/N ratio for S^0 accumulation followed by its recovery and its separation. This paper also presents the techniques that cause the desired operational results, maximum S^0 productivity, stability, acceptable biomass dewaterability, and some kinetic studies for project application. Moreover, kinetic studies were crucial in this research; therefore, MATLAB software version 17b was used to estimate bio-kinetic parameters in order to compare experimental and calculated values.

2. Materials and methods

2.1. Microbial source and the nutrient culture medium

The microbial source was obtained from a synthetic consortium which is obtained from desulfurizing strains, and developed by the Research Institute of Petroleum Industry (RIPI) of Iran. This microbial source is known as an efficient domestic mixture to successfully remove sulfide from oil industry waste streams and was also adapted to nitrate (KNO_3) for 6 weeks. The major distinction between the oiled-field consortium and other conventional biomass (sludge) is that this biomass is synthesized for the fastest sulfide removal accompanied by the highest elemental sulfur production. The next-generation sequencing (NGS) analysis that was performed by BGI (China) in 2017 was done to detect the DNA sequencing of microbial resource. The results showed that the main members of the community were *Pseudomonas*, *Thermodesulfobacteria*, *Thiomonas*, *Thioalkavibrio supfidophilus*, and *Desulfovibrio*. A nutrient culture containing 2 g/L KH_2PO_4, 0.4 g/L NH_4Cl, 0.2 g/L $MgCl_2{\cdot}6H_2O$, 0.159 g/L $NaNO_3$, and 0.4 g/L $NaHCO_3$ was made.

2.2. Sample preparation and the batch experiments

4,800 mL of the nutrient medium consisting of 10 g/L $Na_2S_2O_3{\cdot}5H_2O$, 20 g/L Na_2CO_3, 10 g/L $NaHCO_3$, 5 g/L NaCl, 1 g/L K_2HPO_4, 0.5 g/L KNO_3, and 0.1 g/L $MgCl_2{\cdot}6H_2O$ was prepared and divided into the three 2 L Erlenmeyer Flasks, each 1,600 mL. Three amounts of thoroughly dried KNO_3, that is, 221.64, 193.44, and 171.74 mg/L (for nitrate concentration), were weighed and added to each flask to prepare three cultures having initial S/N ratios of 1.75, 2.0, and 2.25 (mol/mol). This arrangement allowed 16 culture samples (each 100 mL) to be decanted into sixteen 200 mL serum bottles (half-full); this configuration also provided more exact data for mass and kinetic determination. The philosophy of S/N ratios will be explained in the Results and Discussion section. To reach anoxic circumstance, the concentration of dissolved oxygen (DO; Hach) was kept under 0.10 mg/L by stripping nitrogen gas for 10 min.

After the culture medium was prepared, the bottles were autoclaved for 30 min at 121°C. Then, a filter-sterilized high-concentration sodium sulfide solution was added to the bottles. These sterilized samples were cooled down to room temperature and then inoculated by the microbial source at 10% v/v.

Some preliminary tests on the microbial source that were previously conducted (data not shown) demonstrated that all sulfide was consumed and disappeared in a maximum time interval of 8 h. The pre-experimental analysis helped us to narrow molar S/N ratio values. For each 30 min interval, one of the sampling containers was taken and analyzed for the experimental data. An incubator Climo-Shaker ISF1-X was used to supply the intended conditions for each batch experiment. The operational situations of the incubator were 140 rpm stirrer, and the temperature was kept at 30°C.

2.3. Chemical and biological analyses

Nitrogen nitrate (NO_3^-) was assessed by ultraviolet spectrophotometric screening according to the Standard Methods for the Examination of Water and Wastewater APHA Method 4500 NO_3^- [24]. Sulfate (SO_4^{2-}) was measured by the ultraviolet spectrophotometric HACH method 8051 [25]. Sulfide was measured by the methylene blue spectrophotometric HACH method 8131 [26]. The Laurie modified protein metric test was used for protein concentration (Sanderman and Strominger, 1972). A mini-SHIMADZU UV-VIS spectrophotometer 1240 was used for all experiments and metric methods. Elemental sulfur was determined by the Sörbo method, and sulfur mass balance confirmed our results [27,28]:

$$\left[S^0\right]=\left[S^{2-}\right]-\left[HS^-\right]-\left[SO_4^{2-}\right] \tag{4}$$

Thiosulfate does not form in anaerobic conditions, so it was not measured according to the reliable references [29].

2.4. Kinetic study

Sulfide was the substrate (S), and elemental sulfur was the product (P). All numerical calculations on suggested kinetic models were done using the software MATLAB version 17b. The kinetic parameters were calculated and compared to the results of a data fitting problem using nonlinear least-squares different lsqnonlin algorithms. The model computed a vector of deviations between predicted and observed values. The default model Trust-Region-Reflective (TRR) algorithm was used to calculate kinetic parameters and coefficients. The Levenberg–Marquardt algorithm was used as an alternative in cases in which calculations performed by the TRR could not be converged. To understand the biochemical mechanisms of the reaction, biochemical kinetic mechanisms were deeply studied. Eqs. (5)–(7) represent inhibition-free substrate limitations, whereas Eqs. (8)–(10) include substrate inhibition and Eqs. (11)–(13) include product inhibition (Table 1).

