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Sulfur Production by Hydrogen Sulfide Biological Removal from Pollutants

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ABSTRACT

Hydrogen sulfide (H_2S) is one of the polluting gases that enter the atmosphere during the natural gas processing of coal and furnace oil consumption. One of the best ways to remove H_2S is to absorb H_2s in the liquid phase and remove it biologically by sulfur bacteria in the liquid phase. This process considers the transfer of H_2S and O_2 between liquid and gas phases, biological oxidation of H_2s to sulfate and elemental sulfur, and chemical oxidation to thiosulfate in the liquid phase. Due to the presence of sulfur bacteria in natural sulfur sources, the sulfur sources of sewage of Shahid Tondgooyan Oil Refining Co. in Tehran, Iran, and Mahallat Hot Spring in Iran, which contain sulfur compounds, were sampled in this study and were transferred to the laboratory for examination. Thiobacillusthioparus – one of the significant bacteria consuming sulfur compounds – was evaluated as a control sample. Further, the performance of bacteria in different culture conditions (carbon source and aeration conditions) was evaluated, and suitable conditions for their growth were determined. Sodium sulfide was used to create the sulfide medium. Next, sulfide consumption was evaluated by bacteria, and appropriate bacteria were selected. Finally, the production of sulfur during the process was evaluated using the ANOVA data analysis method. Then, the optimal points for sulfur production were predicted using the Response Surface Methodology (RSM).

Highlights

- Due to the presence of sulfur bacteria in natural sulfur sources, the sulfur sources of sewage
- Thiobacillus thioparus – one of the significant bacteria consuming sulfur compounds – was evaluated as a control sample.
- The performance of bacteria in different culture conditions (carbon source and aeration conditions) was evaluated, and suitable.
- Conditions for their growth were determined.
- Sodium sulfide was used to create the sulfide medium.
- The production of sulfur during the process was evaluated using the ANOVA data analysis method.
- The optimal points for sulfur production were predicted using the Response Surface Methodology (RSM).

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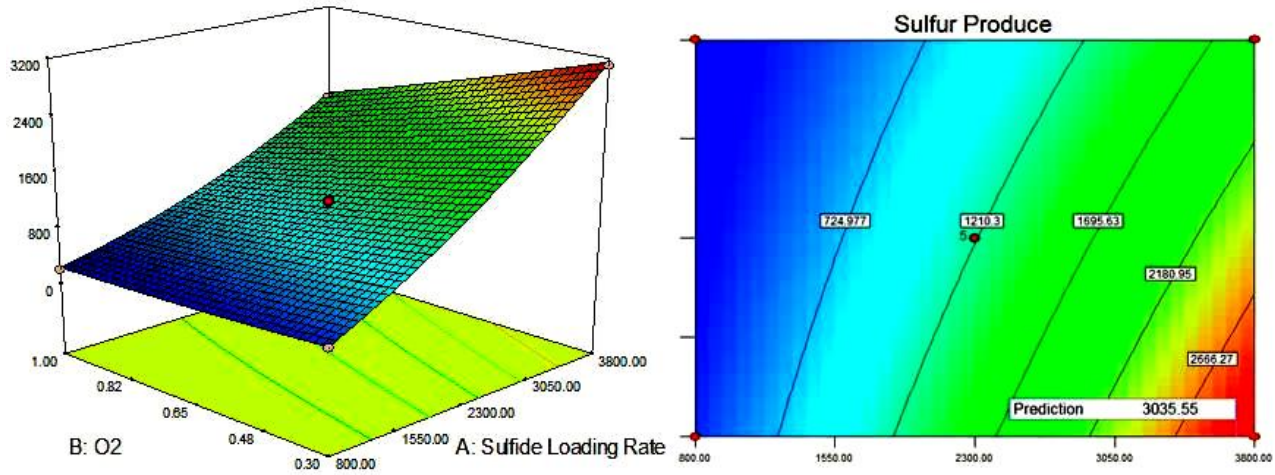
GRAPHICAL ABSTRACT



Introduction

With the advancement of human civilization and the development of technology and the increasing population, the world is currently facing a problem called air pollution. Because environmental protection, both human and natural, against pollutants requires the identification and recognition of pollutants and sources of production, environmental research groups have focused their training and research on these issues. These pollutants, one of the most important of which is hydrogen sulfide, enter the atmosphere during the process of refinement of fossil fuels, wastewater treatment processes, and the use of fossil fuels in industry. Hydrogen sulfide is a colorless, toxic, and flammable gas with a pungent odor of rotten egg that is naturally present in crude oil, natural gas reservoirs, volcanic gases, and hot springs. Short-term contact with this gas irritates the throat, nose, eyes, and lungs. This gas also hurts the environment, so it is crucial to remove hydrogen sulfide from gaseous pollutants and effluents[1, 2]. The most important sources of bacterial hydrogen sulfide reducing sulfate (SBR) are the thermal decomposition of sulfuric organic matter in crude oil, thermochemical regeneration of sulfate (TSR). In general, the sources of hydrogen sulfide emissions in industries are crude oil refineries, paper industry, sewage facilities, and industrial activities, such as food processing [3, 4].

Today, various methods, such as physical and chemical methods are used to remove polluting gases from the environment. However, disadvantages such as high energy consumption and high operating costs have led researchers to look for ways to compensate for these disadvantages [5, 6]. Therefore, methods that have environmental and economic benefits and, at the same time, have high efficiency are always developing and progressing. One of these developing methods that have received much attention today is biological methods. In 1980, research at Wageningen University in the Netherlands examined the biological cycle of sulfur to reduce the environmental problems caused by the release of hydrogen sulfide. This research led to the development of new processes for the refinement of sour gases such as biogas and gases from waste burial[7, 8]. Biological processes not only show a high rate of H_2S removal but also produce elemental sulfur. It will also recover mineral resources from waste.

Therefore, elemental sulfur formation is preferred in biological treatment plants because sulfur granules formed by microorganisms are non-toxic and non-edible and have a high sulfur

content, which can be widely used in the manufacture of raw materials. It is also used in bio-leaching processes, agricultural products, and as an adsorbent for the removal of heavy metals from waste [9-11]. Also, in 1990, environmentally friendly and cost-effective desulfurization processes based on biotechnological knowledge were developed to substitute conventional chemical and physical methods such as the Amine Clous process[12]. Another biological process used is the SHELL-PAQUES Process, in which sulfide is rapidly absorbed from a gas into an alkaline liquid in an absorption tower. The alkaline liquid is automatically collected at the end of the tower and directly enters the second stage of the process (bioreactor). Inside the bioreactor, the adsorbed sulfide is converted to sulfur and sulfate. Biologically produced sulfur particles are used after dewatering (sulfur sludge). The biological process of removing sulfur compounds is based on the biological cycle of sulfur production[13, 14].

