ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Kinetics of Copper Removal from Electro Coating Industrial Sludge through Biosolubilization: Effects of Sulfur Concentration.

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ABSTRACT

In this study, typical characteristics and kinetics of biosolubilization of copper from the electro coating industrial sludge using microorganism, *Acidithiobacillus ferrooxidans* were investigated. Since elemental sulfur (S^0) is key nutrient and chief energy source for *A. ferrooxidans*, it important to study the effect of S^0 concentration on biosolubilization to optimize the S^0 requirement. The experiments were carried out in 250 mL Erlenmeyer flasks with S^0 concentration ranging from 0.2 to1.0% (w/v) at temperature 30 °C. Each flask contained electro coating sludge of 0.5% (w/v) and was shaken at 200 rpm. The attainment of Cu biosolubilization was inspected over the period of 20 days. It was observed that the high pH reduction, absence of lag phase and improved Cu solubilization were obtained in the experiment with 0.6% (w/v) S^0 concentration. At this S^0 concentration, the efficiency of biosolubilization of Cu from the sludge was 54.28% after 20 days. The pseudo-first order kinetic equation was used to determine the rate-constant of Cu solubilization. The kinetic study indicated that the rate-constant of Cu solubilization was observed to be maximum while using 0.6% (w/v) S^0. Using shrinking core model kinetics, it was also observed the rate of solubilization was controlled by the chemical reaction step.

Keywords: electro coating sludge, biosolubilization, pseudo-first order, shrinking core model.

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INTRODUCTION

Mechanization and technology evolution results in boost of waste products in the environment and tons of retained heavy metal laden sludge was dumped in enormous quantity around the world. Before disposal of such sludge, removal of heavy metals as waste treatment is immense important. It involves high cost and human effort for the administration of sludge detoxification [1]. In process like electro coating and metal finishing industries, heavy metal hydroxides and toxic metal cyanides are generated in enormous quantities during industrial activity as sludge [2]. Heavy metals are more toxic and non-biodegradable in the soil matrix when disposed in land. Improper treated disposal of heavy metal laden sludge in the land for a long period of time which circulates through the food chain causes hazardous issues to the humans. [3,4]. So, the eradication of heavy metals from the sludge is one of the important things to sustain the contentment of human being.

The heavy metal concentration can be reduced either by pretreatment methods such as alkaline-chlorination-oxidation, electro coagulation, adsorption, membrane process, reverse osmosis, evaporative recovery, ion exchange, and electrochemical treatment [5,6].The major constraint by this method are demand of huge amount of chemicals, high process cost, complication in the operational procedure and release of harmful gases in the atmosphere [7- 9]. In the last decade, heavy metal removal by biological method studies had been employed in sludge treatment in different countries due to its low cost when compared to chemical solubilization. The effectiveness of this method is very high and it follows ecofriendly approach [10,11]. Generally, sulfur-oxidizing bacteria such as *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Acidithiobacillus caldus, Acidithiobacillus albertensis and Acidiphilium acidophilum* are the most important strains for biosolubilization process [12]. The utilization of sulfur, bio-acidification, and metal solubilization during the process can be represented by the following equations.

$$S^o + H_2O + (3/2)\ O_2 \rightarrow H_2SO_4 \text{ ………… (1) (by bacterial catalysis)}$$

$$H_2SO_4 + \text{Sludge-M} \rightarrow \text{Sludge-2H} + MSO_4 \text{………. (2) (by reaction)}$$

When elemental sulfur (S^0) provided to microorganism as a substrate, the bio-oxidation of sulfur drives the production of sulfuric acid (Eq.1) which is liable for metal solubilization (Eq.2). The S^0 concentration in biosolubilization process is one of the key aspects for the sulfuric acid production which determines the rate and efficiency of metal solubilization. Low concentration of S^0 results in inadequate production of acid which could not provide satisfactory metal removal by biosolubilization. Very high concentration of S^0 results in insoluble surplus S^0 which inhibits growth of microorganism. Therefore, the treatment with necessary optimum S^0 concentration is quite important for biological treatment of sludge. Although, several studies have been disclosed elsewhere, yet there is still a lag of kinetic information on biological regimen of industrial sludge treatment.