3. Results and discussion

3.1. Sulfide, sulfur, and sulfate balance

The microbial consortium removed sulfide rapidly, and the sulfide concentration dropped drastically. During autotrophic denitrification, sulfide converts to elemental sulfur and sulfate; however, the nature of this conversion and the products' stability depend strongly on the initial amount of molar S/N ratio. Sulfide is definitely oxidized; however, there are two destinies for this rapid oxidization: sulfate and elemental sulfur. As this study focused on the production of elemental sulfur, it looked for the best sulfide removal efficiency and optimum point for maximum elemental sulfur accumulation as well as stability. In addition, the rapidness of the reaction caused us to study its kinetic features comprehensively.

Research has been done to find effective parameters on autotrophic sulfide conversion [23]. Most previous works focused for the molar ratio of sulfur to nitrogen as a key parameter, and nitrate/sulfide loading has been stated as the parameter effective on sulfide conversion to sulfate and sulfur recovery in anaerobic autotrophic denitrification and desulfurization [30].

Reliable scientific sources indicate that although sulfide oxidization occurs in a vast range of S/N, significant amounts of elemental sulfur would appear when the molar S/N ratio exceeds 1.3, and the increment of this value up to 2.4 would lead to its accumulation [10]. A significant number of investigations have reported that the principal sign of elemental sulfur production inside the reactor is the appearance of a milky white color [22]. This milky colloidal elemental sulfur would disappear at a molar S/N ratio >2.8 [31]. Therefore, following the previous research, the molar S/N ratio of $1.75 < S/N < 2.25$ was chosen for this work. The pretests evidenced significant elemental sulfur production in the range as mentioned-above (data not shown). Oxidation of elemental sulfur could be easily extended to sulfate; this states the unstability of this helpful element as well as the fact that in all S/N ratios, a variation of sulfate and sulfur concentrations can be seen. This unstability entirely depends on the

Table 1
Kinetic models equations

Equation number	Equation name	Equation formula
(5)	Monod	$\mu=\frac{\mu_{max}S}{K_S+S}$
(6)	Tessier	$\mu=\mu_{max}\left(\frac{S^n}{K_S+S^n}\right)$
(7)	Moser	$\mu=\mu_{max}\left(1-\exp\left(-\frac{S}{K_S}\right)\right)$
(8)	Andrew	$\mu=\mu_{max}\left(\frac{S}{1+\frac{K_S}{S}+\frac{S}{K_I}}\right)$
(9)	Aiba[I]	$\mu=\mu_{max}\left(\frac{S}{K_S+S}\right)\exp\left(-\frac{S}{K_I}\right)$
(10)	Edwards	$\mu=\mu_{max}S\left[\exp\left(\frac{-S}{K_I}\right)-\exp\left(\frac{-S}{K_S}\right)\right]$
(11)	Aiba[II]	$\mu=\mu_{max}\left(\frac{S}{K_S+S}\right)\exp\left(-K_PP\right)$
(12)	Hinshelwood	$\mu=\mu_{max}\left(\frac{S}{K_S+S}\right)\left(1-K_P\right)$
(13)	Jerusalimsky	$\mu=\mu_{max}\left(\frac{S}{K_S+S}\right)\left(\frac{K_P}{K_P+P}\right)$

presence of various forms of oxygen that react with sulfide and convert it to sulfur and sulfate.

This study, attempted to reach the highest amount of sulfide-to-elemental sulfur conversion and find a way to alter sulfur to a stable and recoverable product. An operational technique that could be sought here might be defined as an optimum S/N ratio point where all sulfide transforms to elemental sulfur. During this conversion, no sulfate is generated. From the viewpoint of industrial application, because elemental sulfur sediments are much faster than biomass, in applying the optimum S/N ratio, neither sulfate nor biomass will exist in the stream that drains the elemental sulfur [32]. Elemental sulfur remains stable and pure because of the absence of dissolved oxygen and sulfate, respectively.

At a molar S/N of 1.75, 90% of the initial 200 mg/L sulfide was removed in 4.5 h (Fig. 1a); however, sulfide still existed in the reaction even at trace concentrations. After 0.5 h, sulfide concentrations were 145 and 135 mg/L at the molar S/Ns of 1.75 and 2, respectively, which indicates that by increasing the molar S/N from 1.75 to 2, the sulfide removal rate increased consequently. Compared to the S/N of 1.75, 90% of sulfide was consumed in 3 h at S/Nb2, and this phenomenon proved that the sulfide removal rate was greater at S/N 2 than at S/N 1.75 (Fig. 1b).

The investigation of sulfide removal efficiency at molar S/N 2.25 demonstrated that unlike molar S/N 2, sulfide removal performance was decreased at a higher rate compared to the molar S/N ratios of 1.75 and 2. Sulfide concentration declined to 170 mg/L after 0.5 h, a significantly lower amount than in S/N 1.75 and 2.0 after the same period. By observing Fig. 1a–c, one can conclude that tuning S/N for amounts between 1.75 and 2 may achieve the desired results, that is, (1 maximum and fast sulfide removal, and 2) exhausting sulfide without sulfate production. Experimental results convinced us to study in depth to determine the best optimum S/N, and it was found to be 1.9 (tuning data not shown).

The sulfide removal rate at this S/N molar reached the highest value, and the sulfide was entirely consumed after 3 h (before the end of the reaction), which is earlier than the other molar S/N ratios. Sulfide was removed steeply after 2 h, and 200 mg/L initial of sulfide was converted to 24 mg/L. After 2.5 h, the sulfide concentration was 12 mg/L; it took 3 h for the trace amounts to be excluded. Moreover, no sulfate was produced until the elemental sulfur concentration reached its maximum level (Fig. 1d).