The potential and ability of the hydrogen sulfide biological removal process are enhanced by the presence of microorganisms, including a wide range of bacteria, which are classified according to different criteria, and they are called sulfur-oxidizing bacteria (SOB). Categorical criteria include the source of carbon used, how oxygen is consumed, the source of hydrogen consumed, as well as the color of bacteria, the shape, and temperature of the growing medium[15, 16]. Besides the fact that SOB plays an essential role in H2S removal, adjusting the pH to about 8 can prevent the growth of sulfate-reducing bacteria, which plays an essential role in H2S production[17]. One of the most critical bacteria used for sulfur oxidation is thiobacillus. Thiobacillus is acid-friendly bacteria that oxidize iron or sulfur. This type of bacterium is the most crucial sulfur oxidizer in the soil, and their oxidizing ability has made it possible to use them in various industries to remove sulfide in various processes. All thiobacillus bacteria can oxidize sulfide to sulfate, producing elemental sulfur as an intermediate[18, 19]. The most important factors influencing biological treatment processes are the aeration rate, the number of nutrients required by the bacterial culture medium, temperature, and pH. Among these, the aeration rate is one of the vital factors because oxygen plays the primary role as the electron receptor for the metabolism of sulfur-oxidizing bacteria (SOB)[20].

Since many countries have to import large amounts of sulfur to meet industrial needs, which in turn increases environmental pollution from sulfur, researchers are not only concerned about removing sulfide from pollutants in waste to protect the environment but are also concerned about recovering the sulfur resources in the wastewater. Many researchers have studied the removal of sulfide in waste. Nevertheless, given the little research that has been done on the process of biological removal of sulfide and elemental sulfur recovery, in this study, the performance of different bacteria for sulfide removal during various processes is measured to achieve bacteria with high biological oxidation ability of sulfide, after cultivation and proliferation of used bacteria. After selecting the appropriate bacterium, different operating conditions for the selected bacterium are evaluated. Then, the process of removing hydrogen sulfide was performed on a laboratory scale, and its results were analyzed using Design Expert 10.1.1 through Central Compound Design (CDD) method.

Materials and methods

Materials

Standard samples were used to evaluate natural samples. The standard sample used in this study was the Thiobacillusthioparus bacterium. It is the best species used in industrial applications in sulfur environments. This bacterium was present in the microbial collection of Iran Scientific and

Industrial Research Organization and was prepared from this bank. To obtain two other microbial strains from the sulfur sources of Mahallat Hot Spring, Iran, and the effluent of the wastewater treatment plant of Shahid Tondgooyan Oil Refining Company in Tehran was sampled. To test the performance of the species, they should be cultured in a suitable culture medium. For this purpose, a unique culture medium was used for the Thiobacillusthioparus, the values and compounds of which are shown in Table.1, and also for metabolism, reproduction, and better growth of bacteria, the rare elements were used, whose compounds are shown in Table.2.

Methods of analysis

Sorbo method is applied for identifying elemental sulfur used in aquatic environments containing sulfur. This method is designed based on the conversion of sulfur to thiocyanate.

Table .1 Culture medium compounds

Components	Quantity(g/L)
KH_2PO_4	2
K_2HPO_4	2
NH_4Cl	0.4
Na_2CO_3	0.4
$MgCl_2.6H_2O$	0.2
$Na_2S_2O_3.5H_2O$	1

Table.2 Compounds of rare elements

Matter	Quantity(g/L)
Na_2-EDTA	50
$CaCl_2.2H_2O$	34.7
$FeSO_4.7H_2O$	5
$MnCl_2.4H_2O$	5.2
$ZnSO_4.7H_2O$	2.2
$(NH_4)_6Mo_7O_{24}.4H_2O$	5.0
$CaSO_4.5H_2O$	2.0

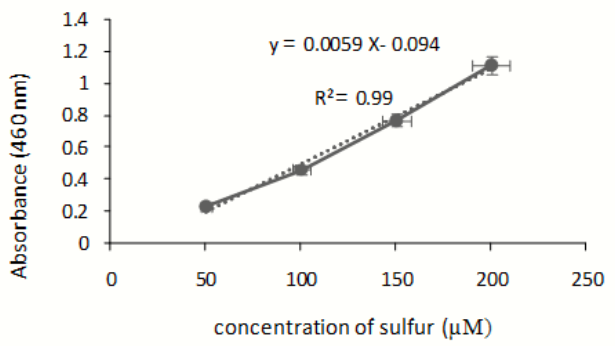


Fig.1. sulfur calibration diagram using by Sorbo method

In the next step, thiocyanate with 3-capacity iron is given in complex acid conditions, which has a maximum absorption at a wavelength of 460 nanometers, that the complex absorption is considered as a measurement tool[21]. **Fig.1** shows the sulfur calibration diagram using the above method.

The methylene blue method was used to measure the amount of sulfide and polysulfide ions in the culture medium containing bacteria. This experiment is based on the deposition of sulfide (or polysulfide atoms) with ZnAc (acetate zinc) and the conversion to 2-Methyl-p-phenylenediamine sulfate to the medium and reaction with iron (III) atom and production of the final methylene blue product in an acidic environment. This method applies to a medium containing sulfide ions with concentrations between 0.1 mg/Lit to 20 mg/Lit[22]. Figure2 shows the calibration of sulfide ions using the above method.

A Chemometrics measurement kit was used to measure thiosulfate. The thiosulfate concentration is expressed as mg/L using the iodometric method and spectrophotometric device. Measurement of sulfate ions was performed by turbidity measurement at a wavelength of 420 nm. In this way, the sulfate ion with barium chloride precipitates as barium sulfate.

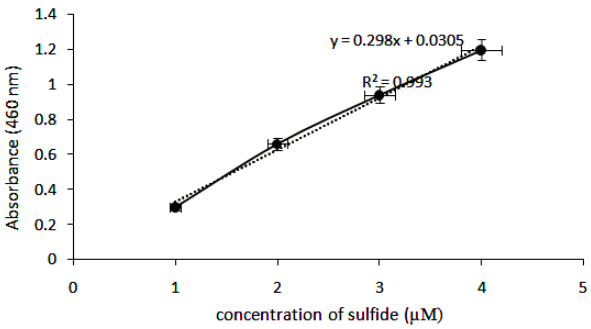


Fig.2. the calibration of sulfide ions using the methylene blue method

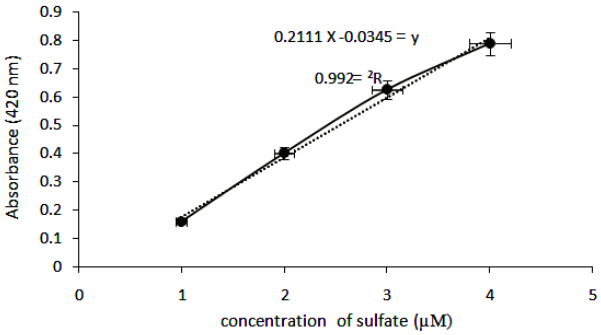


Fig. 3. Calibration diagram of sulfate measurement using by turbidity method

The precipitate is re-suspension with a viscous solution containing glycerol, ethanol, and sodium chloride. Suspension turbidity is measured at 420 nm. The basis of the analysis is based on the production of barium sulfate [23, 24]. Figure 3 shows the calibration diagram of sulfate measurement using the above method.