In this work, experimental studies have been carried out on removal of copper by biosolubilization from electro coating industrial sludge propagated in typical effluent treatment plant in Chennai, Tamil Nadu by employing sulfur adapted *A. ferrooxidans*. Experiment was performed to determine the optimum S^0 concentration and Cu removal by biosolubilization. In addition, the kinetic study for rate of biosolubilization of Cu based on pseudo-first order model was explored. The rate-controlling step was also identified using the shrinking core model (SCM).

MATERIALS AND METHODS

Characterization of Electro Coating Sludge

Heavy metal enriched sludge was obtained from electro coating industrial sector situated in Chennai, India. It was collected from the sludge bed in the effluent treatment section and stored at temperature of 4 °C using polythene sterilized bags. It was then air dried at room temperature for overnight. In order to assess the total Cu content in the sludge, a sample was digested with nitric acid: per chloric acid: sulfuric acid at the ratio of 8:1:1. Dissolved Cu concentration was analyzed by using atomic absorption spectrometer (Perkin Elmer, AA200 model). The pH was interpreted by using 10:25 dry sludge/water extract through calibrated pH meter (Eutech Instruments, Singapore). To figure out the organic matter present in the sludge, Walkely-Black method (with standard 1N $K_2Cr_2O_7$ and Ferroin indicator) was used. Micro-Kjeldahl distillation procedure was used to scrutinize the total nitrogen content. Total available phosphorus in the sludge was estimated by using Micro-vanadate-molybdate method after the extraction with 0.5M sodium bicarbonate. Calcium, magnesium and potassium content in the sludge were determined by using a flame photometer (Elico, CL378 model) after the ammonium acetate extraction. Total soluble sulfate was determined by using UV-visible spectrophotometer (Hitachi, U2900 model) after precipitation as barium sulfate as out lined in APHA [13]. A number of five replicates were employed for sludge characterization and mean values of replicates were considered.

Bacterial Strain and Adapted Culture Enhancement

The sulfur-oxidizing bacterial strain, *A. ferrooxidans* was obtained from National Collection of Industrial Microorganism, Pune, India (NCIM, No: 5371). The strain was grown in 9K synthetic medium (pH 2.5) with S^0 as key nutrient. The medium contained the following chemical compositions: S^0 (sterilized by tyndallization), 2g/L; $MgSO4.7H_2O$, 0.5 g/L; $(NH4)_2SO_4$, 3 g/L; K_2HPO_4, 0.5 g/L; $Ca(CO_3)_2$, 0.01 g/L; and KCl, 0.1 g/L. To develop sludge adapted culture, 90 mL sterilized media supplemented with 0.3% (w/v) of electro coating sludge along with 10% (v/v) of inoculum and incubated at 30 °C in a rotary shaker at 180 rpm for two weeks. From this culture, 10 mL culture medium was transferred to the fresh media containing 0.5% (w/v) of sludge. In this way, further step-wise transfers were made to the fresh media containing sludge levels (w/v) of 0.8% and 1.0%. It was used as the working cell culture for the biosolubilization experiments which having increased resistance activity of the culture against sludge toxicity.

Biosolubilization Assay

Biosolubilization experiment was performed in 250 mL Erlenmeyer flasks, each flask had working volume of 100 mL containing 90% (v/v) of 9K medium and 10% (v/v) adapted inoculum along with dry sludge concentration of 5% (w/v). Predetermined sulfur concentrations of 0.20, 0.40, 0.60, 0.80, and 1% (w/v) were used for determining the effect of S^0 concentration. Biosolubilization process was carried out at agitation speed of 200 rpm and temperature 30 $^\circ$C. All experiments were performed as triplicate and the mean values were taken in to account. A control experiment was carried out with 5% (w/v) of sludge concentration and 0.2 % (w/v) of sulfur without inoculum to compare with biological process. During the biosolubilization, pH was measured every day by using a calibrated pH meter (Eutech Instruments, Singapore). Oxidation-reduction potential (ORP) of the medium was measured using a calibrated ORP meter (Eutech Instruments, Singapore) for every day. At every two days of interval, the samples (5 mL) were collected and the dissolved Cu concentration was analyzed by atomic absorption spectrometry. The volume depletion in working solutions by the sample collection and the deficit due to evaporation were remitted by the addition of a parallel volume of fresh nutrient solution (9K medium without elemental sulfur). Metal solubilization efficiency, denoted by η_{Cu} (%), was calculated as the ratio between the solubilized Cu and total Cu present in the primary sludge as given in equation(3).

