Stimulatory Effect of Chemical Nutrients on Desulfurization of Indial Coal by a Mutant \textit{Thiobacillus ferrooxidans} X\textsubscript{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).

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\textit{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\textsubscript{4}Cl served as the best carbon and nitrogen sources respectively. Optimum concentration of both MgCl\textsubscript{2} and KH\textsubscript{2}PO\textsubscript{4} in fermentation medium was 0.025% supplementation of medium with 10 µg/ml Fe\textsubscript{2}(SO\textsubscript{4})\textsubscript{3}.H\textsubscript{2}O 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 \textit{Thiobacillus ferrooxidans} X\textsubscript{200} as against parent strain (55.1%).

Key words: \textit{Thiobacillus ferrooxidans}, Desulfurization, Optimization, Chemical nutrients.

Coal deposits of North-Eastern India have a high sulfur content (2-6\%). During the combustion of these sulfur-bearing coal emissions of volatile sulfur compounds like SO\textsubscript{2}, SO\textsubscript{3} and H\textsubscript{2}S cause air pollution as well as corrosion in boilers\textsuperscript{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 \textit{Thiobacillus ferrooxidans}, \textit{Thiobacillus thiooxidans}, \textit{Thiobacillus acidophilus}, \textit{Bacillus brevis} etc.\textsuperscript{3-17}.

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

The present study deals with the nutritional requirements of the bacterium, viz. concentrations of the suitable carbon source, nitrogen source, MgCl\textsubscript{2} and KH\textsubscript{2}PO\textsubscript{4}. The effect of supplementation of the medium by Fe\textsubscript{2}(SO\textsubscript{4})\textsubscript{3}.H\textsubscript{2}O 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 and 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 FeSO₄ medium which was a 7:3 (v/v) mixture of two media of following composition:

- **Medium A** – (NH₄)₂SO₄, 0.43%; MgSO₄.7H₂O, 0.07%; KH₂PO₄, 0.07%; KCl, 0.17; (NH)₄H₂SO₄, 0.03 ml in 100 ml deionized double distilled water.
- **Medium B** – FeSO₄.7H₂O, 10.2%; (NH)₄H₂SO₄, 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* X₂₀₀ which had a higher sulfur removing capacity (32%) from coal.

Fermentation medium and cultural conditions
The fermentation medium for desulfurization of coal by *Thiobacillus ferrooxidans* X₂₀₀ was selected by substituting glucose and NH₄Cl 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 treated coal samples were further, dried and analysed for total sulfur content.

Estimation of sulfur content in coal
Total sulfur of coal was determined by the conventional Eschka’s method. Pyritic sulfur and sulfate sulfur was also estimated according to the standard procedures. 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 further, 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 Dunnett’s post hoc multiple comparison test using “prism 4.0” software (Graphpad Inc., USA). A “p” value less than 0.05 was considered significant 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. Carbon dioxide serves as the major source of cell carbon for growing *Thiobacillus ferrooxidans* X₂₀₀. Carbonate minerals are also used as a substitute carbon source. But addition of CaCO₃ may inhibit the bacteria to oxidize pyrite due to the acid neutralizing 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 concentration of glucose
The dependence of desulfurization capacity of *Thiobacillus ferrooxidans* X₂₀₀ 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 NH₄⁺ ion, is essential for
Fig. 1. Effect of different carbon sources on desulfurization of Coal by the mutant *Thiobacillus ferrooxidans* X200

(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 desulfurization of Coal by the mutant *Thiobacillus ferrooxidans* X200

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

Fig. 3. Effect of different nitrogen sources (0.5%) on desulfurization of Coal by the mutant *Thiobacillus ferrooxidans* X200

(values were expressed as Mean ± SEM, where n = 6; * p < 0.05, ** p < 0.01 when compared to control)
Fig. 4. Optimization of NH₄Cl for desulfurization of Coal by the mutant *Thiobacillus ferrooxidans* X₂₀₀ (values were expressed as Mean ± SEM, where n = 6; * p < 0.05, ** p < 0.01 when compared to control)

Fig. 5. Effect of different Concentration of MgCl₂ (%) on desulfurization of Coal by the mutant *Thiobacillus ferrooxidans* X₂₀₀ (values were expressed as Mean ± SEM, where n = 6; * p < 0.05, ** p < 0.01 when compared to control)

Fig. 6. Effect of different Concentration of KH₂PO₄ (%) on desulfurization of Coal by the mutant *Thiobacillus ferrooxidans* X₂₀₀ (values were expressed as Mean ± SEM, where n = 6; * p < 0.05, ** p < 0.01 when compared to control)
bacterial growth. The nitrogen requirement of *Thiobacillus ferrooxidans* X200 was examined using different nitrogen sources (Fig.3). Most of the ionic salts proved out to be good nitrogen source for *Thiobacillus ferrooxidans* X200, among which NH4Cl is most effective. Urea and ammonium acetate proved out to be less effective as nitrogen source.

**Determination of optimum concentrations of NH4Cl**

Fig.4 shows that maximum desulfurization of coal by *Thiobacillus ferrooxidans* X200 has occurred when the concentration of NH4Cl is 0.07%. Desulfurization capacity of the bacteria decreased in both lower (0.04%) and higher (0.15%) range of concentrations.

The composition of the fermentsations medium after optimization of the chemical nutrients is glucose, 2%; NH4Cl, 0.07%; MgCl2, 0.025%; KH2PO4, 0.025%; Fe2(SO4)3.H2O 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* X200.

**Determination of optimum concentrations of MgCl2 and KH2PO4**

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* X200 as MgCl2. The effect of different concentrations of MgCl2 is given in Fig.5, which shows that optimum concentration for MgCl2 is 0.025%.

KH2PO4 was used as the source for both K+ ion and phosphorus in the leaching medium. Fig.6 shows that optimum concentration of KH2PO4 is 0.025%. Higher phosphate concentration inhibited the desulfurization process by formation of insoluble iron phosphates with the pyritic particles of coal.

The precipitation of the complex was found to be minimum at the optimum N/P ratio of 7.07 where NH4+ ion counteract the apparent phosphate inhibition.

**Effect of different concentration of Fe2(SO4)3.H2O**

Supplementation of fermentation medium for desulfurization of coal by *Thiobacillus ferrooxidans* had the general effect of increasing the removal of pyrite as Fe3+ 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 rate. Fig.7 shows that the optimal concentration of Fe2(SO4)3.H2O is 10 µg/ml and addition of Fe+++ in excess of the optimal concentration decrease bioleaching rate.
REFERENCES