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Optimization of Factors Affecting on Sulfide Oxidation from Synthetic Spent Caustic by Haloalkaliphilic *Thioalkalivibrio versutus*: Application of Response Surface Methodology

***H. Kalantari*[1], *M. Nosrati*[1*], *S. A. Shojaosadati*[1], *M. Shavandi*[2]**

[1] *Biotechnology Group, Faculty of Chemical Engineering, Tarbiat Modares University, P.O. Box 14115-143, Tehran, Iran*
[2] *Environment and Biotechnology Group, Research Institute of Petroleum Industry, P.O. Box 1485733111, Tehran, Iran*

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ABSTRACT

In the present study, the effects of four factors including initial sulfide concentration, agitation speed, amount of inoculum and sodium concentration on removal efficiency (R %) and yield of sulfate production by Thioalkalivibrio versutus from synthetic spent caustic were investigated. For this purpose, experiments are designed by DOE and Response Surface Methodology uses results of experiments to determine the relationship between experimental factors and measured responses. The coefficient of determination (R^2) was calculated as 0.9012 and 0.9544 for removal efficiency (R %) and yield of sulfate production ($Y_{SO4/S}$), respectively. The best local maximum was found to be at initial sulfide concentration 1500 mg/l, agitation speed 180 rpm, inoculum 8 %, Na concentration 1.38 M, removal efficiency 96.99 %, yield of sulfate production 2.65 and desirability of 0.909.

1. Introduction

One of the most problematic wastewater streams is originated in the process to extract hydrogen sulfide, mercaptans and organic acids from hydrocarbon streams. Caustic (NaOH) is utilized in petrochemical plants for the removal of hydrogen sulfide (H_2S) from a variety of natural gas streams. The use of caustic leads to the formation of a waste product referred to as sulfidic spent caustic (SSC). SSC contains a high concentration of hydrogen sulfide, alkalinity resulting from NaOH solutions, and certain non-biodegradable organics, including benzene, toluene, and phenoles [1-3]. These spent

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[*] Corresponding author: mnosrati20@modares.ac.ir

caustic solutions have high pH (pH>12) and high salinity (Na of 5-12 %) [4-6]. Due to the characteristics of SSC, environmental problems are expected. Therefore, severe environmental regulations have been considered regarding the treatment of this wastewater [7-8]. A wide range of physico-chemical processes such as disposal in a deep well, wet air oxidation (WAO), Fenton's oxidation, and incineration with auxiliary fuel, most of which occurred in extreme temperature and pressure, have been developed currently for the treatment of SSCs [9-10]. However, these methods of treatment are quite expensive and produce secondary pollution problems.

Biological treatment of hazardous SSCs happens not only in ambient pressure and temperature, but could also be considered as an inexpensive alternative instead of physicochemical treatments [11]. Furthermore, SSCs biological treatment converts sulfide that is a very detrimental form of sulfur into sulfate or biosulfur, both have a much less unfavorable effect on surroundings. Although biological treatment can be an inexpensive disposal option, many refineries do not have the wastewater treatment capacity to treat the entire amount of spent caustic generated. Conventional biological treatment processes are not generally designed for such an ability to receive large amounts of spent caustic streams as these conventional biological processes could be easily damaged by extreme increases in pH values, increasing salt concentrations, and the accumulation of toxic compounds [12-13].

The high pH value and high sodium concentrations that are usual to our new biological process for sulfide removal from SSC, requires sulfur oxidizing bacteria (SOB) that can survive at these extreme conditions. Microorganisms that live and grow under these conditions are generally classified as halo-alkaliphiles, indicating their predominance on high pH and high salt environments [14]. Members of the genus *Thioalkalivibrio* are aerobic chemo-lithoautotrophic sulfide oxidizing bacteria that are the most widely distributed including several described species [15-16]. Different *Thioalkalivibrio* species are able to oxidize a wide range of sulfur compounds including sulfide, $S_2O_3^{2-}$, S^0, SO_3^{2}, tetrathionate ($S_4O_6^{2-}$) and thiocyanate (SCN^-) [14, 17]. *Thioalkalivibrio* species have the accessibility of several enzymes for the oxidation of sulfide to S^0 and SO_4^{2-} [18-21].