$$\eta_{Cu}\,(\%) = [(M^{t}_{sol}) / (M_T)] \times 100 \;\ldots\ldots\ldots.. (3)$$

M^{t}_{sol} is the solubilized Cu concentration at the time t during the operation, and M_T is the total Cu concentration in the primary sludge.

Kinetic Procedure

Based on the pseudo-first order kinetics, the metal dissolution can be described using empirical equation [14],

$$\text{Rate of solubilization of Cu} = \frac{dC_{Cu}}{dt} = k(C - C_{Cu,t}) \ldots\ldots\ldots\ldots. (4)$$

(dC_{Cu}/dt) is the rate of solubilization of Cu with inflation in time. k is defined as the rate constant of Cu solubilization. By integrating the equation (4) between initial and final limits of metal concentration (at t = 0, $C_{Cu,t}$ = 0 and t = t d, $C_{Cu,t} = C_{Cu,t}$) equation (5) can be obtained.

$$\ln\left(\frac{C}{C - C_{Cu,t}}\right) = kt \ldots\ldots\ldots (5)$$

In equation (5), C and $C_{Cu,t}$ are the initial Cu concentration present in the original sludge accessible to leach and leached Cu concentration in aqueous phase at the specific time 't'. The slope obtained from the plot of $\ln[C/(C-C_{Cu,t})]$ *vs* time gives the first order reaction rate constant. In order to get clear interpretation of metal biosolubilization reaction mechanism, the kinetics of fluid–particle reaction was resolved [15] by using shrinking core model (SCM). According to SCM, the step diffusion through ash layer or chemical reaction may have impact in the overall metal biosolubilization rate. The developed equation in terms of conversion and time for the mentioned steps are $1+2(1-X_{Cu})-3(1-X_{Cu})^{2/3} = K_{obs}t$, and $1-(1-X_{Cu})^{1/3} = k_{obs}t$, respectively. X_{Cu} is the fraction of Cu solubilized at the aqueous phase during the time 't' and k_{obs} is observed kinetic constant applicable to the respective model. Based on the significance of regression correlation analysis from the plots of $1+2(1-X_{Cu})-3(1-X_{Cu})^{2/3}$ *vs* time and $1-(1-X_{Cu})^{1/3}$ *vs* time, the rate controlling step was determined.

RESULTS AND DISCUSSION

Characteristics Of Sludge

The physico-chemical characteristics of dry electro coating industrial sludge sample are enlisted in Table 1. The sludge characterization demonstrated that it was in alkali condition (pH 9.1). The total available Kjeldahl Nitrogen in the sludge found to be 2,505 mg kg^{-1}. The presence of considerable amount of phosphorus (1,509 mg kg^{-1}) and moderate level of potassium (356 mg kg^{-1}) were also found. These could be used by the culture, *A. ferrooxidans* during its growth as nutrients. The presence of calcium and magnesium were found as 20,210 and 8,610 mg kg^{-1}, respectively. Since the amount of organic content in the sludge was very low (0.9 mg kg^{-1}), the risk due to inhibition for the microbial growth from organic carbon can be ignored. The heavy metal assessment of the sludge indicated the presence of total copper in the primary sludge was 3,540 mg kg^{-1}.

