CHEMICAL ENGINEERING TRANSACTIONS
VOL. 45, 2015
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Guest Editors: Petar Sabev Varbanov, Jiří Jaromír Klemeš, Sharifah Rafidah Wan Alwi, Jun Yow Yong, Xia Liu

ISBN 978-88-95608-36-5; **ISSN** 2283-9216
DOI: 10.3303/CET1545102

Optimisation Process of Sulphur Recovery Using a Two-Step Biological System

Francine Dimite Alesia, Evans M.N. Chirwa*

Water Utilisation and Environmental Engineering, Department of Chemical Engineering, University of Pretoria, 362 5089, Pretoria 0002, South Africa
Evans.Chirwa@up.ac.za

The production of elemental sulphur (S^0) during treatment of Acid Mine Drainage is regarded as a sustainable solution since the produced elemental sulphur can be re-used for bioleaching processes or as fertilisers. Biological treatment of AMD primarily relies on the activity of sulphate reducing bacteria which reduce sulphate to sulphide in the presence of organic matter thus allowing the precipitation of the metals and increase in pH. However, excess of sulphide remains in the system and if not removed, can be oxidised to sulphate. The objective of the study was to optimise the oxidation process of sulphide to elemental sulphur in a batch reactor with glucose as the supplied carbon source. The study also describes the sulphide oxidation process to elemental sulphur as a Monod-type model with best-fit model parameters estimated. Up to 95 % of elemental sulphur was formed at pH 8 and Eh -80 mV. The mass balance calculations confirmed the results. The redox potential was observed to have a greater effect to the oxidation process compared to pH. This was later confirmed by the modelling results.

1. Introduction

Acid mine drainage is a significant problem to the mining industries worldwide. Various methods have been established to remediate and treat acid mine drainage. Among the proposed treatment methods, the biological sulphate removal system is seen to be most favourable. In this system, sulphate reducing bacteria (SRB) are used to convert sulphate to sulphide which is then either removed as a precipitate or as the acid gas, H_2S (Jiang et al., 2009). The limitation to this method is that the sulphide is precipitated together with toxic heavy metals forming a toxic sludge (Kai-Guang et al., 2007). An interesting way to recover the precipitated metals is the use of sulphide material produced by the bacteria which acts as an absorbent for the heavy metals present in acid mine drainage (Jencarova, 2012). As much as this method is operated at low cost and is quite effective, the residual sulphide left in the system needs to be abstracted to prevent the re-oxidation of sulphide back to sulphate or prevent the discharge of sulphide to the environment because of its toxic, corrosive and odorous properties. Quite a few strategies have been put in place to remove the sulphide.

Oxidation to sulphur, also known as partial oxidation of sulphide has been the most promising method and the use of bacteria known as sulphide oxidizing bacteria (SOB) has made the system more effective and cheaper compared to physic-chemical methods (Rose, 2002). It was reported that a heterotrophic condition was suitable to generate elemental sulphur at a great capacity (Rein, 2002). Different sulphide oxidizing bacteria were identified to generate elemental sulphur. Unfortunately, the conditions under which sulphur production is optimal remain unknown. However, what has been observed is that production of elemental sulphur requires fine tuning of the pH and redox conditions to a very narrow range (Middelburg, 2000).

The aim of this study was to determine the optimal conditions for sulphur recovery using sulphide oxidizing cultures obtained from a local wastewater treatment plant. Sulphide oxidizing bacteria were isolated to determine the most appropriate loading conditions for sulphide removal and if possible sulphur production. The results obtained can potentially provide a reference for sulphur recovery using a bacterium for future research.

Please cite this article as: Alesia F.D., Chirwa E.M.N., 2015, Optimisation process of sulphur recovery using a two-step biological system, Chemical Engineering Transactions, 45, 607-612 DOI:10.3303/CET1545102

2. Materials and Methods

2.1 Isolation of sulphide oxidizing bacteria (SOB)

A modified medium of Rajkumar et al (2012) was composed of different solutions known as A, B, C and D in 250 mL distilled water and the pH adjusted to 7 at 25 ºC. Solution A was prepared from $Na_2S_2O_3.5H_2O$-5.0 g, KNO_3-2.0 g and NH_4Cl-1.0 g in 250 mL of distilled water; solution B was prepared from KH_2PO_4-2.0 g in 250 mL of distilled water; solution C was made up of $NaHCO_3$-2.0 g in 250 mL of distilled water; and solution D was composed of $MgSO_4.7H_2O$-0.8 g, $FeSO_4.7H_2O$ (2% w/v in HCl) -100.0 mL and trace metal Selection of the most appropriate SOB solution -1mL. The trace metal solution consisted of (g/L) EDTA-50.0 g, $ZnSO_4$-22.0 g, $CaCl_2$-5.54 g, $MnCl_2$-5.06 g, $FeSO_4.7H_2O$ -4.99 g, $CoCl_2$-1.11 g, $CuSO_4$-1.57 g and $(NH_4)2MoO_4$-1.1 g; the pH was adjusted to 6. $FeSO_4.7H_2O$ Solution per 100 mL was made up of $FeSO_4.7H_2O$-2 g and HCl. 1N solution - 100.0 mL. The 4 solutions were sterilised then aseptically combined to make the main solution and the pH was adjusted to 7.