The highest amounts of elemental sulfur accumulation at molar S/N 2.25 and 1.75 was close to each other at 149 and 146 mg/L, respectively, after 4 h. However, elemental sulfur accumulation at S/N 1.9 was greater than at the other S/N ratios, reaching 89 mg/L after half an hour, and the highest amount was 197 mg/L after 3 h. Moreover, during this period, no sulfate was produced. This was an essential and very critical result.

At 3 h, sulfate was observed first, and its concentration grew to 200 mg/L right after 6 h. Conversely, when S/N ratios were 1.75 and 2.25, sulfate production began at 0.5 h and increased gradually to 200 mg/L at the end of the reaction. This process continued as long as elemental sulfur existed in the reaction and was converted to sulfate.

Measurements showed that in all experiments at high concentrations of sulfide, ORP was approximately –320 mV. When the S/N ratio was 1.9, this value was steeply increased to near zero, at about –20 mV. This value appeared when elemental sulfur was at the maximum point and denitrification occurred. As long as elemental sulfur converts to sulfate and the denitrification rate decreases, ORP values decrease to –110 mV when all the elemental sulfur has been converted to sulfate. As sulfate is reduced in ORPs less than –350 mV and this range of potentials does not exist in SDD, it never converts to other forms of sulfur again once it is produced. For that reason, our experiments on the S/N ratio were very keen not to meet sulfate till all sulfide was converted to elemental sulfur.

3.2. Autotrophic denitrification at the various molar S/N ratios

Autotrophic denitrification occurs when external organic carbon has not been used to supply sufficient energy for the reaction. Thus in this process, sulfide as an electron donor is oxidized to inorganic sulfur and sulfate [33]. Bio-oxidation of sulfide to elemental sulfur and sulfate can be done by direct oxygen stripping in aerobic conditions or using oxygen-containing components such as nitrate as an electron acceptor under anaerobic conditions [31]. In the absence of oxygen and anaerobic conditions, nitrate can provide the required oxygen for SOB's to oxidize sulfide to sulfur and then sulfate. This process is called autotrophic denitrification, and nitrate is converted to nitrogen (gas).

Nitrate consumption and denitrification rates are directly related to the sulfide removal rate. In other words, when the highest rate of sulfide removal is observed inside the reactor, the denitrification process rapidly occurs correspondingly. According to the different molar S/N ratios and constant initial sulfide concentrations, four different nitrate concentrations were used in various molar S/N ratios. 172.2, 193.75, 203.94, and 221.14 mg/L initial nitrate concentrations were used for S/N ratios 1.75, 1.90, 2.00, and 2.25, respectively.

At molar S/N 1.75, the rate of nitrate consumption was 82% (Fig. 1a); nitrate removal at the end of the reaction was 87% when S/N was both 1.9 (Fig. 1d), and 2 (Fig. 1b). Therefore, it is obvious that the nitrate removal percentages for molar S/N 1.9 and 2 are close to each other according to the molar S/N ratios. Eventually, when molar S/N was 2.25, the initial nitrate ran out, because it was near stoichiometric values and was used as a substrate for reacting with sulfur and sulfide.

3.3. Kinetic results and biomass growth

Table 2 presents the results of the kinetic study on autotrophic simultaneous desulfurization and denitrification. Because there is a first-order stoichiometry relation between nitrate and sulfide conversion (as S/N ratios regulate), one of them can be taken as substrate concentration to analyze the given kinetic models illustrated in Table 2. As mentioned in the Introduction, the basis of this research was formed for attention to sulfide oxidation to elemental sulfur; therefore, we took sulfide as the main substrate for the kinetic models. Regarding their specifications on determining the substrate and product inhibition and substrate limitation, nine kinetic models [Eqs. (5)–(13)] with a high impact in scientific citations were considered for kinetic calculations in this study [34,35].