Table 1: Physico-chemical properties of sludge sample (all values are expressed mg per kg of dry sludge)

Sl. No	Selected parameters	Compositions
1	pH	9.1 ± 0.14
3	Total Nitrogen	2,505 ± 67
4	Total available phosphorus	1,509 ± 46
5	Sulfate	520 ± 68
6	Organic mater	0.9± 0.14%
7	Calcium	20,210± 176
8	Magnesium	8,610± 243
9	Potassium	356 ± 38
10	Copper	3,540± 72
11	Lead	32 ± 12
12	Zinc	47± 02
13	Nickel	36± 9

± indicates the standard deviation of data represent mean value of five samples

Effect of S^0 Concentration on Media pH During Biosolubilization

During biosolubilization the S^0 oxidation by *A. ferrooxidans* was assessed by determination of medium pH. The variation in medium pH at different S^0 concentrations was presented in Figure 1. In control experiment, without addition of bacteria, absence of significant change in pH value was found. However, a small drop in pH value (from 4 to 3.6) occurred due to chemical oxidation of S^0. In the experiments at different S^0 concentrations [0.2, 0.4, 0.6, 0.8, and 1% (w/v)], a drastic decrease in the pH values were observed in two weeks. This pointed out good adaptation and rapid growth of microorganisms accompanied by the better of S^0 oxidation. Then a gradual decrease in the pH value was observed till the 20th day. Although the decrease in pH value increases with S^0 concentration, no appreciable decrease was found in beyond 0.6 % of S^0. After the 20th day, the pH values of the biosolubilization media with 0.2 and 0.4 % S^0 reduced to 2.57 and 2.45, respectively, from initial value of 4.0. The least value of pH 2.08 was observed in the experiment with 0.6 % S^0. In the experiments with 0.8 and 1.0% S^0, pH values observed were 2.38 and 2.51, respectively after 20th day. Therefore, it is clear that 0.6% S^0 concentration is enough to bring down the pH value about 2.0, which is needed for heavy metal solubilization.

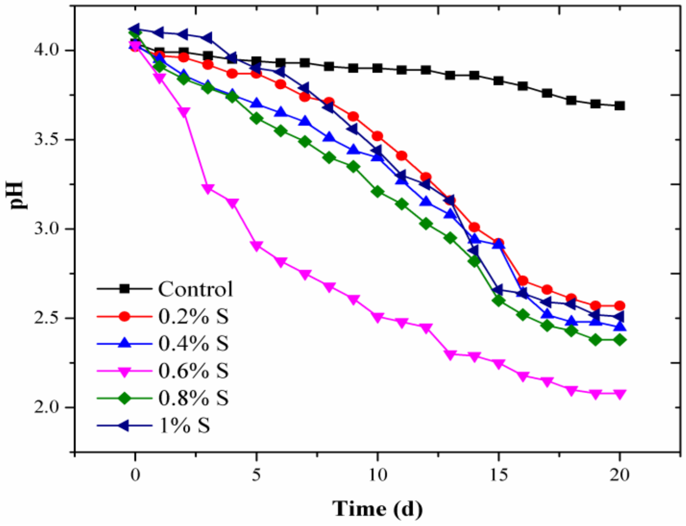

Figure 1: Change in pH value during biosolubilization of Cu at different sulfur concentrations

Effect of S^0 Concentration on Oxidation Reduction Potential of the Medium

The bacterial oxidation of S^0 by *A. ferrooxidans* is collaborated with change in oxidation reduction potential (ORP) of biosolubilization medium. The change in ORP during biosolubilization is presented in Figure 2. At the end of 20th day, the ORP observed in control experiment was about 196 mV due to absence of bacterial activity. The ORP attained in the experiments with 0.2, 0.4, 0.8 and 1% for 20 days were about 229, 237, 322, 381 mV respectively. A significant evolution in ORP from 162 to 466 mV was found in the experiment 0.6 % S^0, which indicates the best oxidizing environment that provides effective condition for metal biosolubilization. This high order of ORP can be contributed by the increase in $[SO_4^{2-}]/O_2$ ratio which are attributed by the oxygen utilization and sulfate production due to bacterial oxidation of S^0.