Response surface methodology (RSM) is a set of mathematical techniques that describe the relation between several independent variables and one or more responses. This procedure evaluates interactive effects of independent variables. RSM is a more effective technique for optimization of process. The design experiment and optimization by means of RSM approach could be divided into six stages: (1) selection of independent variables and possible responses, (2) selection of experimental design strategy, (3) execution of experiments and obtaining results, (4) fitting the model equation to experimental data, (5) obtaining response graphs and verification of the model (ANOVA), and (6) determination of optimal conditions [22].

In this study, the effects of four factors on the removal of sulfide and yield of sulfate production (as the desired product) were investigated and optimal conditions were determined using numerical optimization methodology.

2. Materials and methods

2.1. Microorganism and growth conditions

Thioakalivibrio versutus DSM 13738 was used throughout this study. It is an obligate haloalkaliphilic, obligate chemolithoautotrophic, mesophilic, gram-negative bacterium able to use sulfide, polysulfide, thiosulfate, elemental sulfur, and tetrathionate as energy source and oxygen as electron acceptor. For routine batch cultivation a mineral medium buffered with a sodium carbonate/sodium bicarbonate mixture containing 0.6 M total Na^+ at pH 10.1 (after sterilization) was used [14]. The culture medium included 20 g/1 Na_2CO_3; 10 g/1 $NaHCO_3$; 5 g /1 NaCl; 1 g/l K_2HPO_4; 0.5 g/1 KNO_3; 0.1g/1 $MgCl_2.6H_2O$, 2 ml/1 trace elements solution containing (mg/l): EDTA, 5; $FeSO_4.7H_2O$, 2; $ZnSO_4.7H_2O$, 100; $MnCl_2.4H_2O$, 30; $CoCl_2.6H_2O$, 200; $NiCl_2.6H_2O$, 20; $Na_2MoO_4.2H_2O$, 30; $CuCl_2.2H_2O$, 10; H_3BO_3, 300; and 40 mM thiosulfate as energy source. $MgCl_2$, trace elements, and thiosulfate were added after sterilization from concentrated stock solutions. Culture was incubated on a rotary shaker at 170 rpm and 30 ^oC. This culture was used as inoculum for experiments. For synthetic wastewater preparation, instead of thiosulfate, $Na_2S.9H_2O$ was used as a sulfur source.

2.2. Experimental design and optimization

In this study, the optimal conditions for sulfide oxidation by *Thioakalivibrio versutus* were determined by means of central composite design (CCD) under response surface methodology (RSM). CCD is a second order model that is the most commonly used method under RSM design. First, experiment is designed by DOE and then, RSM uses results of experiments to determine the relationship between experimental factors and measured responses.

Optimization studies were carried out by studying the effect of four factors including, initial sulfide concentration, amount of inoculum, agitation speed and sodium concentration. All of the factors used in this study were coded according to Eq. (1):

$$\chi = \frac{\xi - \eta}{d} \tag{1}$$

Where ξ and η are defined by the following equations:

$$\xi = \frac{X_{Hi} + X_{Low}}{2} \tag{2}$$

$$d = \frac{X_{Hi} - X_{Low}}{2} \tag{3}$$

Where X_{Hi} and X_{Low} are the high and low values of factors, respectively, and χ is the dimensionless coded value of the factor.

The full second order used to explain behavior of the system is given by Eq. (4):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j>i}^{k} \beta_{ij} x_i x_j \tag{4}$$

Where y is the predicted response, x_i is the input variable that affects the response y and is named "*main effect*". x_i^2 is the square effect, $x_i x_j$ is the interaction effect, β_0 is the intercept term, β_i is the linear effect, β_{ii} is the square effect and β_{ij} is the interaction effect.

Design Expert 7.0 software was used for the regression and graphical analysis of the data. According to the CCD method, factors of experiment were coded at five levels: -α, -1, 0, 1, +α. From Plackett-Burman tests (data not shown) it was found that sulfide concentration (C_s), biomass inoculation (%), sodium concentration (Na), and agitation speed that serves as the rate of oxygenation and dissolved oxygen had significant effects on the sulfide oxidation by *Thioakalivibrio*. The factors in coded units are given in Table 1. Synthetic spent caustic effluent was prepared in the lab and at different concentrations of sulfide by using sodium sulfide.

