BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2011. Vol. **8**(2), p. 729-734

Stimulatory Effect of Chemical Nutrients on Desulfurization of Indial Coal by a Mutant *Thiobacillus ferrooxidans* X_{200}

D. Sengupta, S. Ganguly* and A.K. Banik

Department of Chemical Engineering Biochemical Engineering Division, Biotechnology Laboratory, University of Calcutta, 92, A. P. C. Road, Kolkata - 700 009 (India).

(Received: 25 June 2011; accepted: 15 August 2011)

Thiobacillus ferrooxidans **treated with ethylmethane sulfonate and UV rays was incubated with coal sample taken from Assam, India for its desulfurization capacity. 2% glucose and 0.7% NH_4Cl served as the best carbon and nitrogen sources respectively. Optimum concentration of both $MgCl_2$ and KH_2PO_4 in fermentation medium was 0.025% supplementation of medium with 10 μg/ml $Fe_2(SO_4)_3.H_2O$ had marked positive effect on desulfurization. Optimization of chemical nutrients resulted in a significant ($p<0.05$) impact on desulfurization (66.1%) by the mutant strain *Thiobacillus ferrooxidans* X_{200} as against parent strain (55.1%).**

Key words: *Thiobacillus ferrooxidans,* Desulfurization, Optimization, Chemical nutrients.

Coal deposits of North-Eastern India have a high sulfur content (2-6%)[1]. During the combustion of these sulfur-bearing coal emissions of volatile sulfur compounds like SO_2, SO_3 and H_2S cause air pollution as well as corrosion in boilers[2]. These problems need an efficient and economic method of coal desulfurization at its source. Precombustion removal of sulfur from coal by microbial action presents an alteractive alternative to the existing physical or chemical methods because it is cost-effective and runs under ambient temperature and pressure conditions. Microorganisms frequently used for this purpose are *Thiobacillus ferrooxidans, Thiobacillus thiooxidans, Thiobacillus acidophilus, Bacillus brevis* etc.[3-17].

The organism used in our study for desulfurization of coal is *Thiobacillus ferrooxidans* which may be described as an iron and sulfur oxidizing bacterium that catalyzes the removal of inorganic (mainly pyritic) sulfur from Coal[3]. We have already developed a mutant strain of *Thiobacillus ferrooxidans* by using multistep mutagenic agents like ethyl methane sulfonate an UV-irraditions. The mutant Strain *Thiobacillus ferrooxidans* X_{200} has a higher sulfur removing capacity (32%) than the parsent strain (10%). The optimum conditions of Physical parameters for desulfurization of coal by *Thiobacillus ferrooxidans* X_{200} have been also developed earlier[4].

The present study deals with the nutritional requirements of the bacterium, viz. concentrations of the suitable carbon source, nitrogen source, $MgCl_2$ and KH_2PO_4. The effect of supplementation of the medium by $Fe_2(SO_4)_3$, H_2O was also studied.

* To whom all correspondence should be addressed.
E-mail: subhadeepgangulyphysiol@rediffmail.com

MATERIAL AND METHODS

Source and composition of coal

Coal samples used in this experiment were obtained from North Eastern Coal fields, Coal India Ltd., Margherita, Assam. The sample was found to contain 3.03% total sulfur in which 14.7% was pyritic sulfur, 20.5% was sulfate sulfur an 64.7% was organic sulfur.

Microorganism

The present strain of *Thiobacillus ferrooxidans* was obtained from the Department of Molecular Biology, Biophysics and Genetic Engineering, University of Calcutta. The culture was maintained in $FeSO_4$ medium which was a 7 : 3 (v/v) mixture of two media of following composition : Medium A – $(NH_4)_2SO_4$, 0.43%; $MgSO_4.7H_2O$, 0.07%; KH_2PO_4, 0.07%; KCl, 0.17; 10(N)H_2SO_4, 0.03 ml in 100 ml deionized double distilled water. Medium B – $FeSO_4.7H_2O$, 10.2%; 10(N) H_2SO_4, 0.2 ml in 100 ml deionized double distilled water. The pH of the medium was adjusted to 4.0. The parent strain on exposure to ethyl methane sulfonate [0.2 (M) for 120 mins] and UV rays 15 watt Hanovia germicidal lamp for 30 mins) gave the mutant strain *Thiobacillus ferrooxidans* X_{200} which had a higher sulfure removing capacity (32%) from coal.

