

## REMOVAL OF INORGANIC AND ORGANIC SULPHUR FROM FOSSIL FUELS BY BACTERIA

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### ABSTRACT

Some soil-coal moist samples collected from a coal mine were checked for sulphur oxidisers using the recommended liquid media for acidophilic and non-acidophilic thiobacilli, of pH 2.5, 4.5 and 7.5 with ferrous sulphate, sulphur and sodium thiosulphate, separate or in combinations as energy sources were tried. The coal mine samples were found devoid of such bacteria (even after 28 days of incubation) indicating that this coal mine in its surrounding does not have sulphur source for energy for such bacteria.

Isolation of some bacteria capable of utilizing thiophene as carbon, sulphur and energy source and producing sulphate as end product was carried out. Oil-soil samples collected previously were used to isolate these cultures using modified Bushnell and Haas (BH) medium and thiophene (5% v/v). Modified BH medium was prepared by using chloride salts of the components instead of sulphate salts and the sulphate produced was analysed spectrophotometrically. In two samples out of 12 tested, sulphate ions were formed to a level significantly higher than in control flasks as well as other tested flasks.

The inorganic sulphur present in three Japanese coal samples was found to be amenable to attack by both *T. thiooxidans* and *T. ferrooxidans* strains, previously isolated from local ecosystems. Coal sample No.1 and No.2 are better oxidised by *T. ferrooxidans* whereas for sample No. 3, *T. thiooxidans* were found better. This behaviour may be contributed to higher ash contents in the first two samples: more metals present were directly attacked by *T. ferrooxidans*, while *T. thiooxidans* lack the ability to attack the metals directly.

### INTRODUCTION

The demand for low sulphur and low nitrogen fossil fuels has been intensified by increasingly stringent regulatory standards for reduced levels of sulphur- and nitrogen oxides in released atmospheric emissions. High sulphur and/or nitrogen fossil fuel combustion results in the generation of sulphur and nitrogen oxides which appear to be involved in the formation of atmospheric acid deposition. Such acid depositions produce detrimental effects on complex ecosystems and has become an issue of international significance. Low quality (high sulphur and/or nitrogen) fuels need to be subjected to either pre- or post- combustion desulphurization.

Most crude oils contain between 0.1 and 3% sulphur, both inorganic and organic in nature, whereas in few cases such as bitumens and oils may contain more than 14% sulphur (Orr, 1978). Non biological methods for coal desulphurization include both physical and chemical techniques, but being energy intensive and corrosive in nature, have very little application. Physical methods (Kargi, 1984) take advantage of the significant difference in densities of coal ( $1.1-1.3 \text{ g cm}^{-3}$ ) and pyrite ( $4.8-5.3 \text{ g cm}^{-3}$ ) and the paramagnetic properties of pyrite but results in both material and energy losses. Chemical methods involve conversion to volatile products ( $\text{H}_2\text{S}$  and  $\text{SO}_2$ ) or soluble sulphate. No chemical method has been developed to specifically solubilize the organic sulphur in coal. Microbiologically, the removal of inorganic sulphur is being carried out by sulphur oxidizing bacteria; *Thiobacillus ferrooxidans* (Myerson and Kline, 1984; Beyer et al 1987) and *Sulfolobus acidocaldarius* (Kargi and Robinson, 1985). Some investigators have used combination of *Thiobacillus ferrooxidans* and *T. thiooxidans* for more effective desulphurization (Dugan and Apel 1978; Andrew and Maczuga 1982). These were found to be capable of utilizing elemental sulphur and sulphur produced through acid reaction on metal sulphides.



The sulphur so produced, if not removed, forms a passive layer resulting in cessation of further attack by acid or bacteria. *T. ferrooxidans* have ability to attack the pyrite and other metal sulphide directly, or indirectly by acid and/or ferric iron production, thus solubilizing the undesired sulphides in coal. *T. thiooxidans* have better enzyme system for sulphur oxidation (Lundgren et al, 1986).