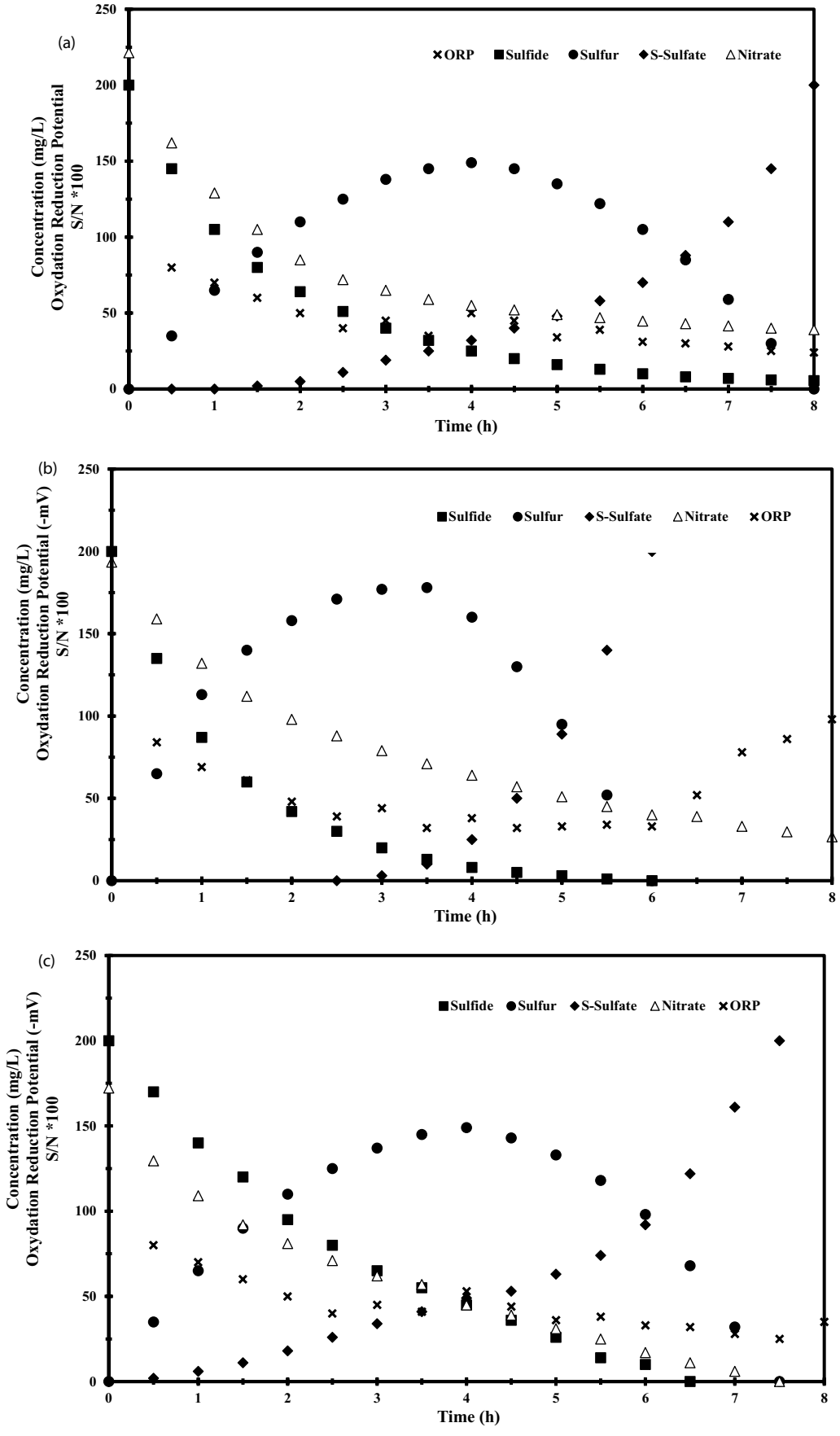

Fig. 1 (Continued)

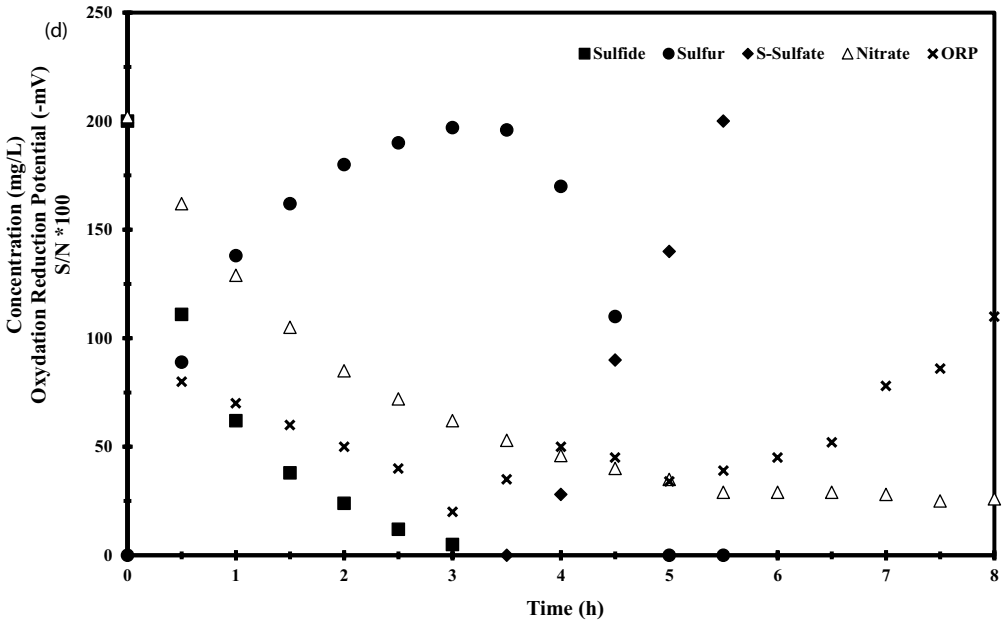

Fig. 1. Profile of sulfide, sulfate, elemental sulfur and nitrate concentrations during the batch reactions with different molar S/N ratios. (a) Molar S/N ratio 1.75, (b) molar S/N ratio 2, (c) molar S/N ratio 2.25, and (d) molar S/N ratio 1.9.

μ is the specific growth rate (h^{-1}) and μ_{max} is maximum specific growth rate (h^{-1}) at the time (h). Also, K_S, K_I, and K_P are the half velocity, inhibition, and product constants, respectively (all in mg/L). The first three models, that is, Eqs. (5)–(7) addressed in Table 2 were developed according to the inhibition-free substrate limitation, whereas the second and third three groups of models are given for substrate and product inhibition [Eqs. (8)–(10) and Eqs. (11)–(13), respectively].