Table 1

Factors and levels used in the CCD design.

	-2	-1	0	1	2
Sulfide concentration (mg/l) –X1	200	525	850	1175	1500
Agitation speed (rpm)- X2	20	60	200	140	180
Biomass inoculation (%)- X3	2 %	4%	6 %	8 %	10 %
Sodium concentration (M)- X4	0.6	1.45	2.3	3.15	4

DOE gave 30 experiments for four factorials design (2^4) on CCD method with 6 central points. These 30 experiments contained 16 corner points (fractional factorial points; +1,-1), 6 replicates at center point (0) and 8 star (or axial) points were employed for the quadratic model. Table 2 shows the experiments and levels of factors. Two responses are considered, including removal efficiency (R %) and yield of sulfate production ($Y_{SO4/S}$) defined as follows:

$$R\ \% = \frac{(Cs_{in} - Cs_f)}{Cs_{in}} \times 100 \qquad (5)$$

$$Y_{SO4/S} = -\frac{\Delta SO_4}{\Delta S} \qquad (6)$$

Analysis of data was performed by RSM and a quadratic model was fitted in the data. The significance of each coefficient was distinguished by p-values and F-values. The smaller the p-value and the larger the F-value, the more significant the corresponding coefficient is.

2.3. Analytical methods

Total sulfide was measured by the iodometric method following Standard Method for Examination of Water and Wastewater [23]. Sulfate was analyzed by using a turbidimetric method [23].

Table 2
Full factorial central composite design matrix of four factors.

No.	X1	X2	X3	X4
1	-1	-1	-1	-1
2	-1	-1	1	1
3	-1	-1	1	-1
4	-1	1	-1	1
5	1	-1	-1	-1
6	-1	1	1	1
7	1	1	-1	-1
8	1	1	1	-1
9	1	1	-1	1
10	1	-1	1	-1
11	-1	1	-1	-1
12	-1	1	1	-1
13	-1	-1	-1	1
14	1	-1	1	1
15	1	-1	-1	1
16	1	1	1	1
17	2	0	0	0
18	-2	0	0	0
19	0	2	0	0
20	0	-2	0	0
21	0	0	2	0
22	0	0	-2	0
23	0	0	0	2
24	0	0	0	-2
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0

3. Results and discussion

3.1. Fitting the model

Experiments were performed according to the CCD experimental design given in Table 2. Table 3 shows the observed value of sulfide removal efficiency and yield of sulfate production as the responses and predicted values from fitted model. The evaluated models were represented by the following equations:

For R %:

$$Y=21.48+11.79x_1+7.54x_2+3.93x_3-4.10x_4+3x_1^2-1.48x_2^2-1.13x_3^2+2.98x_4^2+0.697x_1x_2-2.18x_1x_3+1.41x_1x_4-0.298x_2x_3-0.37x_2x_4-1.24x_3x_4 \tag{7}$$

For $Y_{SO4/S}$:

$$Y=1.597+0.16x_1+0.21x_2+0.12x_3-0.065x_4+3.92x_1^2-0.047x_2^2-6.83x_3^2-0.049x_4^2+0.04x_1x_2+0.044x_1x_3+0.027x_1x_4+0.0199x_2x_3-0.065x_2x_4+0.015x_3x_4 \tag{8}$$

Model summary and analysis of variance for the quadratic model for R % and $Y_{SO4/S}$ are represented in Table 4. The fit of the model is checked by the determination coefficient (R^2). The R^2 value is always between 0 and 1. The closer R^2 is to 1.0, the stronger the model and the better it predicts the response. The coefficient of determination (R^2) was calculated as 0.9012 for removal efficiency, indicating that the statistical model can explain 90.1 % of variability in the response. In fact, the value of R^2 indicates that only 9.9 % of the total variations are not explained by the model. The adjusted R^2 value corrects the R^2 value for the sample size and for the number of terms in the model. The adjusted R^2 for R % is 0.81 that is high for supporting the significance of the model.