Bacteria isolation

To detect the presence or absence of sulfur bacteria and evaluate them, after sampling the mentioned sulfur sources, the bacteria must be cultured in the culture medium. Since the activity and ability of the Thiobacillusthioparus bacterium have been proven, it was removed from the experiments. After preparing the culture medium, 90cc of the culture medium was first poured into a 250 cc Erlenmeyer flask (pH = 7-7.2). Then, 10 cc of each bacterial sample (refinery wastewater sample and hot mineral water sample) was poured into the Erlenmeyer flask, and the specimens were placed in a 150 rpm incubator shaker. Since at the time of sampling the desired sources, the temperature of the source is measured with a thermometer, so the temperature of the incubator shaker should be set at that temperature so that the temperature of the sampled medium is provided for bacteria to grow. For this purpose, two incubator shakers were used. The first incubator shaker temperature for hot spring bacteria, which were thermophiles, was 55°C, and the temperature of the second incubator shaker for refinery sewage bacteria, which were mesophiles, was 25 (ambient temperature). To measure the growth of microorganisms daily, the method of measuring the amount of thiosulfate and solution turbidity was used. This procedure was repeated several times for both species. At each inoculation, the turbidity rate was measured using a Spectrophotometer at 600 nm wavelength, and the thiosulfate of each Erlenmeyer was measured using the methylene blue method. The turbidity of the culture medium also indicated an increase in the bacterial population and the production of sulfur, and a decrease in the amount of thiosulfate also indicated that it was consumed by bacteria. This medium continued until it reached a medium rich in one or more species capable of oxidizing thiosulfate. Thiobacillusthioparus were also cultured separately for further testing. The optimum temperature for the growth of this bacteria was also 30° C and pH = 7.1.

Investigating the growth of bacteria in different conditions

Bacterial culture conditions in the culture medium were evaluated in both aerobic and anaerobic conditions. To investigate this, 10 cc of inoculation medium, which had been enriched several times after inoculation, was added to 90 cc of culture medium. For this purpose, two Erlenmeyer flasks were considered. In one Erlenmeyer, the aeration was performed with a flow rate of 200 ml/min, and in the next

Erlenmeyer, no aeration was performed. To measure bacterial growth in aerobic and anaerobic conditions, optical density measurement at 600 nm was used. Then a test was designed for autotrophy or heterotrophy. In this experiment, the carbon in the air carbon dioxide was used once, and organic carbon was once again used as a carbon source for bacteria. The organic carbon used was sodium bicarbonate, which was dissolved in 1000 cc as provided in the culture medium. After checking the suitable conditions for aerobic or anaerobic and the presence of bacteria, air conditions are usually applied to them. For this purpose, two identical Erlenmeyer flasks were used. For one Erlenmeyer flask, an organic carbon source was used, and for the other, without an organic source and carbon dioxide in the air was used. Aeration was done during incubation to examine the effect of this type of carbon.

Investigating the performance of bacteria in alkaline PHs

The performance of the two bacterial species was evaluated in different alkaline pH. In this way, the pH of the culture medium was increased to 1 molar by NaOH, and in each pH, the same conditions were created for both, such as agitation and aeration. In this experiment, two 250 cc Erlenmeyer flasks were used. Each Erlenmeyer flask used 100 cc of culture medium and 10 cc of inoculation medium and used 200 ml/min aeration and 150 rpm agitation. This test was performed for both species in pH 7, 9, and 10.

Investigation of the initial sulfide density in the performance of bacteria

The tolerability of bacteria to the amount of sulfide in the environment is one of the most important factors in choosing the type of bacteria in the laboratory and an industrial scale. Therefore, one of the parameters that should be considered is the ability of bacteria in sulfide removal. Accordingly, the tolerability of bacteria or the amount of sulfide shock in these experiments was investigated[25].

After increasing the bacterial population and examining the suitable conditions for creating a sulfide medium, the amount of thiosulfate gradually decreased. Then, sodium sulfide was added to the culture medium as a sulfide source with a concentration of 250 mg/L to examine the yield of bacteria in exchange for the presence of sulfide as the source of sulfur. To further investigate the effect of the initial sulfide level on bacterial yield, based on desk studies, the amount of sulfide in Erlenmeyer (containing 90cc of culture medium and 10cc of homogeneous biomass) was selected to be250-500-1000-2000 mg/ml, respectively. All four Erlenmeyer had the same aeration, and a 150 rpm mixer was used. The solution was sampled during different stages. The amount of sulfide in the solution was measured immediately after sampling to prevent possible error.

Continuous operation to remove sulfide

The 5-liter mixer-controlled bioreactor was used to simulate the biodegradation of hydrogen sulfide on an industrial scale. One of the features of this bioreactor is the increasing level of contact between liquid and gas and transferring the appropriate mass of oxygen into the liquid phase due to the presence of sparger, proper agitation, and non-accumulation of bacteria in some parts of the bioreactor. First, 1 liter of culture medium was prepared without sulfide source and poured into the bioreactor. The culture medium containing sulfide prepared by the peristaltic pump was then pumped into the bioreactor at a constant speed. This process was done until the bacteria reached a stable state, which usually took 2 to 10 hours. The stable state as a complete sulfide conversion in all parts of the photobioreactor or a sulfide concentration in two or three times was constant. Then the amount of input flow gradually increased from

40cc to 3000cc per hour. All tests were performed at room temperature. The pH of the solution in the range of 8-9was kept constant by the alkaline solution. After sampling, the amount of sulfide was measured immediately. All species were sampled at steady-state, and experiments were performed three times to reduce error. A steady-state in continuous operation is a state in which the concentration throughout the system remains constant; in other words, the input concentration is equal to the concentration consumed by the bacteria.

Sulfide volume loading rate

The peristaltic pump (constant speed) was used to investigate the effects of volumetric sulfide loading rate. The constant speed of this pump caused a constant flow. Also, a culture medium of 1500 mg / L sulfide was used to enter the sulfide medium. The feed flow inserted by this pump was50 cc/min. Different amounts of input current were used to load sulfide into the bioreactor. Each flow was kept constant until it reached a constant state value. The values of input flow and input sulfide flow are given in Table.3. The output value inside the bioreactor was the same as the input value after the input sulfide remains constant or completely removed which would keep the culture medium volume constant and prevent it from increasing.

