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Kinetics of SO_4^{-2} reduction under different growth media by sulfate reducing bacteria

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Abstract

Sulfate Reducing Bacteria (SRB) were used to reduce the SO_4^{-2} concentration in waste water. The growth pattern of SRB was found by varying the concentration of nutrients and the biomass. The specific reaction constant was evaluated in each case.

Introduction

The presence of SO_4^{-2} in the natural stream is due to the pollution caused by the mine water and industrial waste water. The occurrence of SO_4^{-2} in mine water is due to the bacterial action on sulfidic ore. Industrial effluents contain considerable amounts of SO_4^{-2} due to presence of heavy metal ions and spent sulfuric acid. The SO_4^{-2} in the solution is the main cause of excess mineralisation of surface water. It can be neutralised by lime but due to non-stochiometric reaction the excess Ca ion increases the hardness of water (Mishra & Roy Chaudhury 1995). To overcome the shortcoming of lime treatment various alternatives have been developed such as ion exchange, adsorption, reverse osmosis and biological methods (Rich *et al.* 1987, Dean *et al.* 1972, Mishra *et al.* 1994, Lapakko *et al.* 1988).

SO_4^{-2} can be reduced by anaerobic sulfate reducing bacteria (SRB) belonging to genera *Desulfovibrio* and *Desulfotomaculum* (Solzhenkin & Lyubamina 1985). The SRB use SO_4^{-2} as an electron acceptor and the end product being hydrogen sulfide. The biological hydrogen sulfide then reacts with metal ions to form the respective metal sulfides (Imai 1986). The main problem of SRB is the super sensitivity towards metal ions. It was reported (Hammck *et al.* 1993) that metal ions concentration more than 1 ppm adversely affect the growth of said microorganism. Therefore the application of SRB for treatment of waste waters containing both metal and SO_4^{-2} have limited use specifically when the metal ions concentration is more. The use of SRB for treatment of waste water containing heavy metal ions can be possible only when the bacteria was grown separately and the biogenic product formed by the reduction of SO_4^{-2} is then used subsequently to remove metal ions from the solution. So the efficiency of metal ion removal depends on the kinetics of SO_4^{-2} reduction by SRB. Therefore to utilise the SRB in a fruitful way the first step is to evaluate the SO_4^{-2} reduction kinetics. So in the present work some attempts have been initiated to find out the kinetics of SO_4^{-2} reduction under various conditions.

Materials and methods

Microorganism

Sulfate reducing bacteria were isolated from Aska Sugar Mill effluent water. The isolated SRB were grown in a medium having following composition (g/l) KH_2PO_4 – 0.5; NH_4Cl – 1.0; $CaSO_4$, $2H_2O$ – 1.0; $MgSO_4$, $7H_2O$ – 2.0; Sodium lactate – 3.5; $FeSO_4$, $7H_2O$ – 0.5 and Yeast extract – 1.0. The pH of the medium was adjusted to 7.5. The growth studies were carried out in airtight stoppered bottle at 37 °C. The SO_4^{-2} concentration in the solution was analysed by

Table 1. Sulfate reduction rate and dependence factors of SRB for different parameters studied

Experiment Number	Parameters studied	$k \times 10^3$ (h^{-1})	SO_4^{-2} reduction rate (mg l^{-1} h^{-1})	Dependence factor
	Biomass conc. $\times 10^{-5}$ (cells/ml)			
1	1.6	0.7	1.18	
2	3.2	1.0	1.23	
3	3.9	1.3	1.39	0.56
4	4.5	1.4	1.65	
5	6.9	1.5	1.74	
	SO_4^{-2} concentration (g/l)			
6	1.33	0.17	0.96	
7	2	0.1	0.82	
8	2.27	0.06	0.68	−1.78
9	3.27	0.05	0.49	
10	3.6	0.023	0.42	
	N_2 concentration (g/l)			
11	0.34	0.301	0.58	
12	0.7	0.31	0.63	
13	1	0.338	0.72	0.13
14	1.5	0.356	0.93	
15	2	0.376	1.05	
16	3	0.096	0.58	
	PO_4^{-3} concentration (g/l)			
17	0.14	0.10	1.23	
18	0.7	0.09	1.08	
19	2.5	0.07	0.51	−0.26
20	4.0	0.05	0.43	
21	8.0	0.04	0.37	
22	12.0	0.03	0.24	

Initial cell concentration (cells/ml)
Experiment No. 6–10 → 2.2×10^4,
11–16 → 3×10^4,
17–22 → 1.5×10^4.

$BaCl_2$ method (Vogel 1959). Bacterial concentration was measured under microscope using Petroff Hauser counting chamber.

Results and discussion

From the media composition it is understood that the growth of bacteria depend on PO_4^{-3}, N_2, SO_4^{-2} and organic acid concentration. The SO_4^{-2} reduction kinetics were determined by varying the concentration of PO_4^{-3}, N_2, SO_4^{-2} and biomass concentration.