Fermentation medium and cultural conditions

The fermentation medium for desulfurization of coal by *Thiobacillus ferrooxidans* X_{200}. The fermentation was carried out for 8 days.

Determination of optimum concentration of suitable chemical nutrients

The suitable carbon and nitrogen source for *Thiobacillus ferrooxidans* X_{200} was selected by substituting glucose and NH_4Cl with equivalent amount of different carbon and nitrogen sources respectively. The optimum concentration of the suitable carbon and nitrogen source was determined by performing fermentation experiments with different concentrations of most the most effective carbon and nitrogen source. The optimum concentrations of $MgCl_2$ and KH_2PO_4 were also determined by conducting experiments with different concentrations of $MgCl_2$ and KH_2PO_4 respectively. To study the effect of $Fe_2(SO_4)_3.H_2O$ all the media components were made free from mineral impurities by the process of solvent extraction where chloroform was used as the solvent and 8-hydroxyquinole as the chelating agent[8]. Thereafter, fermentation experiments were performed with different concentrations of $Fe_2(SO_4)_3.H_2O$.

Estimation of sulfur content in coal

Total sulfur of coal was determined by the conventional Eschka's method[18]. Pyritic sulfur and sulfate sulfur was also estimated according to the standard procedures10. The amount of organic sulfur on coal was obtained by subtracting the amount of pyritic sulfur and sulfate sulfur from the total sulfur content. The treated coal samples were futered, dried and analysed for total sulfur content.

Estimation of pH

pH of the fermentation broth was estimated by electronic pH meter.

Statistical analysis

Values were expressed as mean ± SEM, where n=6. The data were analysed using one way ANOVA followed by Dunett's post hoc multiple comparison test using "prism 4.0" soft ware (Graph pad Inc., USA). A "p" value less than 0.05 was considered significan and less than 0.01 was considered highly significant.

RESULTS AND DISCUSSION

Effect of different carbon sources

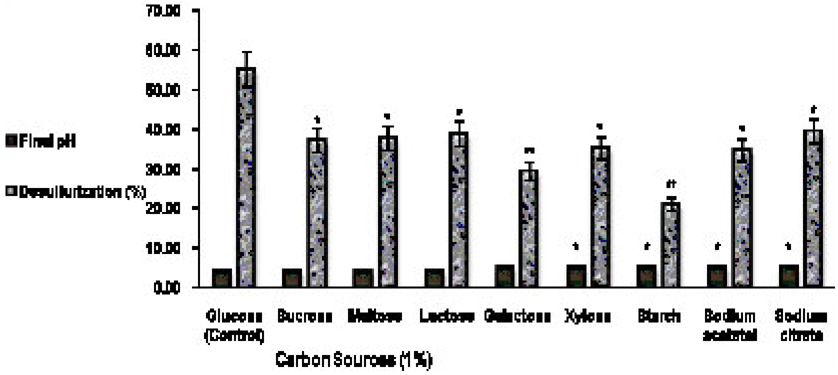
Carbon is required by the bacterium for cellular growth[6]. Carbon dioxide serves as the major source of cell carbon for growing *Thiobacillus ferrooxidans* X_{200}. Carbonate minerals are also used a substitute corbon source[2]. But addition of $CaCO_3$ may inhibit the bacteria to oxidize pyrite due to the acid nutrilizing effect of the carbonate mineral which raise the pH of the leach solution above the upper limit of bacterial activity. Studies with different carbon sources revealed that glucose was the best utilizable carbon (Fig.1).

Determination of optimum concentraton of glucose

The dependence of desulfurization capacity of *Thiobacillus ferrooxidans* X_{200} on glucose concentration is depicted in Fig.2 which shows that the optimum glucose concentration is 2%. Retardation of desulfurization process at higher glucose concentration may be explained by increased substrate concentration above the critical value.