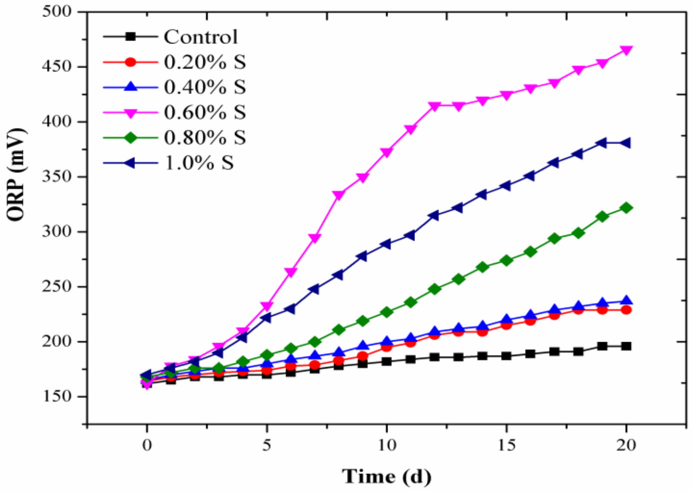

Figure 2: Change in ORP value during biosolubilization of Cu at different sulfur concentrations

Removal of Copper through Biosolubilization

The efficiency of Cu solubilization with time at different S^0 concentration was depicted in Figure 3. Control experiment showed 5.7% solubilization of Cu. This was occurred mainly due to the added sulfuric acid for initialization of pH value to 4. In the treatments with 0.2, 0.4, and 0.6% S^0 concentrations, the Cu solubilization efficiencies were 31.53%, 37.93%, and 54.28%, respectively. With further increase in S^0 concentration to 0.8 and 1.0% S^0, the solubilization of Cu reduced to 42.06 and 32.53%, respectively. The biosolubilization of Cu ranging 80–90% reported elsewhere which depends on the sludge type and the solid concentration of the sludge. While considering the total Cu present in the primary sludge, removal of 54.28% Cu by biosolubilization is quite good. The profiles of Cu solubilization observed in the present study were well assisted by the decrease in pH coupled with increase in ORP for biosolubilization operation.

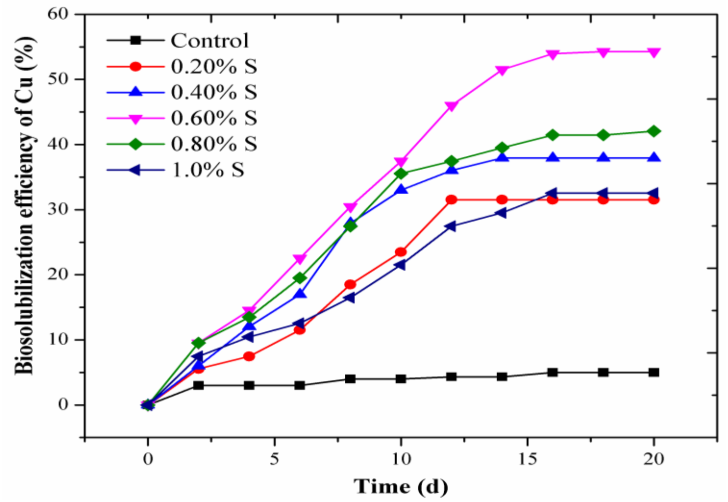

Figure 3: Biosolubilization efficiency of Cu at different sulfur concentrations

Kinetics on Rate and Rate-Controlling Step of Biosolubilization

The pseudo-first order empirical equation (Eq.5) was used to resolve the rate kinetics of copper biosolubilization from electro coating industrial sludge. Figure 4 show plotting of solubilization data to eq. (5). It is clear that the pseudo-first-order rate kinetic model is well fitted to the experimental data and the correlation coefficients were high ($R^2 > 0.9$). The rate constant values increased with S^0 concentration up to 0.6%. The rate constant values were 0.0232 d^{-1} at 0.2% (w/v) sulfur. With further increase in the S^0 concentration to 0.4%, the rate constant values increased to 0.0297 d^{-1}. The maximum rate constant value of biosolubilization was found to be 0.0452 d^{-1} with 0.6% S^0. However, increase in sulfur concentration beyond 0.6% resulted in a decline in the rate constant value. The rate constant value dropped to 0.033 d^{-1} at 0.8% S^0 and further dropped to 0.0229 d^{-1} at 1% S^0 concentration. This decline was because of stress load and less oxygen mass transfer caused by the presence of excess insoluble S^0 in the system. It becomes apparent that S^0 concentration of 0.6% (w/v) is quite enough to achieve enhanced pH reduction, heavy metal solubilization, and metal removal rate for the type of microbe and sludge used in this process.