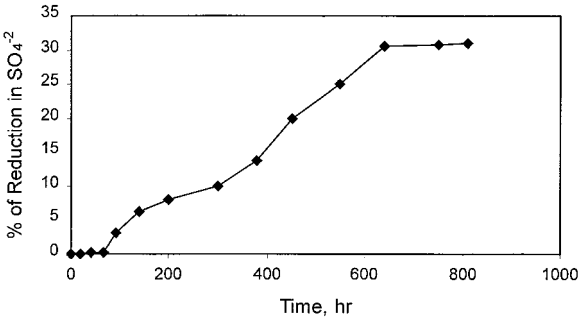

Figure 1. Growth curve for sulfate reducing bacteria.

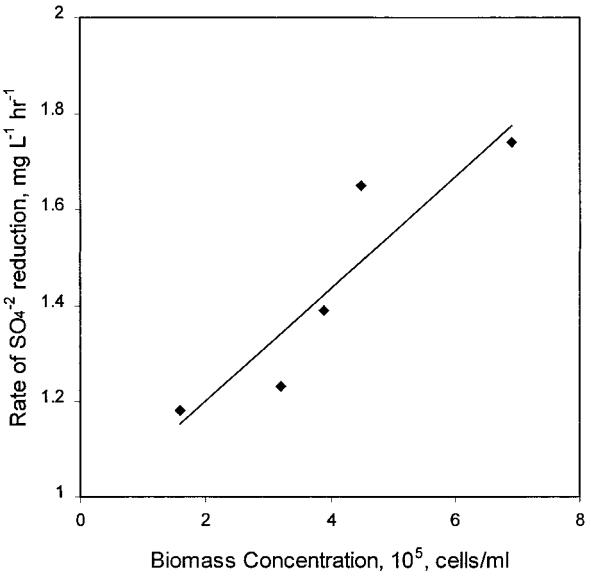

Figure 2. Effect of biomass concentration on the SO_4^{-2} reduction rate.

Effect of time

The SRB is allowed to grow for 1000 h. The growth of the bacteria was measured in terms of SO_4^{-2} (Maree & Strydom 1987) as SRB produce H_2S as end product which is directly associated with the reduction of SO_4^{-2} concentration. Figure 1 shows the SO_4^{-2} reduction at different time intervals. It was observed that the lag and log period continued for 70 and 600 h, respectively. After 600 h it attained a stationary phase. Therefore subsequent kinetics studies were carried out for 600 h.

Effect of biomass concentration

The biomass concentration was varied from 1.6 to 6.9×10^5 cells/ml. It was observed that SO_4^{-2} reduction rate increased with increase of biomass concentration as shown in Figure 2. If the SO_4^{-2} reduction reaction is assumed to be of first order, then a plot of $\ln[(SO_4)_t/(SO_4)_o]$ versus time(h) would give a straight line and the specific reaction rate constant (k) can be calculated from slope. Figure 3 shows the first order plot of various biomass concentrations. The k values are shown in Table 1.

Effect of SO_4^{-2} concentration

The SO_4^{-2} concentration was varied from 1.3 to 3.6 g/l at an initial biomass concentration of 2.2×10^4 cells/ml. It was observed that the increase of SO_4^{-2} concentration decrease the rate of reduction as shown in Table 1 may be due to increase toxicity of SO_4^{-2} ion towards SRB. The specific reaction rate constant was calculated from first order plot and shown in Table 1.

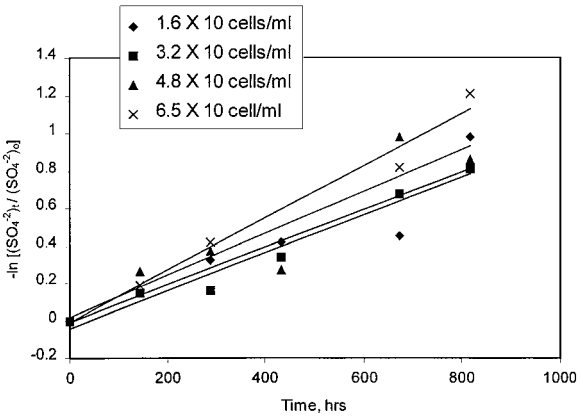

Figure 3. First order plot for different biomass concentration (initial).

Effect of N_2 concentration

The N_2 concentration was varied between 0.34 to 2 g/l at an initial bacterial concentration of 3×10^4 cells/ml. The SO_4^{-2} reduction rate, and specific reaction rate constants are shown in Table 1. It was observed that increase in N_2 concentration increases the reduction rate upto N_2 concentration of 2g/l and thereafter it decreased. The decrease of reduction rate beyond N_2 concentration of 2g/l may be due to bacterial inhibition at higher N_2 concentration.

Effect of PO_4^{-3} concentration

PO_4^{-3} concentration was varied from 0.14 to 12 g/l at an initial biomass concentration of 1.5×10^5 cells/ml. The SO_4^{-2} reduction rate and specific reaction rate constants are shown in Table 1. It was observed that the PO_4^{-3} concentration was very sensitive towards the bacterial growth as the reduction rate decreased with the increase of the same.