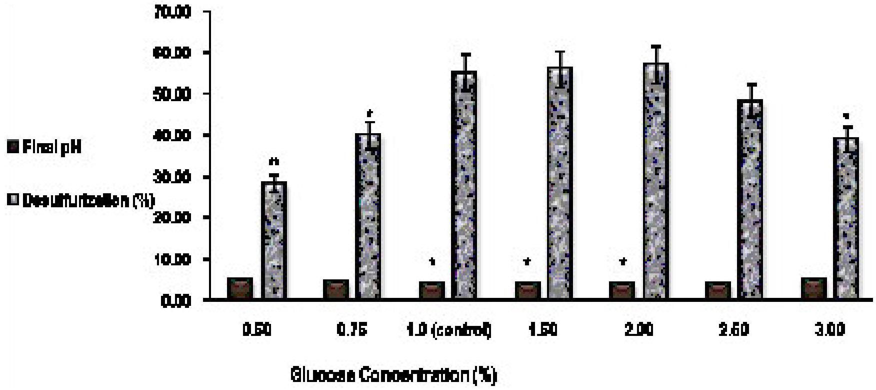
Effect of different nitrogen sources

Nitrogen, as NH_4^+ ion, is essential for



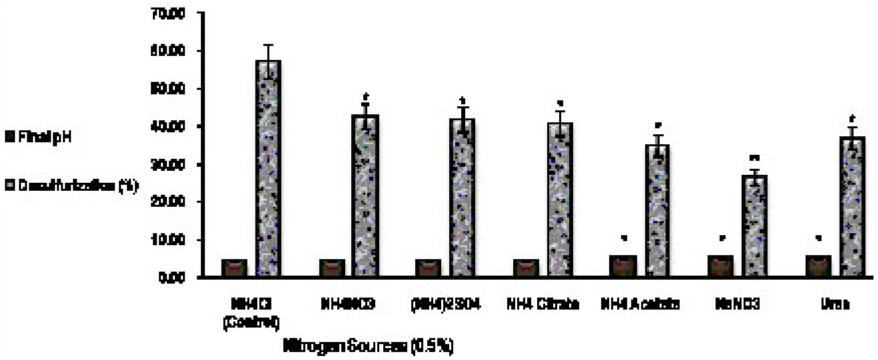
(values were expressed as Mean ± SEM, where n = 6; * $p < 0.05$, ** $p < 0.01$ when compared to control)

Fig. 1. Effect of diffeent carbon sources on desulfurizatin of Coal by the mutant *Thiobacillus ferrooxidans* X_{200}



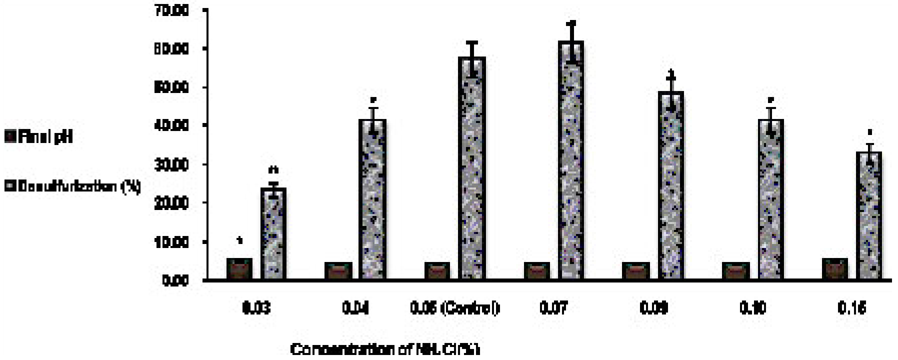
(values were expressed as Mean ± SEM, where n = 6; * $p < 0.05$, ** $p < 0.01$ when compared to control)

Fig. 2. Optimization of glucose concentration for desulfurizatin of Coal by the mutant *Thiobacillus ferrooxidans* X_{200}



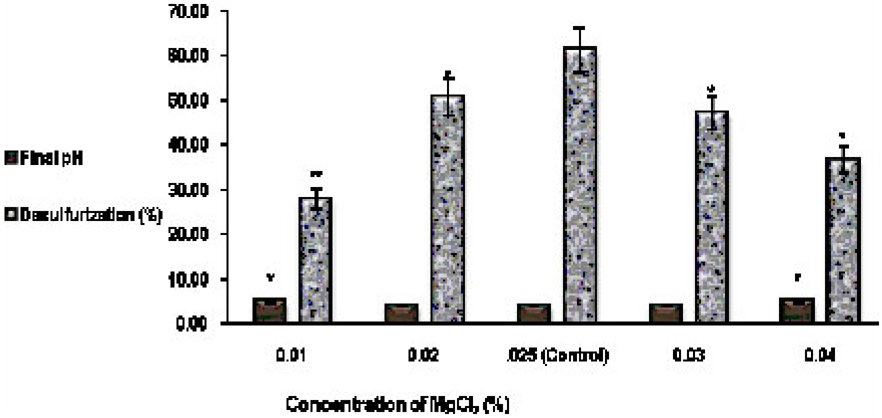
(values were expressed as Mean ± SEM, where n = 6; * $p < 0.05$, ** $p < 0.01$ when compared to control)