The statistical significance of the quadratic model was evaluated by the analysis of variance (ANOVA) and checking F-value and p-value. This analysis was carried out to decide the significant and insignificant factors and effects. The Model F-value of 9.85 (for R

%) implies the model is significant. There is only a 0.01 % chance that a model F-value this large could occur due to noise. P-values less than 0.05 indicate that the model is significant. In this case, p-value is <0.0001 that shows significance of the model for R %. The C.V. % (Coefficient of Variation), calculated by dividing the standard deviation by the mean and multiplying by 100, was 14.26, which is a relatively low value and indicates good precision and reliability of the experiments. The "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable and the ratio of 12.83 indicates an adequate signal. This model can be used to navigate the design space.

Table 3

Uncoded values of the independent variables and experimental and predicted values of the response.

					Experimental		Predicted	
Std No.	Cs (X1)	Rpm (X2)	ino % (X3)	Na (X4)	R %	$Y_{SO4/S}$	R %	$Y_{SO4/S}$
1	525	60	4	1.45	23.30	1.29	26.79	1.16
2	1175	60	4	1.45	56.75	1.26	50.54	1.26
3	525	140	4	1.45	36.92	1.60	41.77	1.58
4	1175	140	4	1.45	66.06	1.86	68.30	1.85
5	525	60	8	1.45	41.40	1.30	42.10	1.24
6	1175	60	8	1.45	60.8	1.50	57.11	1.50
7	525	140	8	1.45	51.15	1.67	55.89	1.73
8	1175	140	8	1.45	74.82	2.30	73.69	2.18
9	525	60	4	3.15	20.45	1.04	19.00	1.08
10	1175	60	4	3.15	48.69	1.38	48.39	1.28
11	525	140	4	3.15	24.36	1.28	32.49	1.24
12	1175	140	4	3.15	67.96	1.63	64.67	1.61
13	525	60	8	3.15	27.17	1.24	29.36	1.21
14	1175	60	8	3.15	57.45	1.65	50.02	1.58
15	525	140	8	3.15	38.04	1.53	41.66	1.45
16	1175	140	8	3.15	64.16	1.912	65.10	1.998
17	200	100	6	2.3	45.29	1.236	32.97	1.291
18	1500	100	6	2.3	69.88	1.87	80.16	1.93
19	850	20	6	2.3	16.26	0.86	23.59	0.99
20	850	180	6	2.3	63.05	1.836	53.65	1.821
21	850	100	2	2.3	35.1	1.26	32.19	1.34
22	850	100	10	2.3	47.06	1.76	47.94	1.799
23	850	100	6	0.6	66.33	1.45	64.66	1.53
24	850	100	6	4	48.65	1.23	48.28	1.27
25	850	100	6	2.3	44.43	1.58	44.57	1.596
26	850	100	6	2.3	44.77	1.612	44.57	1.596
27	850	100	6	2.3	44.29	1.61	44.57	1.596
28	850	100	6	2.3	44.91	1.593	44.57	1.596
29	850	100	6	2.3	44.66	1.597	44.57	1.596
30	850	100	6	2.3	44.5	1.589	44.57	1.596

Similar to R %, for yield of sulfate production ($Y_{SO4/S}$), values of F-value, R^2, adjusted R^2, Adequate Precision and C.V. % are 22.42, 0.954, 0.912, 19.43, and 5.73 respectively. These values (for $Y_{SO4/S}$) imply that the model is significant and indicates good precision and reliability of the experiments.

In Table 5 model coefficients estimated by multiple linear regressions are shown. Values of "P-value" less than 0.05 indicate model terms are significant.

In this case, for sulfide removal efficiency (R %), X_1, X_2, X_3, X_4, X_1^2, X_4^2 are significant model terms. For $Y_{SO4/S}$, terms of X_1, X_2, X_3, X_4, $X_2.X_4$, X_2^2, X_4^2 are significant model terms.Values greater than 0.1000 indicate that the model terms are not significant.

Table 4

Model summary and analysis of variance for the quadratic model for R % and $\boldsymbol{Y_{SO4/S}}$.

	F-value	P-value	Important terms	R^2	Adj. R^2	Adequate precision	C.V. %
R %	9.85	<0.0001	X_1, X_2, X_3, X_4, X_1^2, X_4^2	0.901	0.810	12.83	14.26
$Y_{SO4/S}$	22.42	<0.0001	X_1, X_2, X_3, X_4, $X_2.X_4$, X_2^2, X_4^2	0.954	0.912	19.43	5.73

Table 5

Regression analysis of the effects for R % and $\boldsymbol{Y_{SO4/S}}$.