Model 4 is the well-known Monod equation developed for the biomass growth rate for the mechanism of no substrate inhibition. Moser [Eq. (6)] with a stress on substrate effect by a power "*n*" and Tessier [Eq. (7)] developed according to the diffusion-controlled substrate kinetics, suggested the modified forms taken as alternatives for the Monod equation to predict free inhibitory substrate limitation values. According to the total consumption of sulfide before the end of the reactions, selected models must be represented to support the limitation of the inhibition-free substrate. Monod, Moser, and Tessier are the models that can calculate kinetic parameters in this mechanism [34].

Autotrophs do not use organic carbon for their growth; therefore, their biomass never grows considerably. This weakness in the concentration of biomass compared to other chemical species on the one hand and the toxicity and inhibitory nature of the substrate and product on the other leads one to give special consideration to kinetics models containing efficient substrate and product influences. That is why substrate inhibition models [Eqs. (8)–(10)] and product inhibition ones [Eqs. (11)–(13)] must be reviewed sensibly. Furthermore, because sulfide's initial concentration seemed to cause some inhibition, its effect on substrate needed to be tested.

According to Table 2, for the first group (the substrate limitation models), Monod and Tessier predict very close parameters (i.e., μ_{max} and K_S) for all S/N ratios. As "*n*" in Tessier's model is determined near 1, the calculated results for Monod's model show no significant difference between the parameters of these two models. According to limited and sensitive biomass production, models 8–10 which are Andrew (8), Aiba[I] (9), and Edwards (10), address substrate inhibition and were initially thought to be a supportive mechanism for this study. However, the results of determined kinetic parameters were out of range and had no physical meanings; these results as read in Table 2 are dispersed, negative, or very big parameters. This could be interpreted in another way, too: when the free-inhibition substrate limitation resulted in good agreement, the culture was not under the control of substrate inhibition. Another critical object that confirms this reasoning is that in the selected range of S/N ratios, the range of sulfide concentrations is such that SOBs consume the substrate with no thermodynamic limitation [Eq. (4)].

Elemental sulfur can inhibit microbial growth because of its chemical and physical properties. Its accumulation is produced in the colloidal form, in which elemental sulfur forms the main part of the colloids. However, the core of the colloids, which consists of the autotrophic microbes, makes up only a tiny percentage of the granule weight. Sulfur is secreted from the microbe, accumulates on the outer surface, detaches, and drops down frequently; however, it eventually consists of more than 90% of the weight of the colloids. This composition has previously been reported by others [36]. The flocs were generally fragile, less than 4 mm in diameter, and settled quickly (25–100 m/h).

Cell immobilization by attached sulfur on the one hand and high turbidity caused by detached sulfur on the other resulted in a defined type of inhibition addressed previously [37]. The inhibition could be represented by known models demonstrated by Eqs. (11)–(13).

Table 2
Values of kinetic parameters according to different mechanisms (all data in mg/L)

	Models	S/N 1.75					S/N 1.9					S/N 2					S/N 2.25				
		K_S	K_P	K_I	μ_{max}	n	K_S	K_P	K_I	μ_{max}	n	K_S	K_P	K_I	μ_{max}	n	K_S	K_P	K_I	μ_{max}	n
	Monod	4.20	–	–	0.42	–	4.81	–	–	0.51	–	5.07	–	–	0.59	–	5.57	–	–	0.48	–
S. Limitation	Tiesser	4.40	–	–	0.45	1.0	4.99	–	–	0.52	1.1	5.14	–	–	0.58	1.1	5.99	–	–	0.52	1.1
	Mosser	8.34	–	–	0.36	–	7.68	–	–	0.48	–	7.27	–	–	0.57	–	6.19	–	–	0.49	–
	Andrew	–14	–	1667	512	–	–48	–	988	742	–	–54	–	979	760	–	–424	–	752	861	–
S. Inhibition	Aiba[I]	332	–	452	166	–	–14	–	513	181	–	–18	–	517	182	–	–94	–	703	202	–
	Edwards	527	–	558	228	–	695	–	688	147	–	701	–	434	138	–	877	–	107	115	–
	Aiba[II]	5.00	0.20	–	0.27	–	6.47	0.36	–	0.24	–	9.57	0.66	–	0.14	–	4.99	0.1	–	2.49	–
P. Inhibition	Hinshelwood	4.97	0.36	–	0.22	–	4.12	0.48	–	0.18	–	4.01	0.67	–	0.01	–	10.05	0.75	–	0.19	–
	Jerusalimsky	8.52	0.96	–	0.64	–	7.31	0.77	–	0.52	–	7.29	0.72	–	0.49	–	6.6	6.61	–	0.40	–

Table 3
Summary and comparison of literature and this study on the maximum and fastest elemental sulfur production

Reference	Time reaction (h)	Product	Conversion of $S^{-2} \rightarrow S^0$	Effective factor	Stability*	Range
[40]	124	S^0	83.3% ± 0.7%	O/S ratio	No	0.6 – 0.1 mol L^{-1} h^{-1}/mol L^{-1} h^{-1}
[41]	3.8	S^0	90%	DO	No	0.1–0.15 mg/L
[39]	1.4	S^0	70%	DO	No	2–5 mg/L
[22]	40 ± 1.5	S^0	90%	S/N ratio	No	1–1.25
[42]	8	S^0	79%	ORP	No	–400~ – 360 mV
[23]	24	S^0	19%	S/N ratio	No	1.49
[43]	6	S^0	83.7%	S/N ratio	No	2.85
[44]	24	S^0	84.4% ± 7.7%	S/N ratio	No	5.6
This study	3	S^0	98.5%	S/N ratio and ORP	Yes	S/N ratio 1.9 – ORP – 20 mV

*Stability denotes that this study has reached the highest amount of sulfide bio-oxidation to elemental sulfur considering no sulfate production that the elemental sulfur can be recovered and purified directly by the separation process.