Fig. 3. Effect of diffeent nitrogen sources (0.5%) on desulfurizatin of Coal by the mutant *Thiobacillus ferrooxidans* X_{200}



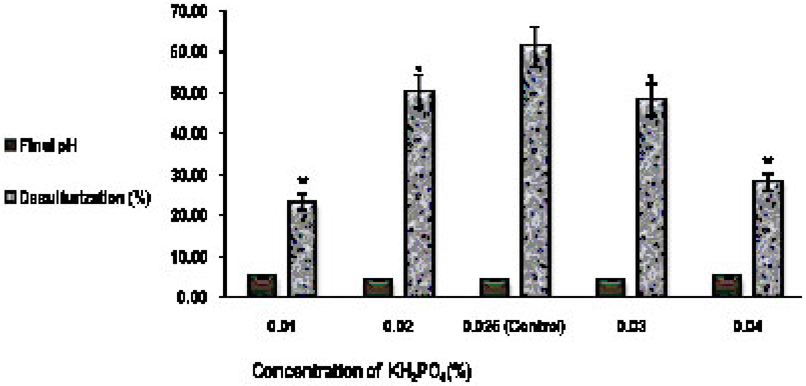
(values were expressed as Mean ± SEM, where n = 6; * $p < 0.05$, ** $p < 0.01$ when compared to control)

Fig. 4. Optimization of NH_4Cl for desulfurizatin of Coal by the mutant *Thiobacillus ferrooxidans* X_{200}



(values were expressed as Mean ± SEM, where n = 6; * $p < 0.05$, ** $p < 0.01$ when compared to control)

Fig. 5. Effect of diffeent Concentration of $MgCl_2$ (%) on desulfurizatin of Coal by the mutant *Thiobacillus ferrooxidans* X_{200}



(values were expressed as Mean ± SEM, where n = 6; * $p < 0.05$, ** $p < 0.01$ when compared to control)

Fig. 6. Effect of diffeent Concentration of KH_2PO_4 (%) on desulfurizatin of Coal by the mutant *Thiobacillus ferrooxidans* X_{200}

bacterial growth[7]. The nitrogen requirement of *Thiobacillus ferrooxidans* X_{200} was examined using different nitrogen sources (Fig.3). Most f the ionic salts proved out to be good nitrogen source for *Thiobacillus ferrooxidans* X_{200} among which NH_4Cl is most effective. Urea and ammonium acetate proved out to be less effective as nitrogen source[8]

Determination of optimum concentrations of NH_4Cl

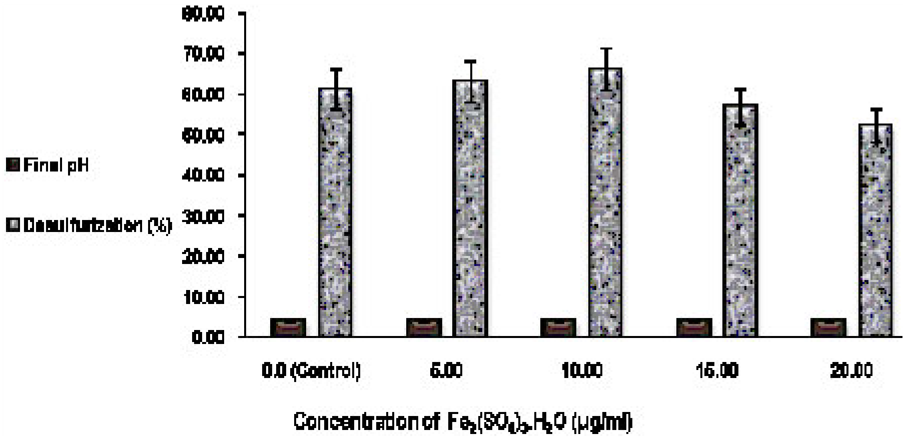
Fig.4 shows that maximum desulfurization of coal by *Thiobacillus ferrooxidans* X_{200} has occurred when the concentration of NH_4Cl is 0.07%. Desulfurization capacity of the bacteria decreased in both lower (0.04%) and higher (0.15%) range of concentrations.