Evaluation of modified rate equation

Usually the bacterial growth kinetics is assumed to be 1^{st} order in nature (Modak et al. 1996). Therefore the rate equation of SRB can be written as:

$$\text{Rate} = -\mathrm{d}[SO_4^{-2}]/\mathrm{d}t = k[SO_4^{-2}]. \quad (1)$$

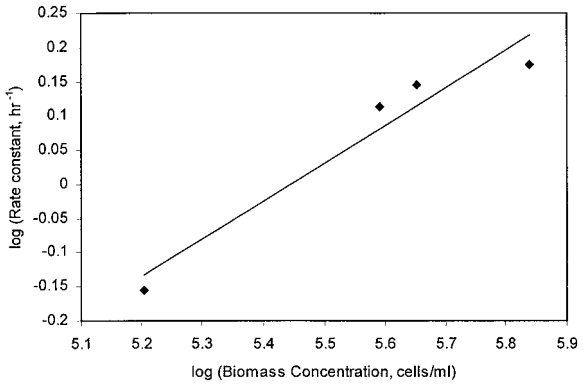

Figure 4. Evaluation of dependence factor for the different biomass concentration

But experimentally it is found that the rate of growth of SRB is also depends on other parameters like biomass, N_2, and PO_4^{-3} concentration. So the growth of SRB is considered to be pseudo first order in nature and the rate equation (1) can be modified by introducing dependence factors and can be written as:

$$\text{Rate} = \text{k[Biomass concentration]}_1^n [N_2]_2^n [SO_4^{-2}]_3^n [PO_4^{-3}]_4^n, \quad (2)$$

Where n_1, n_2, n_3 and n_4 are the dependence factors for biomass, N_2, SO_4^{-2}, and PO_4^{-3} concentration respectively.

The dependence factor of a particular parameters can be calculated from the slope of a graph plotted between log k versus log of that particular parameter. Figure 4 shows the plot of log *k* vs. log (biomass variation) and the slope was found to be 0.56, i.e, the dependence factor for biomass variation. The dependence factors for N_2, PO_4^{-3}, and SO_4^{-2} are found to be 0.13, -0.26, and -1.78 respectively and are shown in Table 1. Therefore the modified rate equation can be written as:

$$\text{Rate} = \text{k[Biomass concentration]}^{0.56} [SO_4^{-2}]^{-1.78} [N_2]^{0.13} [PO_4^{-3}]^{-0.26}. \quad (3)$$

Conclusions

(a) SRB show a lag phase of 70 h and the log phase continue up to 600 h.
(b) SRB show positive growth rate with the increase of biomass and N_2 concentration.
(c) The increase of SO_4^{-2} and PO_4^{-3} concentrations appreciably decrease the bacterial growth kinetics.
(d) Based on dependence factor a unified rate equation have been formulated.

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References

Dean JG, Bosqui FL. 1972 Removing heavy metals from waste water. *Environ Sci Technol* **6**, 518–522.

Hammck RN, Dvorak DH, Edenborn HM. 1993 The use of biogenic hydrogen sulphide to selectively recovery Cu and Zn from severely contaminated mine drainage. *Biohydrometallurgy Technologies, Minerals, Metals and Material Society*, 631–639.

Imai K. 1986 Utilization of Sulfate-reducing and photolithotrophic bacteria in biohydrometallurgy. In: Lawrina RN, Brain RMR, Ebner HG, eds. *Fundamental and Applied Biohydrometallurgy*. Amsterdam, Elsevier, 383–394.

Lapakko K, Eger P. 1988 Trace metal removal from stockpile drainage by peat. *Mine Drainage and Surface Mine Reclamation; Bureau of Mines. IC 9183* **1**, 291–300.

Maree JP, Strydom WF. 1987 Biological Sulphate removal from industrial effluent in an up-flow packed bed reactor. *Wat Res* **21**(2), 141–146.

Mishra SP, Roy Chaudhury G. 1994 Kinetics of zinc adsorption on charcoal, *J Chem Tech Biotechnol* **59**, 359–364.

Mishra SP, Roy Chaudhury G. 1995 Biosorption of copper by penicillium sp. *Mineral Processing Extractive Metall Rev* **14**, 111.

Modak JM, Natarajan KA, Mukhopadhyay S. 1996 Development of temperature tolerant strains of *Thiobacillus ferrooxidans* to improve bioleaching kinetics. *Hydromet* **42**, 51–61.

Rich G, Cherry K. 1987 *Hazardous Waste Treatment Technologies*. Pudvas Publ. Co., 169.

Solzhenkin PM, Lyubanina LL. 1985 In: Karavaiko GI, Groudev SN, eds. *Proceedings of International Seminar on Modern Aspects of Microbiological Hydrometallurgy and International Training on Microbiological Leaching of Metals and Ores.* Centre of International Projects GKNI, Moscow, 409.

Vogel AI. 1959 *A Text Book of Quantitative Inorganic Analysis*. London, Longmans.