Table.3 Sulfide removal rate by thiobacillusthioparus at different concentrations of sulfide

Concentration of sulfide (mg/l)	Sulfide removal rate(mg/L.h)
250	25.34
500	27.56
1000	29.89
2000	7.5

Investigation of sulfur and sulfate production

According to the experiment an appropriate sulfide loading range was selected. The aeration rate was selected according to the values reported in the articles. The rate of sulfide removal, sulfate, and sulfur production was measured. The samples were first centrifuged to measure the amount of sulfate present. Then the surface liquid was removed and stored at -72° C to be measured later. The amount of oxygen in the solution was also measured by the solution's oxygen electrode. This electrode showed the amount of oxygen as a percentage of the saturated oxygen at the test temperature. Since experiments were one at laboratory temperature (25 °C), and the oxygen saturation rate at this temperature was 8.3 mg/L, so the oxygen content was expressed as a percentage of this saturation.

Optimization of sulfur production

The amount of dissolved oxygen and the rate of sulfide loading were factors influencing the production of sulfate and sulfur in the process of biological removal of sulfide[26]. The central compound multi-level test design method was used to begin the work and determine the test levels. Since, in the previous experiment, the appropriate loading rate range was selected for sulfide removal, this range was used. In this experiment, the input sulfide rate of 3800-800 mg/h was used. The amount of dissolved oxygen was also adjustable. According to the values reported in the source, the amount of oxygen in the solution was selected to be 0.1-3 mg / L. The aeration rate varied from 10 to 30 ml/min. By transferring the above information in the Design Expert, the test design table is presented as shown in Table.4.

Table.4 Test design for two factors using a central combination design

Std	Run	A: Loading Rate (mg/h)	B: O2(mg/L)	Sulfur produce(mg/L)	Sulfate produce(mg/L)
13	1	2300	0.65	1205	2775
4	2	3800	1	1803	4890
2	3	3800	0.3	3107	930
1	4	800	0.3	408	975
6	5	4421.32	0.65	3211	2529
11	6	2300	0.65	1184	3168
77	7	2300	0.16	1809	624
10	8	۲۳۰۰	0.65	1213	2853
5	9	178.68	0.65	65	312
8	10	2300	1.14	810	4122
12	11	2300	0.65	1220	2832
3	12	800	1	210	1731
9	13	2300	0.65	1190	2202

Results and Discussion

Primary culture

To conduct the primary culture, thiosulfate was used as a sulfur source, and its amount was measured during the growth period. On the other hand, the optical density for measuring the turbidity of the solution was evaluated, which indicated the growth of biomass. When using the specific culture medium of the Thiobacillusthioparus during conducting primary culture of samples in the culture medium, we excluded this bacterium from this stage of the experiment. Increasing the turbidity of the solution and decreasing the amount of thiosulfate indicated the uptake of sulfur source by bacteria from the refinery wastewater (T.R.W) and the growth of biomass. The results are shown in **Fig.4**.

According to the results displayed in Figure4, the amount of thiosulfate in the medium containing the bacteria of refinery wastewater was reduced from 457 mg/L to 27 mg/L.

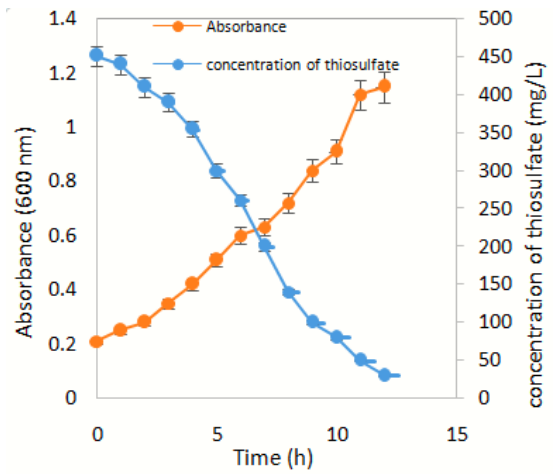


Fig.4. Changes in thiosulfate concentration and optical density for T. R.W bacteria

Also, increasing the turbidity of the solution from 0.2 at the beginning of the process to 1.12 at the end of it indicates the consumption of thiosulfate and biomass growth.

The above experiment was also performed for the sample obtained from the Mahallat Hot Spring (M. H.S). The result is shown in Figure5.

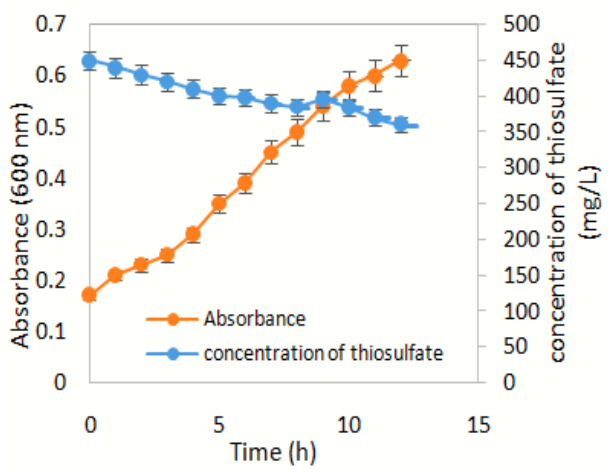


Fig.5. Changes in thiosulfate concentration and optical density for M.H.S bacteria

The results of experiments show that changes in thiosulfate levels in the medium containing hot spring bacteria were not significant, but the increase in solution turbidity from 0.17 to 0.63 at the end of the process indicates its activity and biomass growth.

Investigation of bacterial growth at different conditions

One of the conditions studied was the aerobic or anaerobic mechanism of bacteria. Optical density was evaluated to measure the turbidity of the solution, which indicated the growth of biomass, the results of which are shown in Figure 6.

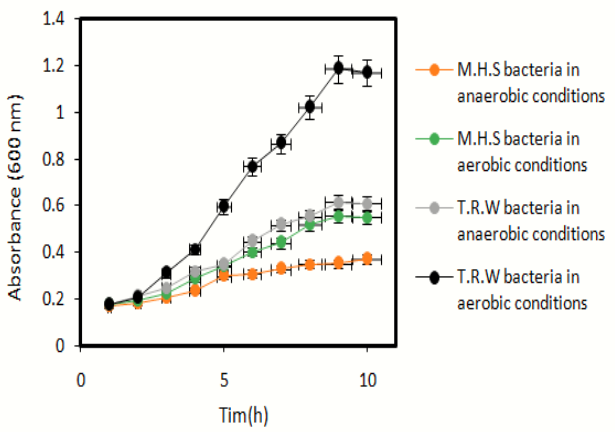


Fig.6. Investigating the growth of T.R.w & M.H.S bacteria in aerobic and anaerobic conditions

As shown in Figure 6, a comparison of the results of aerobic and anaerobic growth tests shows that these bacteria grow better in aerobic conditions so that in the same bacterial samples, this difference is quite apparent and the graphs show a significant difference. They show that the bacteria grow better in the aerobic state than in the anaerobic state. Therefore, the aerobic state should be considered both for bacteria from the sewage of Shahid Tondgooyan Refinery Co. and the bacteria from the Hot Spring. Another condition considered is the bacterial autotrophy or heterotrophy mechanisms, evaluation of bacterial growth in terms of carbon source. After checking the appropriate conditions for aerobic or anaerobic bacteria, aerobic conditions were applied to them. For one Erlenmeyer flask, an organic carbon source, sodium carbonate in the culture medium, was used. For another Erlenmeyer, carbon dioxide was used, and optical density was used to measure the turbidity of the solution, indicating the growth of biomass. The results are shown in **Fig. 7**.