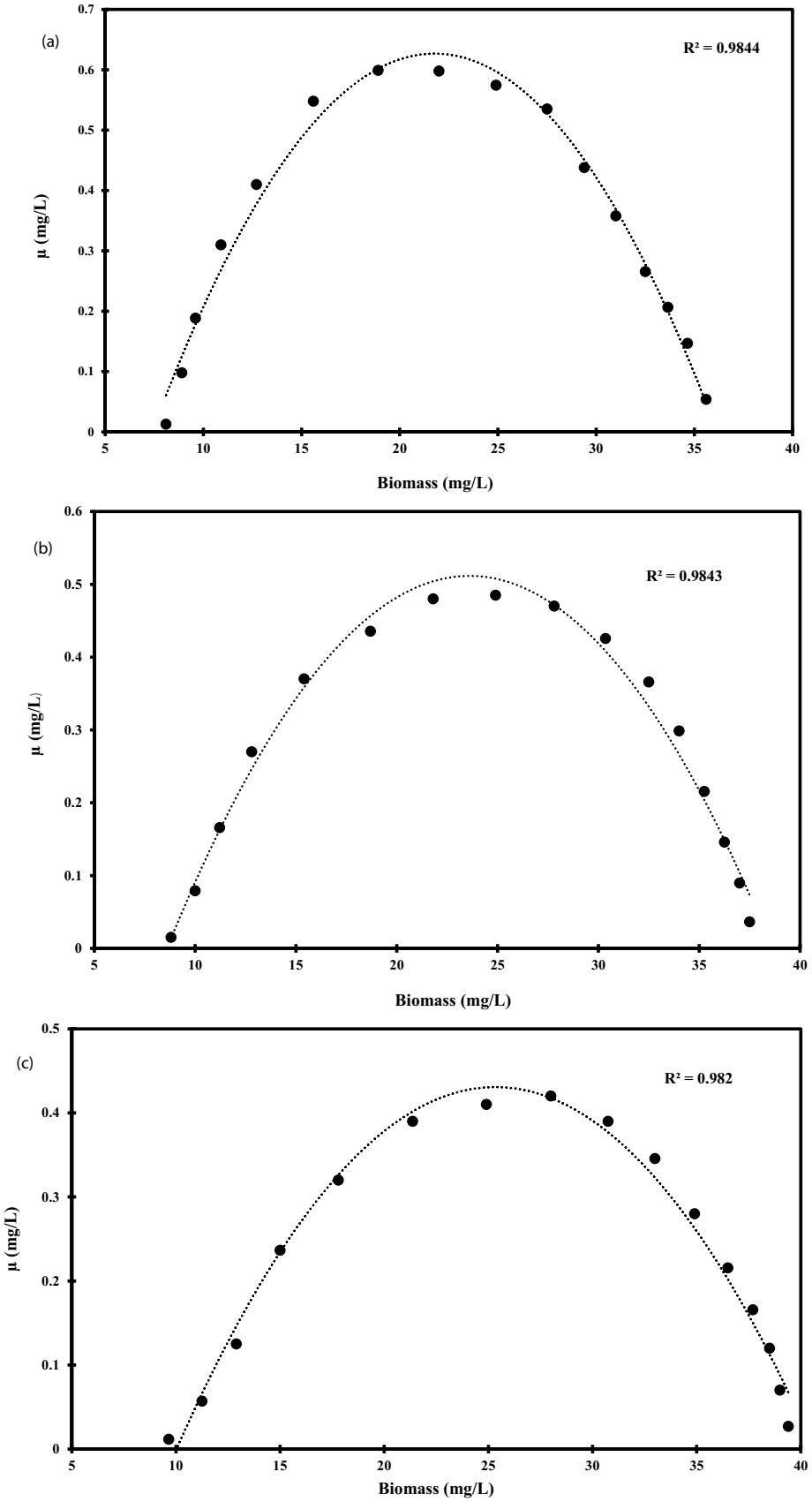

Fig. 2. Calculated (dashed line) and experimental (circle point) variation of maximum growth rate with different molar S/N ratio. (a) Molar S/N ratio 2, (b) molar S/N ratio 2.25 and (c) molar S/N ratio 1.75.

All parameters determined and recorded for the last three models in Table 2 indicate that these models predict inhibition significantly. Their parameters (K_S and μ_{max}), common with the models of inhibition-free substrate limitation [Eqs. (5)–(7)] show that Aiba[II] [Eq. (11)] and Hinshelwood [Eq. (12)] provide better and more reliable values.

Table 3 shows the current results of the bio-oxidation of sulfide to elemental sulfur as the end product compared with those in the literature. The results that distinguish this study include the conversion of sulfide to elemental sulfur, the reaction rate, and the most significant production of sulfur with negligible or zero sulfate production, which differed when various experimental conditions were implied [38]. In more detail, the reaction time was lower than in previous studies. Moreover, sulfide conversion to sulfur reached 98.5%, considering no produced sulfate.