		R %			$Y_{SO4/S}$		
Source	df	Sum of squares	F-value	P-value	Sum of squares	F-value	P-value
Model	14	6268.8	9.85	< 0.0001	2.37	22.42	< 0.0001
X_1	1	3338.9	73.42	< 0.0001	0.61	80.18	< 0.0001
X_2	1	1365.7	30.03	< 0.0001	1.08	142.24	< 0.0001
X_3	1	371.5	8.17	0.0120	0.32	42.29	< 0.0001
X_4	1	402.5	8.85	0.0094	0.10	13.55	0.0022
$X_1. X_2$	1	7.8	0.17	0.6849	0.03	4.074	0.0618
$X_1. X_3$	1	76.4	1.68	0.2146	0.03	4.01	0.0638
$X_1. X_4$	1	31.9	0.70	0.4157	0.01	1.50	0.2395
$X_2. X_3$	1	1.4	0.03	0.8623	0.01	0.84	0.3750
$X_2. X_4$	1	2.2	0.049	0.8287	0.07	8.81	0.0096
$X_3. X_4$	1	24.4	0.54	0.4747	0.001	0.48	0.4972
X_1^2	1	246.9	5.43	0.0342	0.0004	0.055	0.8167
X_2^2	1	60.3	1.33	0.2678	0.06	8.13	0.0121
X_3^2	1	34.8	0.76	0.3957	0.0012	0.17	0.6864
X_4^2	1	243.0	5.34	0.0354	0.07	8.83	0.0095
Lack of fit	10	681.9	1294.75	< 0.0001	0.11	73.82	< 0.0001

3.2. Effect of factors on desired responses

The 3-D response surfaces and contour plots demonstrated the effects of factors on sulfide removal efficiency and yield of sulfate production at six combinations. As it can be seen from Figs. 1 and 2, by increasing the amount of sulfide concentration (Cs) and rpm, both R % and $Y_{SO4/S}$ increase. Increase in the agitation speed causes an increase in the amount of dissolved oxygen available to the microorganisms. This increase in dissolved oxygen evinces bio-oxidation process (providing change in oxidation number from -2 to +6) and both removal efficiency and yield of sulfate production rise. In relation to the combinations of "Cs-ino %" and "ino %-rpm", the same trends (similar to "Cs-rpm" combination) can be seen. In "Cs-Na" combination, by increasing sulfide concentration and decreasing Na concentration, R % and $Y_{SO4/S}$ increase. However, at one Na concentration, between -1 and 0 (coded value), maximum of $Y_{SO4/S}$ and minimum of R % occur. A similar trend is also seen for "Na-rpm" and "Na-ino %" combinations.

As mentioned previously, *Thioalkalivibrio versutus* can grow at high concentration of sodium. Therefore, sodium can be effective on oxidation rate of sulfide to sulfate. Growth in cultures at 4 M NaCl was much slower than at 2 M [24]. Banciu [25] has illustrated the growth yield of *Thioalkalivibrio versutus* strain ALJ 15 decreased with increasing sodium concentration and batch experiments indicated an optimum growth between 0.6 and 1 M Na^+, while at higher sodium concentrations the yield was lower.

3.3. Optimization with desirability function

Response surface methodology approach has been successfully applied for identification of significant factors, modelling and optimization of various chemical and biochemical processes. The Analysis of Variance (ANOVA) provides complete information on model accuracy and significance. In fact, the main purpose of ANOVA is the identification of important factors and the determination which is the most significant; also, whether the experiment results are meaningful.

When multiple responses are treated, the simultaneous optimization of two or more values might be required. The use of desirability function allows determining the most suitable conditions for two or more system responses [26]. Typically, the desirability function is defined with Eq. (9):

$$D = \left(\prod_{i=1}^{m} d_i\right)^{1/m} \tag{9}$$

where d_i stands for desirability of i-th response. The response desirability value ranges from $d_i=0$ for inacceptable value to $d_i=1$ for the single response maximum. To optimize the desirability function, numerical methods are usually applied [22]. In numerical optimization, the desired goal for each factor and response is chosen from the menu. The possible goals are: maximize, minimize, target, within range, none and set to an exact value. A minimum and maximum level must be provided for each parameter included [26]. A weight can be assigned to each goal to adjust the shape of its particular desirability function.