A sample of activated sludge (1 g) was then mixed into 10 mL of the main solution. A serial dilution up to 10^{-3} was done. Simple streaking was done by taking sample from the 10^0 to 10^{-3} dilutions. The petri dishes were incubated at 25 ºC for one week.

Heterotrophic growth of a selected strain was determined using the nutrient broth, in which lactate and glucose were added to determine which one the carbon source is appropriate for sulphide removal. Determination of sulphide oxidation activity was based on observed removal of sulphide in a 50 $mg.L^{-1}$ sulphide solution under incubation at 27 °C over 4 h.

2.2 Analytical Measures

The Merck Spectroquant® system (photometric colour measurement) was used for sulphide determination (Merck, South Africa). Samples were collected in test tubes containing 100 mL of 0.1 M zinc acetate solution. Photometric readings were made using the SQ 118 spectrophotometer (Merck, South Africa).

3. Results and Discussion

3.1 Isolation of SOB Strains

Following above isolation procedure, 6 possible isolates (S_1 to S_6) were identified. The cultures were grown in range of carbon sources to determine the most suitable carbon and energy source for the rest of the experiments. It was observed that the presence of the organic matter was consumed by the bacteria and that helped with their growth. The strains could grow well with the presence of both carbon sources and each of them had a different growth phases which were specific to lactate or glucose (see Tables 1 and 2).

Table 1: Bacterial Growth in the Presence of Lactate

Time (days)	S_1	S_2	S_3	S_4	S_5	S_6
1	9.10	180.3	211.4	68.04	74.35	39.27
2	43.33	495.3	226.0	120.5	279.8	133.8
3	134.6	664.2	723.1	404.4	612.9	528.5
4	857.6	949.8	1,100	714.6	1,100	990.5
5	1,023	1,100	1,100	1,100	1,100	1,100
6	1,100	1,100	1,100	1,100	1,006	1,100
7	1,100	812.3	641.9	951.3	914.2	1,100

Table 2: Bacterial Growth in the Presence of Glucose

Time (days)	S_1	S_2	S_3	S_4	S_5	S_6
1	131.3	71.9	22.68	473.7	81.16	55.33
2	384.9	167.4	157.5	303.6	171.8	112.4
3	819.4	502.2	456.4	440.5	598.2	270.6
4	1100	650.1	464.4	498.1	975.8	420.0
5	1100	896.2	590.4	585.7	1,100	705.2
6	915.2	1,000	787.9	797.3	1,100	1,020
7	875.6	1,100	950.0	1,100	875.6	1,100

Table 3: Sulphide removal after 4 hours incubation in 50 mg/L batches using glucose and lactate as organic carbon sources

Carbon Source	Glucose		Lactate	
Strains	$[S^{-2}]$ (mg/L)	% Sulphide removal	$[S^{-2}]$ (mg/L)	% Sulphide
S_1	3.2	93.6	0	100
S_2	0	100	9.4	81.2
S_3	2.4	95.2	7.8	84.4
S_4	2.8	94.4	3.7	92.6
S_5	1	98	0	100
S_6	2	96	0	100

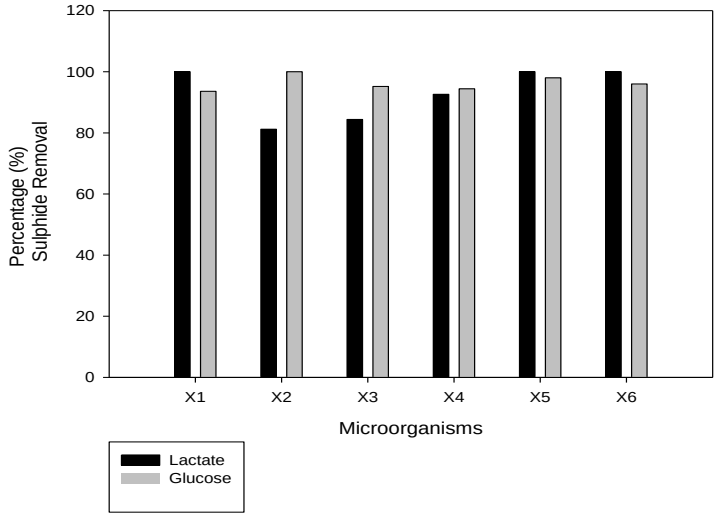

Figure 1: Sulphide removal at 4 h incubation in batches at initial concentration of 50 mg L^{-1}