Te composition of the fermentations medium after optimization of the chemical nutrients is glucose, 2%; NH_4Cl, 0.07%; $MgCl_2$, 0.025%; KH_2PO_4, 0.025%; $Fe_2(SO_4)_3.H_2O$ 10 µg/ml and yeast extract, 0.05%. Further studies are in progress to investigate the effect of other metal ions and complex nutrients on the desulfurization of coal by *Thiobacillus ferrooxidans* X_{200}.

Determination of optimum concentrations of $MgCl_2$ and KH_2PO_4

Magnesium, phosphorus, potassium etc. are essential for bacterial growth and most growth media for *Thiobacillus* sp. contain these substances in various quantities. Mg^{++} was added to the fermentation medium for *Thiobacillus ferrooxidans* X_{200} as $MgCl_2$. The effect of different concentrations of $MgCl_2$ is given in Fig.5, which shows that optimum concentration for $MgCl_2$ is 0.025%

KH_2PO_4 was used as the source for both K^+ ion and phosphorus in the leaching medium. Fig.6 shows that optimum concentration of KH_2PO_4 is 0.025%. Higher phosphate concentration inhibited the desulfurization process by formation of insoluble iron phosphates with the pyritic particles of coal[8].



(values were expressed as Mean ± SEM, where n = 6; when compared to control)

Fig. 7. Effect of diffeent Concentration of $Fe_2(SO_4)_3.H_2O$ (µg/ml) on desulfurizatin of Coal by the mutant *Thiobacillus ferrooxidans* X_{200}

The precipitation of the complex was found to be minimum at the optimum N/P ratio of 7.07 where NH_4^+ ion counteract the apparent phosphate inhibition.

Effect of different concentration of $Fe_2(SO_4)_3.H_2O$

Supplementation of fermentation medium for desulfurization of coal by *Thiobacillus ferrooxidans* had the general effect of increasing the removal of pyrite as $Fe_2(SO_4)_3$ could oxidize pyrite chemically". But high concentration of Fe^{+++} might lead to the deposition of insoluble complexes on the coal surface. Consequently there is an optimal relation between ferric ion concentration and bioleaching ratee[12]. Fig.7 shows that the optimal concentration of $Fe_2(SO_4)_3.H_2O$ is 10 µg/ml and addition of Fe^{+++} in excess of the optimal concentration decrease bioleaching rate.

REFERENCES

1. Jeszczak A., Domka F., Kozlowski M and Wachowska H., Fuel., **74**(5): 725 (1995).
2. Pichtel J. R. and Dick W. A., *Soil Biol. Biochem.*, **23**(2): 101 (1991).
3. Silverman M. P. and Lundgren D. G., *J. Bacteriol.,* **17**: 642 (1959).
4. Silverman M. P., Rogoff M. H. and Wendes I., *Fuel*., **42**: 113 (1963).
5. Indian Standard, I S : 1350 (Part III), 5-3 (1969).
6. Brierley C. L., CRC, *Crit. Rev. Microbiol*., **6**: 207 (1978).
7. Hoffmann M. R., Faust B. C., Panda F. A., Koo H. H. and Tsuchiya H. M., *Appl. Environ. Microbiol*., **42**(2): 259 (1981).
8. Olsen G. J. and Brinckman F. E., *Fuel*., **65**: 1638 (1986).
9. Eligwe C. A., *Fuel*., **67**: 451 (1988).
10. Komishi Y., Kubo H. and Asai S., *Biotechnol. Bioeng*., **39**(1): 66 (1992).
11. Konishi Y., Kawamura T. and Asai S., *J. Chem Eng. Japan*, **26**(1): 83 (1993).
12. Shennan J. L., *J. Chem. Tech. Biotechnol.,* **67**: 109-123 (1996).
13. Monticelo D. J., *Cemtech*., **28**(7): 38-45 (1998).
14. Sengupta D., Das S. K., Banik A. K., *Int. Conf. on Disperse Systems*, 19-21 (1998).
15. Ohshiro T. and Izumi Y., *Biosci. Biotechnol : Biochem*., **63**(1): 1-9 (1999).
16. Olson, U. S. *Patent* 6124130 (2000).
17. Xiong X. C., Li W. L., Li X., Xing J. M. and Liu H. Z., *Wei Sheng Wu Xue Bao.,* **45**(5): 733-737 (2005).
18. Ahmed S. M. and Whalley B. J. P., *Fuel*., **51**(3): 190-193 (1972).