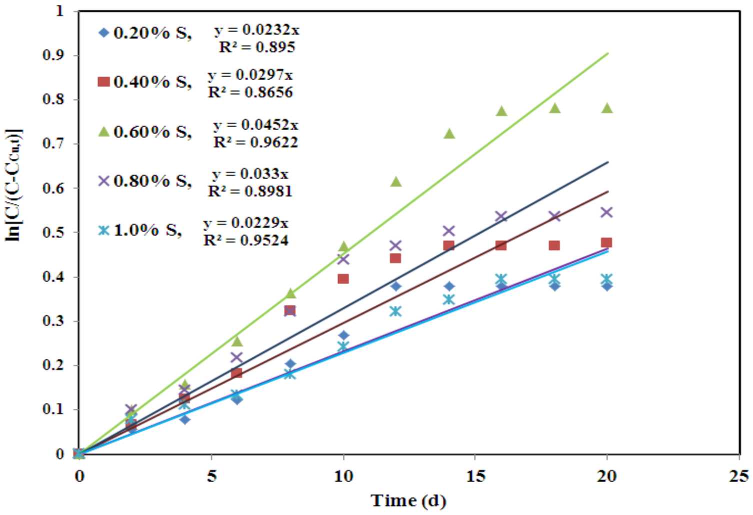

Figure 4: Pseudo-first order kinetic plot for Cu solubilization at different sulfur concentrations

The shrinking core model was used for determining the rate-controlling step. According to this model ash layer diffusion or chemical reaction may control the overall rate of Cu solubilization. Figures 5(a) and (b) show the graphical representation to test the significance of mathematical linear equation of controlling steps mentioned earlier. The correlation coefficients were given in Table 2. It was observed that solubilization data fit better to chemical reaction controlled shrinking core model. The rate controlling factor is chemical reaction between sulfuric acid produced by oxidation of sulfur and metal components present in the sludge. It was observed that there was no effect of sulfur concentration on the rate-controlling step in Cu solubilization.

Table 2: Values of regression coefficient from graphical fitting of Cu solubilization data to rate-controlling step models

Sl. No	S^0 Content (%) w/v	Controlling-step	
		Ash layer diffusion	Chemical Reaction
1	0.20	0.8669	0.8914
2	0.40	0.9003	0.9124
3	0.60	0.9191	0.9552
4	0.80	0.9382	0.9514
5	1.0	0.9199	0.9455