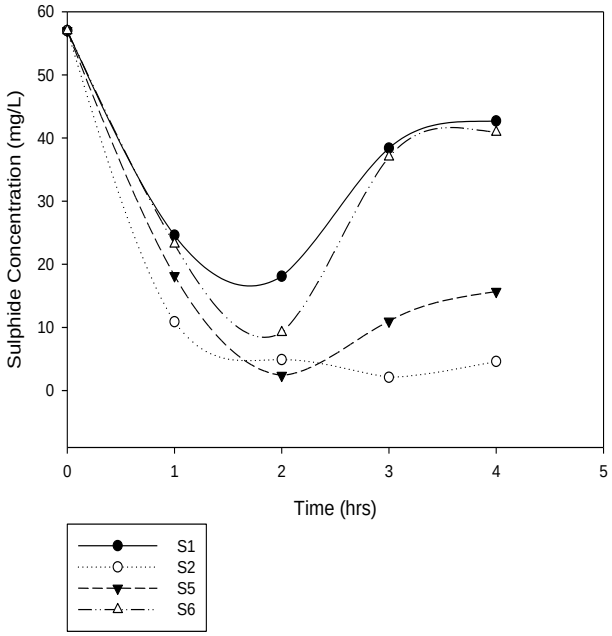

Figure 2: Sulphide removal by reselected sulphide oxidising bacteria (SOB)

Turbidity value of 1,100 was recorded as a higher value than the instrument could measure and this was translated at a high concentration of bacteria in the broths.

3.2 Selection of the appropriate SOB

The sulphide removal percentage was calculated as follows: [(Sulphide $_{in}$ – Sulphide $_{out}$) / Sulphide $_{in}$] * 100 %. After 4 h of incubation, data were obtained as shown in Tables 3 and Figures 1a and b. The preliminary data shown in Figure 3 indicated that Strain 2 was the most efficient isolate under glucose as a sole carbon source whereas sulphide removal under lactate did not show a specific trend (Table 3, Figure 1). After 3 h Sulphide concentration increased in the strains 1, 5 and 6 when even after 4 h increased just increased for a little amount in strain 2. Further experiments tested over a shorter period of incubation showed that the best overall performer was the isolate S_2 (Figure 2).

3.3 Kinetic modelling theory

The quantitative determination of elemental sulphur formation was done by mass balance calculation (Chen et al., 2009) or via sulphite method (Jiang et al., 2009). Xu et al (2013) stated that kinetic models could also assist during the optimization process of elemental sulphur generation. At low and high oxygen concentration, sulphide is oxidised to elemental sulphur (Eq 1) and sulphate (Eq 2), respectively.

$$2HS^- + O_2 \rightarrow 2S^o + 2OH^- \quad (1)$$

$$2HS^- + 4O_2 \rightarrow 2SO_4^{2-} + 2H^+ \quad (2)$$

According to Eq. 2, sulphur production depends on HS^- and dissolved oxygen. Dutta (2008) stated that when two substances limit the biological reaction rate, the rate of the product adopted is the following:

$$\frac{d[HS^-]}{dt} = \frac{X_{op} \cdot k_1 \cdot [HS^-]}{k_2 + [HS^-]} \cdot \frac{O_2}{k_3 + O_2} \quad (3)$$

where: X_{op} = Optical density of the bacteria, k_1= reaction rate constant (mg L^{-1} h^{-1}), k_2 = half velocity concentration (mg L^{-1}), k_3 = oxidative inhibition coefficient (mg L^{-1}), [HS^-]= concentration of sulphide (mg/L) and O_2= concentration of the dissolved oxygen (mg/L). The above reaction rate successfully simulates sulphide oxidation rate in a batch system is shown in Figure 3.

The optimum kinetic parameter values for the data plotted in Figure 3 were k_1 = 9.8 mg L^{-1} h^-, k_2 = 0.01 mg L^{-1}, X_{op} = 619 mg L^{-1}, and k_3 = 0.023 mg L^{-1}, which yielded an average chi-square value – χ^2 = 14.74 (mg L^{-1})$^{-2}$.