As shown in Figure 7, the results of experiments examining the carbon source required by the bacteria indicate that the bacteria can use both carbon sources as a carbon source. However, the rate of growth in media containing organic carbon is much higher than the rate of growth in media containing carbon in the air.

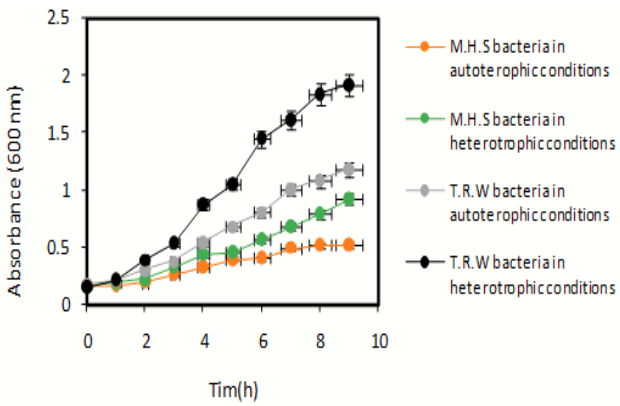


Fig.7. Investigating the growth of T.R.w& M.H.S bacteria in autotrophic and heterotrophic conditions

However, according to the results and consumption of both carbon sources, samples can be considered as mixotrophic (optional) bacteria.

Investigation of the performance of bacteria in alkaline PHs

At neutral and weak acidity pH, sulfide species are often soluble in hydrogen sulfide (H_2S_{aq}, HS-). Because the species are volatile and enter the air due to aeration and contact with the air, the performance of the process at this pH repels the sulfur gas entering the air, and the bacteria do not have access to the sulfide. High pH performance not only causes the generation of dominant species as S^{2-} but also increases the rate of uptake in the adsorption tower. Therefore, performance at high pH is desirable. On the other hand, due to the alkalinity of the medium and poor performance or the inability of many species to operate in it, the possibility of contamination is reduced[27, 28]. The results of these experiments for refinery wastewater bacteria are shown in Figure 8.

As can be seen, refinery wastewater bacteria at pH = 9 perform well, showing that sulfide removal rates at this pH such as pH = 7as the pH increases to 10, and there is a difference in the function of the bacteria.

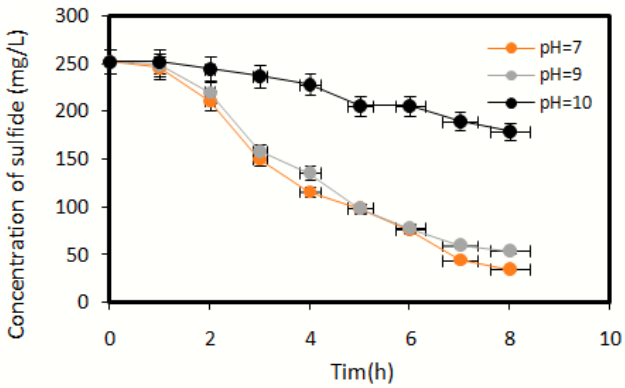


Fig.8. Changes in sulfide concentrations at different pH levels by T.R.W bacteria

At this pH, the performance of bacteria has changed slightly compared with the lower pH, but it can still be claimed that they have a high ability to remove sulfide. The performance of these bacteria at this pH is desirable and can be used on a larger scale. In a similar experiment, the results of thiobacillusthioparusare are shown in Figure 9.

The results show that the thiobacillusthioparus species show good activity and growth only at and pH = 7 and close to it, and with increasing pH, the yield and activity of this bacterium decrease sharply. In other words, at higher pH, the bacterium is inactive and cannot remove the sulfide in the solution. This result is especially noticeable at higher pH such as 10. This pH does not correctly remove sulfide and spends more time annihilating it. Increasing the time will drastically reduce the removal rate and significantly increases operating and process costs, so it is important to use species that have a high sulfide removal rate.

For these reasons, as well as the poor performance of the thiobacillusthioparus bacterium at high sulfide concentrations, the bacterium has been removed from tests, and further testing will be performed for bacteria isolated from the refinery's wastewater.

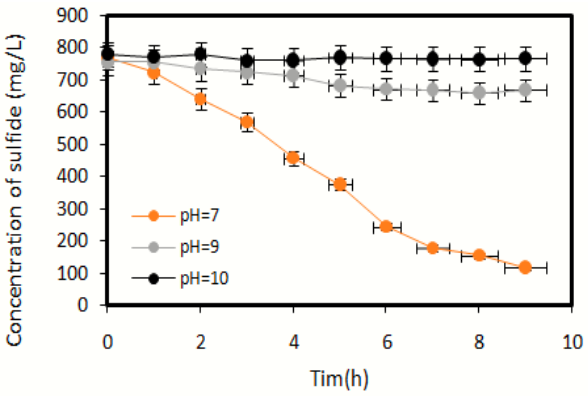


Fig.9. Changes in sulfide concentration at different pH by thiobacillusthioparus bacteria

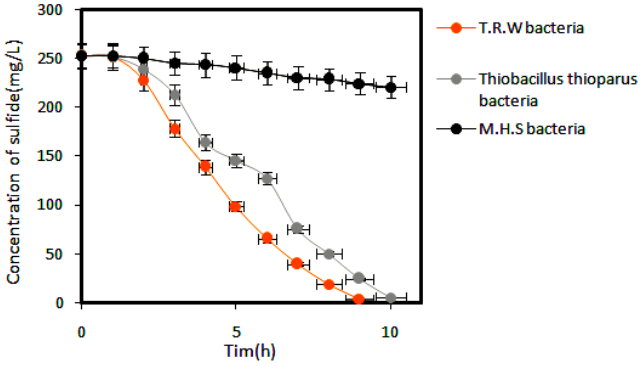


Fig.10. Bacterial function in sulfide consumption

Evaluation of sulfide consumption by bacteria as a source of sulfur

After examining the growth of bacteria in a medium containing thiosulfate, their energy source changed. The energy source changed from thiosulfate to sulfide. Sodium sulfide was used to supply the sulfide source. In the beginning, at the end, and during the culture, the solution was sampled, and sulfide was measured. In this section, the function of thiobacillusthioparus bacterium in sulfide consumption was also evaluated. Decreased sulfide levels indicated the consumption of sulfide source and biomass growth, as shown in Figure 8.