The effect of different molar S/N ratios on maximum growth rate was studied, and the comparison of calculated results is shown by the dashed line with experimental results shown by the black circle illustrated in Fig. 2a–c. The Monod model fitted well with experimental values of μ obtained for each molar S/N ratio. *R*-square values represent good correlations of coefficients of 0.9844, 0.9843, and 0.982, respectively. The values of kinetic parameters and coefficients are reported in Table 2. The maximum specific growth rate of molar S/N ratio 2 is higher than that of molar S/N ratios 1.75 and 2.25, and it is clearly dependent on the K_S, K_P, and K_I.

4. Conclusion

The current study revealed that the fastest and the highest amount of biological elemental sulfur produced by the removal of autotrophic sulfide and nitrate occurs at the optimum molar S/N ratio of 1.9 with no sulfate made. In other words, the complete conversion of sulfide to elemental sulfur with no sulfate production occurred in the minimum time in this study, distinguishing this research from other studies. Concerning the essence of autotroph microbes and the consortium that resulted in no significant change in microbe mass during the process, bio-kinetic studies proved that this process follows the inhibition-free substrate limitation mechanism with a significant physical and chemical product inhibition according to the secreted extracellular sulfur that appears as a colloid within sludge. Studying the transfer phenomenon at the boundary layer of biological elemental sulfur can be a ground breaking topic for further, more in-depth study.

Acknowledgments

This project was financially supported by Biotechnology and Environment research division of Research Institute of Petroleum Industry (RIPI) of Iran.

Declaration of competing interest

The authors declare no conflict of interest.

Ethical statement

Neither ethical approval nor informed consent was required for this study.

References

[1] C. Jing, Z. Ping, Q. Mahmood, Simultaneous sulfide and nitrate removal in anaerobic reactor under shock loading, Bioresour. Technol., 100 (2009) 3010–3014.
[2] Q. Mahmood, P. Zheng, J. Cai, D. Wu, B. Hu, J. Li, Anoxic sulfide biooxidation using nitrite as electron acceptor, J. Hazard. Mater., 147 (2007) 249–256.
[3] E. Can-Dogan, M. Turker, L. Dagasan, A. Arslan, Sulfide removal from industrial wastewaters by lithotrophic denitrification using nitrate as an electron acceptor, Water Sci. Technol., 62 (2010) 2286–2293.
[4] L. Zhang, P. De Schryver, B. De Gusseme, W. De Muynck, N. Boon, W. Verstraete, Chemical and biological technologies for hydrogen sulfide emission control in sewer systems: a review, Water Res., 42 (2008) 1–12.
[5] J.K. Huertas, L. Quipuzco, A. Hassanein, S. Lansing, Comparing hydrogen sulfide removal efficiency in a field-scale digester using microaeration and iron filters, Energies, 13 (2020) 4793, doi: 10.3390/en13184793.
[6] D. Pokorna, J. Maca, J. Zabranska, Combination of hydrogen sulphide removal from biogas and nitrogen removal from wastewater, J. Residuals Sci. Technol., 10 (2013) 41–46.
[7] P. Jenicek, C.A. Celis, L. Krayzelova, N. Anferova, D. Pokorna, Improving products of anaerobic sludge digestion by microaeration, Water Sci. Technol., 69 (2014) 803–809.
[8] H. Kalantari, M. Nosrati, S.A. Shojaosadati, M. Shavandi, Investigation of transient forms of sulfur during biological treatment of spent caustic, Environ. Technol., 39 (2018) 1597–1606.
[9] B. Huber, B. Herzog, J.E. Drewes, K. Koch, E. Müller, Characterization of sulfur oxidizing bacteria related to biogenic sulfuric acid corrosion in sludge digesters, BMC Microbiol., 16 (2016) 1–11.
[10] D. Pokorna, J. Zabranska, Sulfur-oxidizing bacteria in environmental technology, Biotechnol. Adv., 33 (2015) 1246–1259.
[11] W.J. Chung, J.J. Griebel, E.T. Kim, H. Yoon, A.G. Simmonds, H.J. Ji, P.T. Dirlam, R.S. Glass, J.J. Wie, N.A. Nguyen, B.W. Guralnick, J. Park, Á. Somogyi, P. Theato, M.E. Mackay, Y.E. Sung, K. Char, J. Pyun, The use of elemental sulfur as an alternative feedstock for polymeric materials, Nat. Chem., 5 (2013) 518–524.
[12] J. Lim, J. Pyun, K. Char, Recent approaches for the direct use of elemental sulfur in the synthesis and processing of advanced materials, Angew. Chem. Int. Ed., 54 (2015) 3249–3258.
[13] R. de Bello Solcia Guerrero, C.E. Diniz dos Santos, L. Américo Soares, M. Zaiat, Comparison between two different fixed-bed reactor configurations for nitrogen removal coupled to biogas biodesulfurization, Biochem. Eng. J., 162 (2020) 107716, doi: 10.1016/J.BEJ.2020.107716.
[14] D. Andreides, Z. Varga, D. Pokorna, J. Zabranska, Performance evaluation of sulfide-based autotrophic denitrification for petrochemical industry wastewater, J. Water Process Eng., 40 (2021) 101834, doi: 10.1016/j.jwpe.2020.101834.
[15] S. Lin, H.R. Mackey, T. Hao, G. Guo, M.C.M. van Loosdrecht, G. Chen, Biological sulfur oxidation in wastewater treatment: a review of emerging opportunities, Water Res., 143 (2018) 399–415.
[16] L. Zhang, Y.Y. Qiu, Y. Zhou, G.H. Chen, M.C.M. van Loosdrecht, F. Jiang, Elemental sulfur as electron donor and/or acceptor: mechanisms, applications and perspectives for biological water and wastewater treatment, Water Res., 202 (2021) 117373, doi: 10.1016/J.WATRES.2021.117373.
[17] A.S. Jatoi, Z. Hashmi, A. Anjum, Z.A. Bhatti, S.H. Siyal, S. Mazari, F. Akhter, N.M. Mubarak, A. Iqbal, Overview of bioelectrochemical approaches for sulfur reduction: current and future perspectives, Biomass Convers. Biorefin., (2021), doi: 10.1007/S13399-021-02026-3.
[18] Y.X. Cui, B.K. Biswal, M.C.M. van Loosdrecht, G.H. Chen, D. Wu, Long term performance and dynamics of microbial biofilm communities performing sulfur-oxidizing autotrophic denitrification in a moving-bed biofilm reactor, Water Res., 166 (2019) 115038, doi: 10.1016/j.watres.2019.115038.