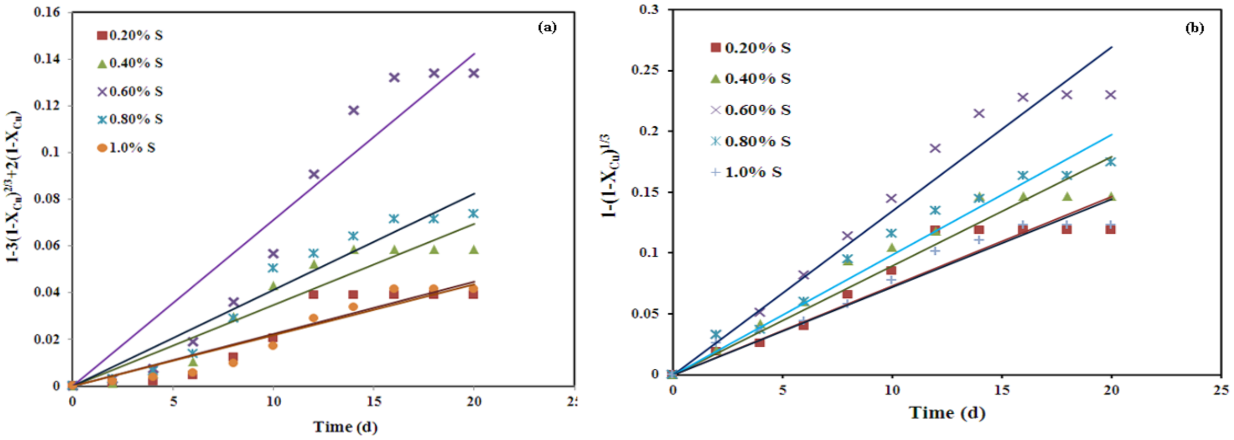

Figure 5: Plot for determination of controlling-step for Cu solubilization. (a) Ash layer diffusion control and (b) Chemical recation control.

CONCLUSIONS

A study on the removal of Cu from the electro coating industrial sludge by adapted, sulfur-oxidizing bacteria, *A. ferrooxidans* through biosolubilization was carried out. The immense importance was given to optimize the S^0 concentration using the following experimental condition: inoculation size; 10% (v/v), working volume; 100 mL, agitation speed; 200 rpm, temperature; 30 °C. From the study, the following conclusions were drawn:

(i) The sufficient S^0 concentration for better solubilization of Cu is found to be 0.6% (w/v) to reach conditions of acidity ($pH \leq 2.08$) and well oxidizing environment ($ORP \approx 466$ mV).
(ii) The rate-constant of Cu solubilization is considerably affected by the sulfur concentration. The experiment with 0.6% of S^0 showed the highest value of rate constant ($0.0452\ d^{-1}$) among the other substrate concentration used.
(iii) Based on SCM, the analysis showed that the rate-controlling step is chemical reaction.

ACKNOWLEDGEMENT

Authors are grateful to the management of K.S. Rangasamy College of Technology (Tamil Nadu, India) for providing infrastructure facilities.

REFERENCES

[1] Sandhya babel, Dominica del Mundo Dacera. Waste Manag 2006; 26: 988-1004.
[2] Venkatesa Prabhu S and Baskar R. Polish J Environ Stud 2015; 24:1–9.
[3] Roska Y. Stefanova. J Environ Sci Health 2001; A36: 1845-1860.
[4] Venkatesa Prabhu S and Baskar R. J Korean Soc App Biol Chem 2015; DOI 10.1007/s13765-015-0027-9.
[5] L Wang, H Chua, PK Wong, WH Lo, and PHF Yu. J Environ Sci Health 2003; A38: 521-531.
[6] Tyagi RD, Couillard D and Tran F. Environ Poll 1988; 50: 295-316.
[7] Klaus Bosecker. FEMS Microbiol Rev 1997; 20: 591-604.
[8] Ashish Pathak, Dastidar MG and Sreekrishnan TR. Journal of Environmental Science and Health, Part A: Toxic /Hazardous substances and Environmental Engineering. 2008; 43: 402-411.
[9] Nareshkumar R, Nagendran R. J Hazard Mater 2008;156:102-107.
[10] Luciene D. Villar and Oswaldo Garcia Jr. Part A:Toxic/Hazardous Substances and Environmental Engineering. 2006; 41: 211-222.
[11] Yun-Guo Liu, Ming Zhou, Guang-Ming Zeng, Xin Li, Wei-Hau Xu, Ting Fan. J Hazard Mater 2007; 141: 202-208.
[12] Donghee park, Dae Sung Lee, Jong Moon Park, Hee Dong Chun, Sung Kook Park, Ikuo Jitsuhara, Osama Miki and Toshiaki Kato. Ind Eng Chem Res 2005; 44:1854-1859.
[13] Yuan-Shan Wang, Zhi-Yan Pan, Jian-Min Lang, Jian-Min Lang, Jian-Miao Xu, Yu-Guo Zheng. J Hazard Mater 2007; 147:319-324.
[14] Venkatesa Prabhu S and Baskar R. Res J Chem Environ 2014; 18:70−76.
[15] Younesi SR, Alimadadi H, Keshavarz Alamdari E and Marashi SPH. Hydrometall 2006; 84: 155–164.