The results of the experiment show that the hot spring bacteria have not grown well in this medium and cannot use sulfide as an energy source. Nevertheless, the other two samples (thiobacillus and bacteria from Tehran's refinery wastewater), as seen in Figure 5, have grown well in an environment containing sulfide.

Investigating the initial concentration of sulfide on the performance of bacteria

Examining the initial concentration of sulfide means obtaining the toxicity threshold for bacteria[25, 29]. According to the results obtained by the bacteria in the sulfide consumption test, it was observed that the bacteria from the hot springs did not grow well in the sulfide-containing medium, so it was omitted from experiments. Subsequently, the presence of different sulfide concentrations was measured as the primary sulfide. After adding the specified amounts of sulfide to the Erlenmeyer during the process, the solution was sampled, and the amount of sulfide in it was measured. The results of the sulfide removal rate process for different amounts of sulfide are shown in Table3 and Table5 for both bacteria from the refinery wastewater and the thiobacillusthioparus.

Table .5: Sulfide removal rate by T.R.W bacteria

Concentration of sulfide (mg/l)	Sulfide removal rate (mg/L.h)
250	28.25
500	39.6
1000	40.75
2000	38.25
3000	39.45
4000	10/78

at different sulfide concentrations

Based on the results shown in Table.5, we see that the Thiobacillusthioparus does not tolerate high levels of sulfide. At concentrations above 1000 mg/L, the bacterium failed to perform well and eliminate high levels of sulfide. However, according to Figure 10, we can see that the bacteria from the refinery wastewater up to a concentration of 3000 mg/L have a high elimination rate. At a concentration of 4000 mg/L, this bacterium did not function properly, and finally, at a concentration of 5000 mg/L function of this bacterium is severely dropped, meaning that the removal of sulfide at a concentration of about 3800 mg/L is stopped by this bacterium. It also has a higher elimination rate than the thiobacillusthioparus bacteria in lower sulfide levels.

Production of sulfur and sulfate

As mentioned, the amount of dissolved oxygen and the rate of sulfide loading are factors

influencing the production of sulfate and sulfur in the process of sulfide removal. The central compound multi-level test design method was used to begin the work and determine the test levels. In this design, 9 experiments were performed as the main experiment of the process, and 4 experiments were performed repeatedly to control the usefulness of the model for the central point. Subsequently, experiments were performed, and samples were measured to produce sulfur and sulfate. In this section, using the data obtained from the experiments, the value of R^2wascalculated for different models, and the appropriate model was selected and suggested using the maximum value for it. In these experiments, the proposed model was a second-order model. The results are shown in Table.6. In the next step, using analysis of the data variance, the importance of the parameters was examined. The results are shown in Table.7.

Table .6: Model Summary Statistics

Source	Std.Dev	R-Squared	Adjusted R-squared	Predicted R-Squared	PRESS	
Linear	241.58	0.9478	0.9374	0.8934	1.192E+006	
2FI	176.74	0.9749	0.9665	0.9508	5.504E+005	
Quadratic	54.51	0.9981	0.9968	0.9873	1.424E+ 005	Suggested
Cubic	59.42	0.9984	0.9962	0.9045	1.067E+006	

Table .7: Analysis of the data variance

Source	Sum of Square	df	F Value	p-value Prob > F
Model	1.116E+007	5	750.97	< 0.0001
A	9.534E+006	1	3208.25	< 0.0001
B	1.062E+006	1	357.21	< 0.0001
AB	3.025E+005	1	101.79	< 0.0001
A^2	2.603E+005	1	87.59	< 0.0001
B^2	5141.98	1	1.73	0.2298

In this section, various parameters were examined. The higher the F-Value and the lower the Prob> F values, the more critical the parameter is, and this parameter can be called the process-affecting parameter. From Table.6, it can be concluded that the loading rate is an influential parameter in this process (sulfur production). Other parameters, such as the amount of oxygen and the interaction of the two factors of loading rate and oxygen, are also useful parameters. However, their importance is not equal to the mentioned parameter.

In the next step, the software provides a second-order equation Eq.1 for analyzing the data, which can predict the amount of sulfur production using a loading rate and the amount of dissolved oxygen with good approximation.

$$\text{Sulfur Produce} = -31.95143 + 0.67280 * \text{Sulfide Loading Rate} - 124.54906 * O_2 - 0.52381 * \text{Sulfide Loading Rate} * O_2 + 8.59722E\text{-}005 * \text{Sulfide Loading Rate}^2 + 221.93878 * O_2^2 \quad (1)$$

In the next step, the results of the prediction and the actual values obtained are evaluated. These results are shown in Fig.11. In Figure 11, the actual values obtained and the predicted values are shown as horizontal and vertical axes.

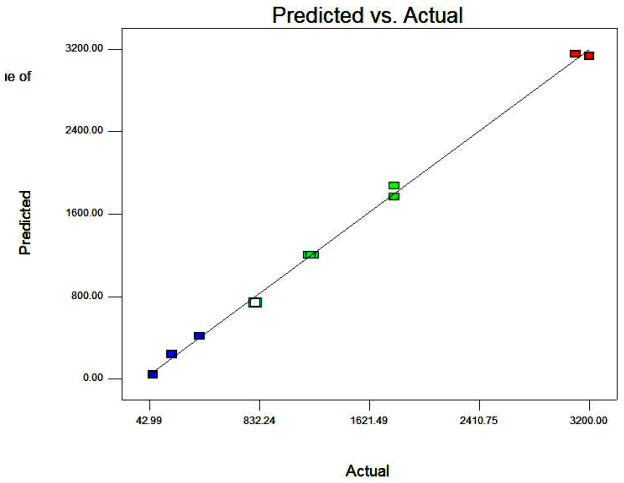


Fig.11. Comparison of real and predicted results

The midline indicates the complete conformity of these two values, and the predicted results can be used with high accuracy to continue experiments.

The results of data analysis are shown as the effect of these two parameters on the amount of sulfur production as a three-dimensional procedure in Figure12. As shown in this Fig.12, this procedure covers all points in the range shown. Red dots are the points where the maximum sulfur is produced. This part of the surface occurs at points with high sulfide loading rate and low oxygen content, which go to blue dots with decreasing loading rate and increasing oxygen rate, which shows sulfide reduction.