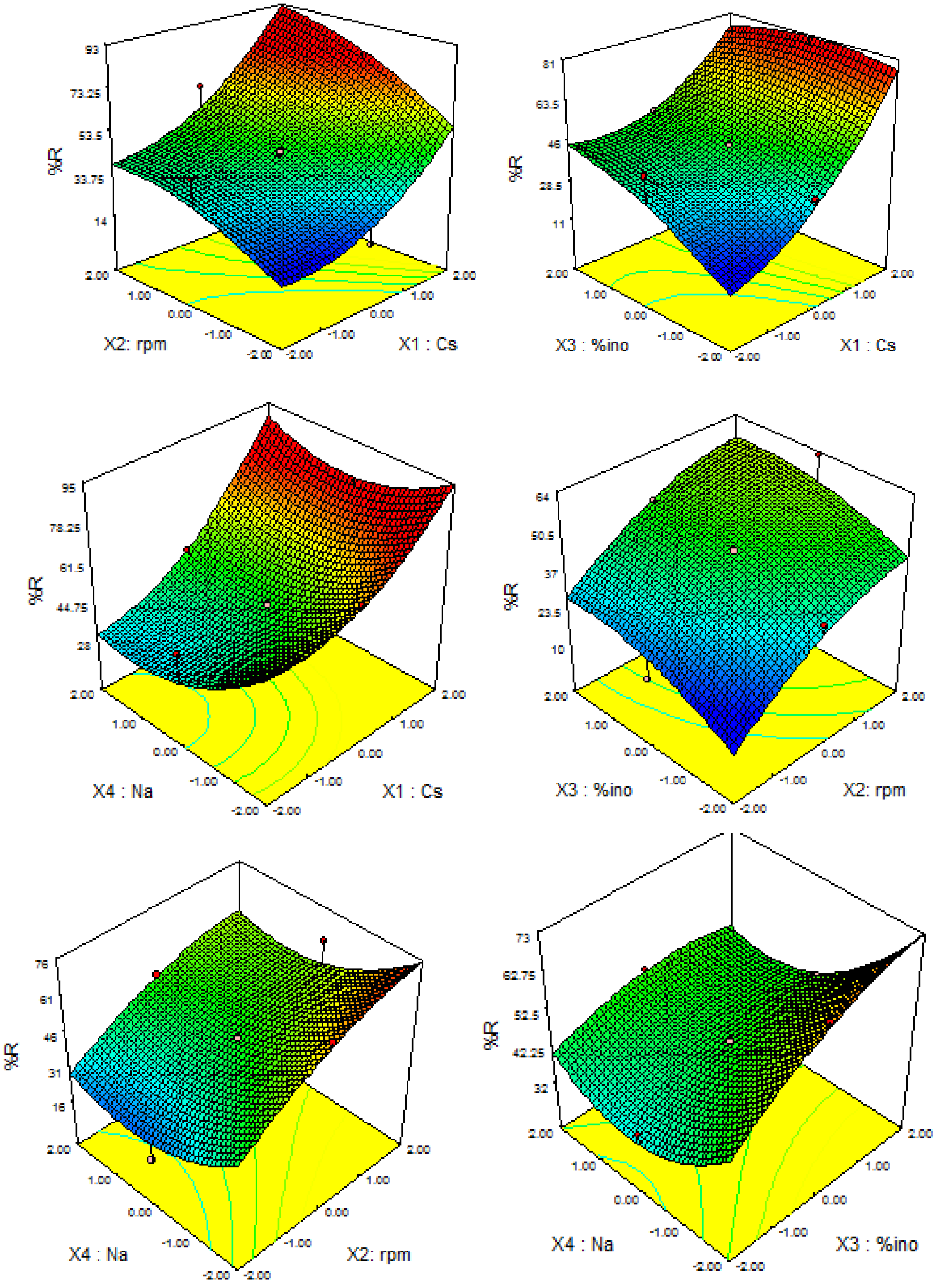

Figure 1. 3D response surface plot for the interaction effects for sulfide removal efficiency (R %).