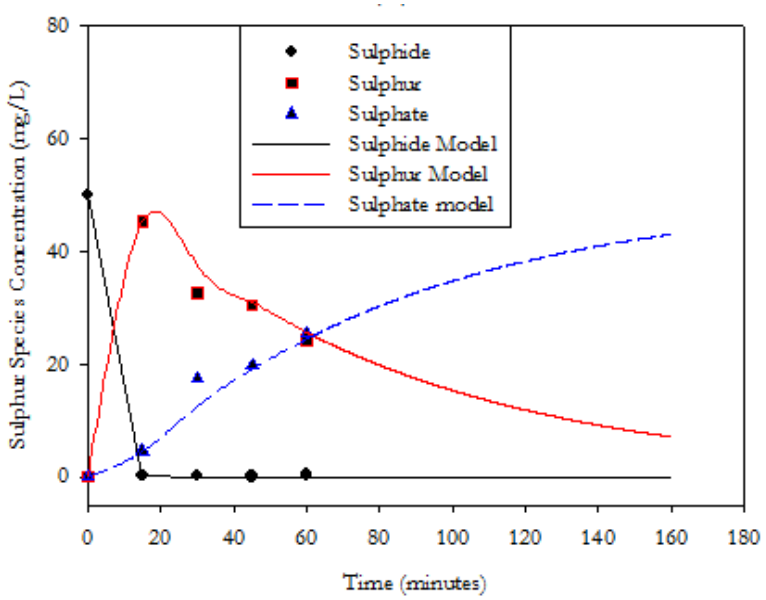

Figure 3: Sulphur species oxidation in batch cultures at pH 6.7 and Eh -130 mV

3.4 Identification of the SOB

Isolate X2 was a gram positive, motile, rod shape like that formed white opaque colonies on a nutrient agar plates. The 16 S rRNA gesne sequence of the isolated X2 (see Figure 3) revealed its phylogenetic relationship to the Bacillus genus. It was observed that X2 has 99 % homology to Lysinicibacillus fusiformis and Lysinicibacillus sphaericae. Apart from being a well-known sulphate reducer, Lysinicibacillus fusiformis

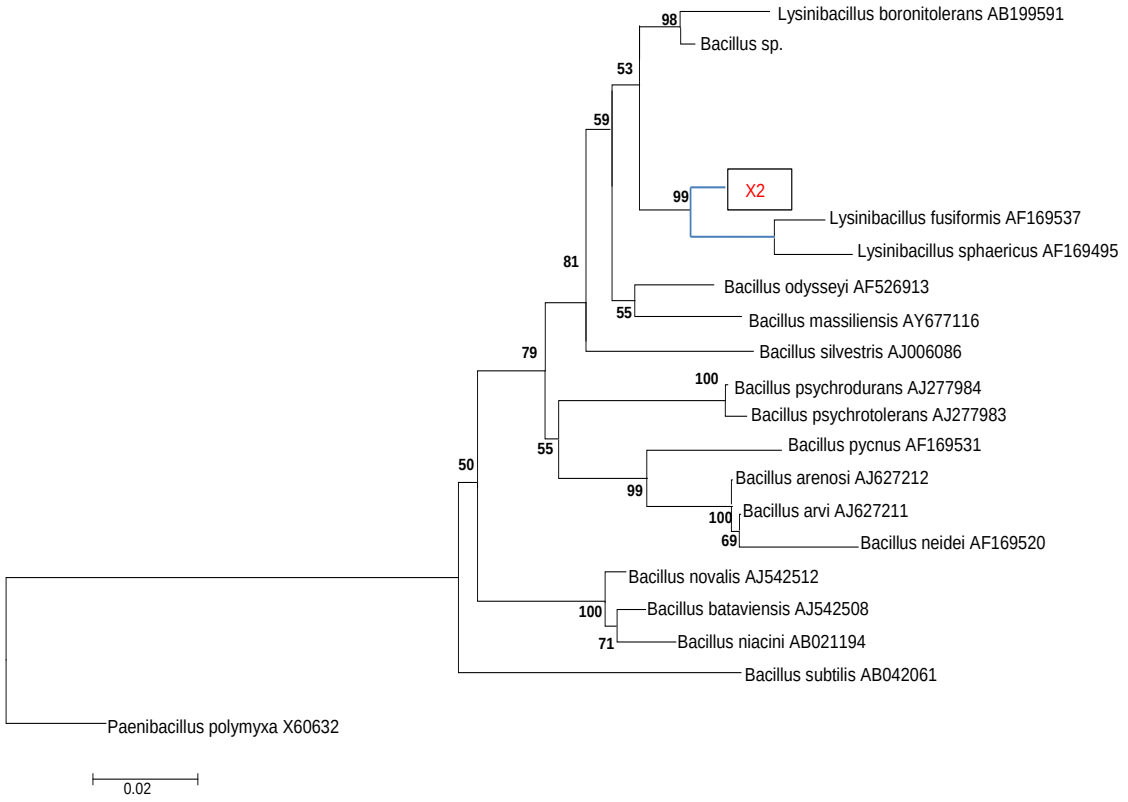

Figure 4: A phylogenetic tree showing the identity of the Bacillus sp. inferred from 16S rRNA gene sequences using the neighbour joining method. 1000 replicates Bootstrap analysis was done to assess the reliability of the groupings and Paenibacillus polymyxa was used as an outgroup