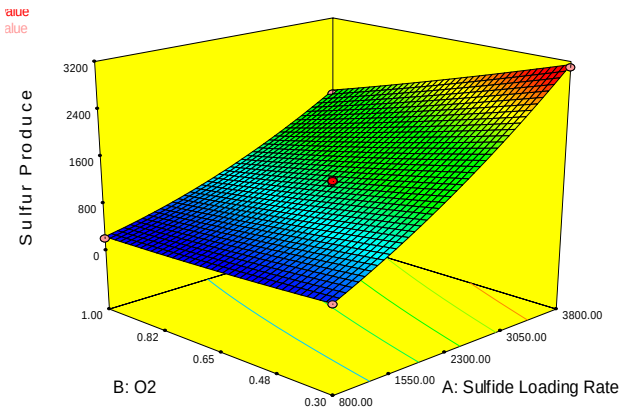


Fig.12. 3D procedure resulting from the effect of parameters

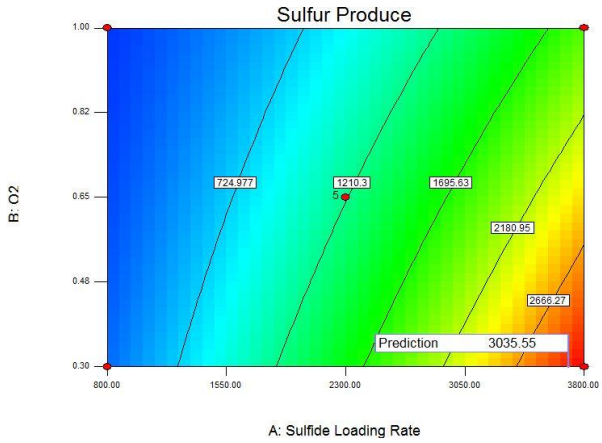


Fig.13. Condition optimization diagram for maximum sulfur production

Finally, the optimal conditions for sulfur production were examined. For maximum sulfur production in this range, optimal conditions were analyzed, at the end of which the sulfide loading rate was 3700 mg/L, and the oxygen content was0.3 mg/L. The amount of sulfur obtained from these conditions was equal to 3035.55mg/L. The optimization results are shown in Figure13.

Conclusion

According to preliminary experiments, some bacteria consume sulfur compounds in both samples from hot springs and sewage of Shahid Tondgooyan Refinery Co. in Tehran. A comparison of the results of bacterial culture experiments in a culture medium containing thiosulfate suggests that the refinery's sewage bacteria performed well in consuming and removing sulfide. Measurement of solution turbidity, as well as microscopic observations, confirm this. In the case of the hot spring sample, although apparently, not much change in the amount of sulfide emerged, the results of measuring optical density and microscopic observations showed the growth and activity of this sample.

Since bacteria are isolated from different sources, they are likely to be of different species and have different nutritional needs. Examining these

conditions can be useful in improving the function of bacteria and identifying their needs. One of the conditions studied is the aerobic or anaerobic mechanisms of bacteria. Some species oxidize sulfur compounds in aerobic conditions, in which oxygen is considered as an electron donor, and some species grow better in anaerobic conditions, in which other compounds act as electron donors. Based on the results of the experiments, it was observed that both samples have the ability to grow in both cases, but their growth is higher in aerobic conditions. Then a test was designed to examine autotrophy and heterotrophy. According to the results of the sample test, the used bacteria are considered to be among the mixotroph (optional) bacteria, after examining the growth conditions of bacteria in the culture medium containing thiosulfate and increasing the population of bacteria, obtaining the desired results. Because the goal was to remove sulfide, to create a sulfide medium, the amount of thiosulfate was reduced, and sodium sulfide was added to the culture medium as a sulfur source. The sulfide consumption by bacteria was then evaluated, and only the Thiobacillusthioparusbacteria and the bacteria from the refinery's wastewater were able to remove the sulfide in the aquatic medium. Due to the need for process performance in alkaline pH to prevent hydrogen sulfide and sulfide removal from the aquatic environment, as well as the benefits of performance in alkaline pH, attempts were made to evaluate performance in this pH. Bacteria from the wastewater of refineries at higher pH did not show a functional difference. This process continued until pH = 10, but in this pH, the activity of bacteria decreased but did not indicate that these bacteria did not work. However, in the thiobacillusthioparus, the increase in pH severely impaired their function so that in pH 9 and 10, it can be argued that these bacteria are incapable of activity. Therefore, according to the results, the bacteria from the refinery's wastewater were used in subsequent experiments.

In the study of the concentration of sulfide on the performance of bacteria, they were exposed to high levels of sulfide concentration. According to the results obtained, bacteria from the refinery's wastewater removed high sulfide concentration well and at the rate of 40 mg/h.L, and the thiobacillusthioparus yielded acceptable rates. Results in amounts were less than 1000 mg/L, whereby the elimination rates were 30 mg/h.L. In lower amounts of sulfide, such as (250-3000 mg), the bacteria isolated from the refinery's wastewater outperformed and had a higher removal rate. In smaller amounts, it could be a good option for sulfide removal operations. At the end of the experiment, sulfur production was analyzed. At this stage, with the experiments and with the help of Design Expert, sulfur production was analyzed. The results showed that the rate of sulfide loading, the amount of oxygen, and the interaction of these two parameters were more active in the movement of the reaction towards sulfur production. In limited amounts of oxygen, such as 0.3 mg/L, and high sulfide loading rate such as 3700 mg/h, we obtained the optimal amount of sulfur, which was 3035/5 mg/L. Besides, according to the results obtained in this paper and investigating the effectiveness of two factors, aeration rate, and sulfide loading rate, it is necessary to provide samples from various other sulfur sources and evaluate their performance in sulfide removal. Among these sources, we can mention the sewage of metal industries and other hot springs with high sulfide content. It can also be suggested that the bacteria obtained from the T.R.W be subjected to genetic engineering operations and that these bacteria be more accurately evaluated. Other operating conditions using these bacteria were evaluated to achieve high yield and removal rates, such as process temperature, the effect of agitation intensity, by these bacteria.

Given that bacteria grew well in organic matter, and functional results were obtained from it, other organic carbon sources can be evaluated to achieve affordable carbon resources ultimately. On the other hand, since in industries, the exhaust gases from the units that use natural gas as fuel contain a high concentration of carbon dioxide, this type of carbon source can also be used. Therefore, the performance of bacteria can be evaluated by performing experiments in the laboratory by combining carbon dioxide in CO_2 and air capsules.

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Conflict of interest

The authors confirm that this article's content has no conflict of interest.

References

[1] R.-C. Jin, G.-F. Yang, Q.-Q. Zhang, C. Ma, J.-J. Yu and B.-S. Xing, The effect of sulfide inhibition on the ANAMMOX process. *water research*, 47 (2013) 1459-1469.

[2] B.R. Gurjar, L.T. Molina and C.S.P. Ojha, *Air pollution: health and environmental impacts*. (2010): CRC Press.

[3] J. Sun, X. Dai, Y. Liu, L. Peng and B.-J. Ni, Sulfide removal and sulfur production in a membrane aerated biofilm reactor: model evaluation. *Chemical Engineering Journal*, 309 (2017) 454-462.