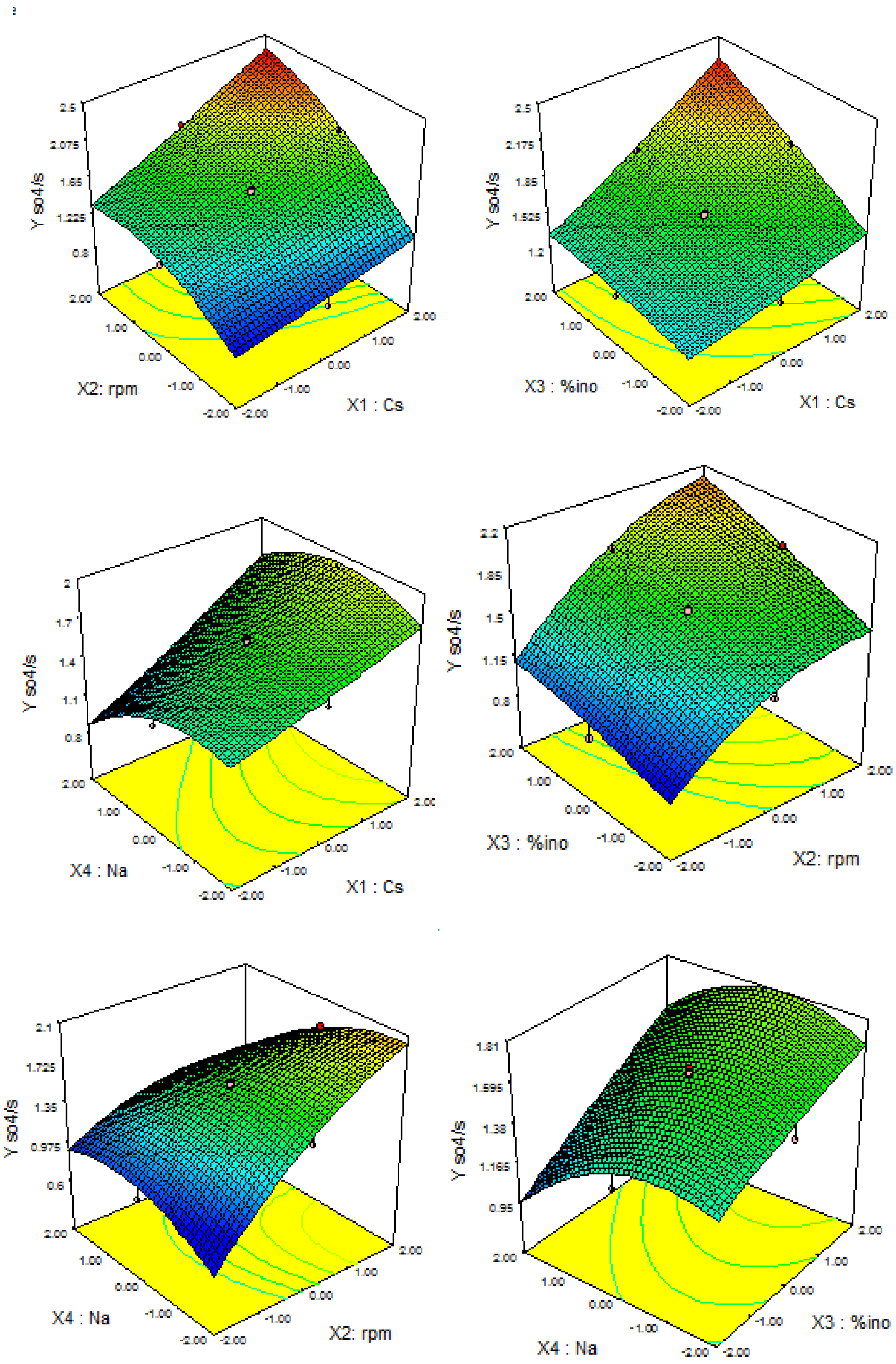

Figure 2. 3D response surface plot for the interaction effects for yield of sulfate production ($Y_{SO4/S}$).