[19] A. Rajta, R. Bhatia, H. Setia, P. Pathania, Role of heterotrophic aerobic denitrifying bacteria in nitrate removal from wastewater, J. Appl. Microbiol., 128 (2020) 1261–1278.
[20] J. Cai, P. Zheng, Q. Mahmood, Effect of sulfide to nitrate ratios on the simultaneous anaerobic sulfide and nitrate removal, Bioresour. Technol., 99 (2008) 5520–5527.
[21] S. An, K. Tang, M. Nemati, Simultaneous biodesulphurization and denitrification using an oil reservoir microbial culture: effects of sulphide loading rate and sulphide to nitrate loading ratio, Water Res., 44 (2010) 1531–1541.
[22] P. Dolejs, L. Paclík, J. Maca, D. Pokorna, J. Zabranska, J. Bartacek, Effect of S/N ratio on sulfide removal by autotrophic denitrification, Appl. Microbiol. Biotechnol., 99 (2015) 2383–2392.
[23] J. Xu, Y. Fan, Z. Li, Effect of pH on elemental sulfur conversion and microbial communities by autotrophic simultaneous desulfurization and denitrification, Environ. Technol. (United Kingdom), 37 (2016) 3014–3023.
[24] Standard Methods for the Examination Water and Wastewater, Am. Public Heal. Assoc. Am. Water Assoc. Water Environ. Fed., 2017. Available at: https://doi.org/10.2105/SMWW.2882.216
[25] Hach Company/Hach Lange GmbH, Method 8051 Powder Pillows, 2018, pp. 1–6.
[26] HACH, Sulfide Method 8131, 2018, pp. 1–6.
[27] B. Sörbo, A colorimetric method for the determination of thiosulfate, Biochim. Biophys. Acta, 23 (1957) 412–416.
[28] X. Xu, C. Chen, D.J. Lee, A. Wang, W. Guo, X. Zhou, H. Guo, Y. Yuan, N. Ren, J.S. Chang, Sulfate-reduction, sulfide-oxidation and elemental sulfur bioreduction process: modeling and experimental validation, Bioresour. Technol., 147 (2013) 202–211.
[29] G. Bitton, Wastewater Microbiology, Third Edition, Wiley Series in Ecological and Applied Microbiology, 2005.
[30] J. Cai, P. Zheng, Y. Xing, M. Qaisar, Effect of electricity on microbial community of microbial fuel cell simultaneously treating sulfide and nitrate, J. Power Sources, 281 (2015) 27–33.
[31] B.S. Moraes, T.S.O. Souza, E. Foresti, Effect of sulfide concentration on autotrophic denitrification from nitrate and nitrite in vertical fixed-bed reactors, Process Biochem., 47 (2012) 1395–1401.
[32] A.J. Janssen, S.C. Ma, P. Lens, G. Lettinga, Performance of a sulfide-oxidizing expanded-bed reactor supplied with dissolved oxygen, Biotechnol. Bioeng., 53 (1997) 32–40.
[33] F. Di Capua, F. Pirozzi, P.N.L. Lens, G. Esposito, Electron donors for autotrophic denitrification, Chem. Eng. J., 362 (2019) 922–937.
[34] G. Birol, P. Doruker, B. Kirdar, Z. İlsen Önsan, K. Ülgen, Mathematical description of ethanol fermentation by immobilised *Saccharomyces cerevisiae*, Process Biochem., 33 (1998) 763–771.
[35] M. Muloiwa, S. Nyende-Byakika, M. Dinka, Comparison of unstructured kinetic bacterial growth models, S. Afr. J. Chem. Eng., 33 (2020) 141–150.
[36] J. Cai, P. Zheng, M. Qaisar, J. Zhang, Elemental sulfur recovery of biological sulfide removal process from wastewater: a review, Crit. Rev. Env. Sci. Technol., 47 (2017) 2079–2099.
[37] A.J.H. Janssen, S.C. Ma, P. Lens, G. Lettinga, Performance of a sulfide-oxidizing expanded-bed reactor supplied with dissolved oxygen, Biotechnol. Bioeng., 53 (1997) 32–40.
[38] V. Midha, M.K. Jha, A. Dey, Sulfide oxidation in fluidized bed bioreactor using nylon support material, J. Environ. Sci. (China), 24 (2012) 512–519.