[4] Q. Mahmood, P. Zheng, J. Cai, Y. Hayat, M.J. Hassan, D.-l. Wu and B.-l. Hu, Sources of sulfide in waste streams and current biotechnologies for its removal. *Journal of Zhejiang University-Science A*, 8 (2007) 1126-1140.

[5] B. Khoshnevisan, P. Tsapekos, N. Alfaro, I. Díaz, M. Fdz-Polanco, S. Rafiee and I. Angelidaki, A review on prospects and challenges of biological H2S removal from biogas with focus on biotrickling filtration and microaerobic desulfurization. *Biofuel Research Journal*, 4 (2017) 741-750.

[6] J. Kanjanarong, B.S. Giri, D.P. Jaisi, F.R. Oliveira, P. Boonsawang, S. Chaiprapat, R. Singh, A. Balakrishna and S.K. Khanal, Removal of hydrogen sulfide generated during anaerobic treatment of sulfate-laden wastewater using biochar: evaluation of efficiency and mechanisms. *Bioresource Technology*, 234 (2017) 115-121.

[7] S. Hall, *Rules of thumb for chemical engineers*. (2017): Butterworth-Heinemann.

[8] S. Mokhatab and W.A. Poe, *Handbook of natural gas transmission and processing*. (2012): Gulf professional publishing.

[9] K. Rabbani, W. Charles, R. Cord-Ruwisch and G. Ho, Recovery of sulfur from contaminated air in wastewater treatment plants by biofiltration: a critical review. *Reviews in Environmental Science and Bio/Technology*, 14 (2015) 523-534.

[10] J. He and J.P. Chen, A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modeling simulation tools. *Bioresource Technology*, 160 (2014) 67-78.

[11] J. Klok, G. van Heeringen, R. De Rink, and H. Wijnbelt, Introducing the next generation of the THIOPAQ O&G process for biotechnological gas desulphurization: THIOPAQ-SQ. *Sulphur*, 18260 (2017) 1-2.

[12] A. ter Heijne, R. de Rink, D. Liu, J.B. Klok and C.J. Buisman, Bacteria as an electron shuttle for sulfide oxidation. *Environmental science & technology letters*, 5 (2018) 495-499.

[13] C. Cline, A. Hoksberg, R. Abry and A. Janssen. *Biological Process for H~ 2S Removal from Gas Streams: The Shell-Paques/THIOPAQ™ Gas Desulfurization Process*. in *Proceedings of the Laurance Reid gas conditioning conference*. 2003.

[14] M. O Brien, C. Wentworth, A. Lanning, and T. Engert. *Shell-Paques® Bio-Desulfurization Process Directly and Selectively Removes H~ 2S from High-Pressure Natural Gas-Start-Up Report*. in *PROCEEDINGS OF THE LAURANCE REID GAS CONDITIONING CONFERENCE*. 2007.

[15] N. Hou, Y. Xia, X. Wang, H. Liu, H. Liu, and L. Xun, H 2 S biotreatment with sulfide-oxidizing heterotrophic bacteria. *Biodegradation*, 29 (2018) 511-524.

[16] H.-K. Namgung and J. Song, The effect of oxygen supply on the dual growth kinetics of Acidithiobacillus thiooxidans under acidic conditions for biogas desulfurization. *International journal of environmental*

research and public health, 12 (2015) 1368-1386.

[17] L. Yan, J. Ye, P. Zhang, D. Xu, Y. Wu, J. Liu, H. Zhang, W. Fang, B. Wang, and G. Zeng, Hydrogen sulfide formation control and microbial competition in batch anaerobic digestion of slaughterhouse wastewater sludge: Effect of initial sludge pH. *Bioresource Technology*, 259 (2018) 67-74.

[18] M. Monachon, M. Albelda-Berenguer, T. Lombardo, E. Cornet, F. Moll-Dau, J. Schramm, K. Schmidt-Ott and E. Joseph, Evaluation of Bio-Based Extraction Methods by Spectroscopic Methods. *Minerals*, 10 (2020) 203.

[19] T. Satyanarayana, B.N. Johri and A. Prakash, *Microorganisms in environmental management: microbes and environment*. (2012): Springer Science & Business Media.

[20] J.-H. Kang, H.-G. Namgung, J.-I. Cho, S.S. Yoo, B.-J. Lee and H.W. Ji, Removal of Hydrogen Sulfide in Septic Tanks for Treating Black Water via an Immobilized Media of Sulfur-Oxidizing Bacteria. *International Journal of Environmental Research and Public Health*, 17 (2020) 684.

[21] B. Sörbo, A colorimetric method for the determination of thiosulfate. *Biochimica et Biophysica acta*, 23 (1957) 412-416.

[22] D.P. Kelly, L. Chambers and P. Trudinger, Cyanolysis and spectrophotometric estimation of trithionate in mixture with thiosulfate and tetrathionate. *Analytical Chemistry*, 41 (1969) 898-901.

[23] M. Fattahi, E. Ezzatzadeh, R. Jalilian, and A. Taheri, Micro solid-phase extraction of cadmium and lead on a new ion-imprinted hierarchical mesoporous polymer via dual-template method in river water and fish muscles: Optimization by experimental design. *J Hazard Mater*, 403 (2021) 123716.

[24] Z. Rezayati zad, B. Moosavi, and A. Taheri, Synthesis of monodisperse magnetic hydroxyapatite/Fe3O4 nanospheres for removal of Brilliant Green (BG) and Coomassie Brilliant Blue (CBB) in the single and binary systems. *Advanced Journal of Chemistry-Section B*, 2 (2020) 159-171.

[25] Y. Cheng, T. Yuan, Y. Deng, C. Lin, J. Zhou, Z. Lei, K. Shimizu and Z. Zhang, Use of sulfur-oxidizing bacteria enriched from sewage sludge to biologically remove H2S from biogas at an industrial-scale biogas plant. *Bioresource Technology Reports*, 3 (2018) 43-50.

[26] J. Cai, P. Zheng, M. Qaisar and J. Zhang, Elemental sulfur recovery of the biological sulfide removal process from wastewater: a review. *Critical reviews in environmental science and technology*, 47 (2017) 2079-2099.

[27] R. de Rink, J.B. Klok, G.J. van Heeringen, K.J. Keesman, A.J. Janssen, A. ter Heijne and C.J. Buisman, Biologically enhanced hydrogen sulfide absorption from sour gas under haloalkaline conditions. *Journal of hazardous materials*, 383 (2020) 121104.

[28] J. Mormontoy and J.E. Hurtado. *Hydrogen sulfide production at alkaline, neutral, and acid pH by a bacterial consortium isolated from Peruvian mine tailing and wetland*. in *Advanced Materials Research*. 2013. Trans Tech Publ.

[29] B. Huber, B. Herzog, J.E. Drewes, K. Koch, and E. Müller, Characterization of sulfur oxidizing bacteria related to biogenic sulfuric acid corrosion in sludge digesters. *BMC microbiology*, 16 (2016) 1-11.

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