A multiple response method was used for optimization of any combination of six goals, namely the initial sulfide concentration, agitation speed, amount of inoculum, sodium concentration, removal efficiency and yield of sulfate production. The numerical optimization found a point that maximizes the desirability function. The goals set for factors were: in range for initial sulfide concentration (850-1500 mg/l), agitation speed (60-180 rpm), amount of inoculum (4 %-8 %), sodium concentration (0.6-3.15 M) and maximization for removal efficiency and yield of sulfate production. The importance of each goal was changed in relation to the other goals. After optimization, 29 optimum points for this experiment were provided via numerical optimization. Among them, 5 first points (Table 6), with the highest desirability, were studied. The best local maximum was found to be at initial sulfide concentration 1500 mg/l, agitation speed 180 rpm, inoculum 8 %, Na concentration 1.38 M, removal efficiency 96.99 %, yield of sulfate production 2.65 and desirability of 0.909 (second point in Table 6). In practice, removal efficiency and yield of sulfate production were obtained 90.3 % and 2.46, respectively. Figure 3 represents a ramp desirability that was generated from the final optimum point mentioned above.

Table 6

Five optimum points with highest desirability.

Number	Cs	rpm	ino %	Na	R %	$Y_{SO4/S}$	Desirability
1	2.00	2.00	1.00	-1.33	99.5251	2.65543	0.910
2	2.00	2.00	1.00	-1.08	96.9925	2.65372	0.909
3	2.00	2.00	1.00	-1.52	101.843	2.65252	0.909
4	2.00	1.99	1.00	-1.13	97.4379	2.65207	0.909
5	2.00	2.00	1.00	-0.99	96.0655	2.65103	0.909

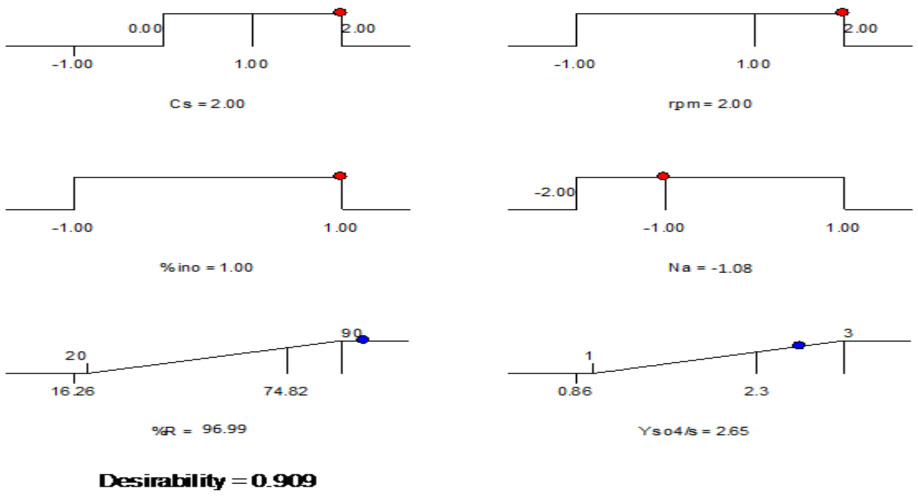

Figure 3. Desirability ramp for numerical optimization of six goals, namely the initial sulfide concentration, agitation speed, amount of inoculum, sodium concentration, R % and $Y_{SO4/S}$.

4. Conclusions

This study shows that *Thioalkalivibrio versutus* as a haloalkaliphilic bacteria can oxidize sulfide to sulfate (even at high concentration of sulfide) at alkalinity conditions in synthetic spent caustic wastewater. Response surface methodology was used in the modelling and optimization of treatment of sulfide-contained wastewater. The results of this methodology represented that four factors including sulfide concentration, agitation speed, amount of inoculum and sodium concentration have important effects on two responses: removal efficiency (R %) and yield of sulfate production. The numerical optimization found a point that maximizes the desirability function. This point applied to maximize removal efficiency (R %) and yield of sulfate production is 1500 mg/l initial sulfide concentration, 180 rpm, 8 % inoculation, 1.38 M Na concentration resulting in desirability of 0.909. According to these observations and results *Thioalkalivibrio versutus* is a suitable bacterium for oxidation of sulfide in spent caustic wastewater.

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