is also known to reduce metals such as Cr(VI) to Cr(III) (He et al., 2011), U(VI) to U(IV) (Kumar et al., 2013), and Mn(IV) to Mn(II) (Carrato et al., 2010).

4. Conclusion

The study demonstrated that sulphide can be removed through oxidation by bacteria called sulphide oxidizing bacteria in the presence of an organic matter. Strain 2 in the presence of glucose was the most efficient bacterium. The 16S rRNA gene sequence has revealed that strain 2 could either be Lysinicibacillus fusiformis or Lysinicibacillu sphaericus. More genetic analysis need to be done to determine the exact identity of strain 2 but however this provide useful information for sulphide removal thus sulphur recovery.

Acknowledgements

The research was funded through the National Research Foundation (NRF) of South Africa through the Focus Areas Programme Grant No. FA2006031900007 and the Incentive Funding for Rated Researchers Grant No. IFR2010042900080 awarded to Evans M.N. Chirwa of the University of Pretoria.

References

Cerrato J.M., Falkinham III J.O., Dietrich A.M., Knocke W.R., McKinney C.W., Pruden A., 2010, Manganese-oxidizing and -reducing microorganisms isolated from biofilms in chlorinated drinking water systems, Wat. Res., 44, 3935-3945.

Chen C., Wang A.J., Ren R.Q., Lee D.J. Lai J.Y., 2009, High-rate denitrifying sulfide removal process in expanded granular sludge bed reactor, Bioresour. Technol., 100, 2316-2319.

Dutta R., 2008. Fundamentals of Biochemical Engineering, Ane Books India, Springer, New Delhi, India, 134.

He M., Li X., Liu H., Miller S.J.. Wang G., Rensing C., 2011, Characterization and genomic analysis of a highly chromate resistant and reducing bacterial strain Lysinibacillus fusiformis ZC1, J. Hazard. Mater., 185, 682-688.

Jencarova J., Luptakova A., 2012, The elimination of heavy metals ions from waters by biogenic iron sulphides, Chemical Engineering Transactions, 28, 205-210.

Jiang Y.R., Zhou D., Rong C.J., Deng X.M., Zhou R.M., Qin M.L., Huang Q.H., 2009, Isolation of high efficient sulphate-reducing bacteria and its biological desulphurization capability. Environ. Sci. Technol., 32, 13-17.

Hu K.G., Wang A.H.., Feng Z.G., 2007, Sulphate-reducing bacteria and dealing with the role of sulphate wastewater. Uran. Min. Metal., 1, 48-52.

Kumar R., Nongkhlaw M., Acharya C., Joshi S.R., 2013, Bacterial community structure from the perspective of the uranium ore deposits of Domiasiat in India, Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci., The National Academy of Sciences, India, DOI 10.1007/s40011-013-0164-z.

Middelburg J.J. 2000, The geochemical sulphur cycle. In:Lens and Hulsoff-Pol(Eds.). Environmental technologies to treat sulphur pollution: Principles and Engineering. IWA Publishing, London. UK, 33-45.

Nagarajan N.R., Sudhakar H., 2012, Isolation of sulfide oxidizing bacteria from sewage - Thiobacillus species. Bull. Environ. Pharm. Life Sci., 1, 40-42.

Rein N.B., 2002, Biological sulphide oxidation in heterotrophic environment. MSc Dissertation, Rhodes University, Grahamstown, South Africa.

Rose P.D., 2002, Overview Volume 1: In: Salinity, Sanitation and Sustainability: A study in environmental biotechnology and integrated wastewater beneficiation in South Africa. WRC Report No.: TT 187/02. Water Research Commission, South Africa.

Xu X., Chen C., Lee D-J., Wang A., Guo W., Zhou X., Guo H., Yuan Y., Ren N., Chang J-S., 2013, Sulphate-reduction, sulphide-oxidation and elemental sulphur bioreduction process: Modelling and experimental validation, Bioresource Technol., 147, 202-211.