For organic sulphur no specific group of bacteria has been recognised. Commonly occurring heterocyclic organic sulphur compounds in oil and oil fractions especially high boiling fractions are thiophenes, phenanthrene, benzothiophenes and dibenzothiophenes. Removal of these compounds without affecting the other hydrocarbons has been tried using various groups of bacteria. Dibenzothiophene degrading bacterial strains have been studied by various workers (Fedorak and Westlake, 1984; Finnerty and Robinson, 1986; Afferden et al, 1988) as model substrate for organic sulphur in oil and coal. Thiophene has also been tested as organic sulphur containing hydrocarbon and as a model substrate for desulphurization (Klubek and Clark, 1987) of coal.

In the present studies coal mine samples were analysed for sulphur oxidizing bacteria. Attempts were also made to enrich oil soil samples for thiophene oxidizing bacteria. The possibility of removing inorganic sulphur in three coal samples was tested using locally isolated strains of *Thiobacillus thiooxidans* and *T. ferrooxidans*.

## MATERIAL AND METHODS

### Sources of Bacteria and Media Employed:

Some soil-coal moist samples collected from a coal mine between Bahl and Minera near Darulurfan, 4 km from Chakwal to Khushab, were checked for the presence of sulphur oxidisers using the recommended liquid media for acidophilic and non-acidophilic thiobacilli, of pH 2.5, 4.5 and 7.5 with ferrous sulphate, sulphur and sodium thiosulphate, separate or in



combinations as energy sources were tried. The media employed for isolation of sulphur oxidizing bacteria were same (9K S, 9K Fe and 9K S+ST) as used in previous studies (Khalid et al 1990). 9K mineral salt medium of pH 2.5 without ferrous sulphate (Silverman and Lundgren 1959) was supplied with either elemental sulphur (2% w/v, 9K S) or ferrous sulphate (5% w/v, 9K Fe). 9K S medium with 1% w/v sodium thiosulphate was termed as 9K S+ST. pH of the media was adjusted using either  $H_2SO_4$  or NaOH.

Isolation of some bacteria capable of utilizing thiophene as carbon, sulphur and energy source and producing sulphate as end product was carried out. Oil soil samples collected previously (Kokub et al 1990) were used to isolate these cultures using modified BH medium and thiophene 5% v/v employing enrichment culture technique. Modified BH (Bushnell and Haas 1941) medium was prepared by using chloride salts of the components instead of sulphate salts and the sulphate produced was analysed spectrophotometrically. Subsamples from these enriched cultures were taken over time and transferred to fresh medium.

*Thiobacillus thiooxidans* and *T. ferrooxidans* previously isolated (Khalid and Malik, 1987) were used for desulphurization studies of coal.

Basal medium described above was modified to contain chloride compounds instead of sulphates and HCl was used to adjust the pH instead of  $H_2SO_4$ . This modification was used to eliminate the sulphates present in the medium which was found necessary for estimation of sulphates produced by bacterial oxidation of inorganic sulphur present in the coal.

For adaptation studies steam sterilized elemental sulphur 1% w/v was used and for biodesulphurization studies 10% w/v of coal was used.

#### Coal Samples and Analyses:

One kg each of three samples (no 1, 2 & 3) were provided by a team of Japanese Scientists who visited NIAB on May 30, 1988. These contained (Table 1); 100-400 ppm of inorganic sulphur and 0.33-2.56 ppm organic sulphur with 0.1-0.2 ppm of sulphur in free form. A representative sample from each coal sample was ground and passed through 40 mesh sieve for subjecting to bacterial attack. For chemical analyses oven dried (70 °C, 6hrs) samples were ground to 100 mesh. Total ash contents (%) were determined by standard method, i.e.; keeping at 750-800°C for 4-5 hrs in muffle furnace. The ash containing various metals was digested in  $HNO_3$  - HF acids and analysed by atomic absorption spectrophotometric technique for various heavy metal contents (Marczenko, 1976). Aliquots from uninoculated and inoculated samples were taken periodically and estimated for sulphate contents (Mateen et al 1984).

## RESULTS AND DISCUSSION

#### Microbial Analysis of Coal Mine Samples:

Soil-coal moist samples collected from a coal mine were checked for sulphur oxidisers using the recommended liquid media for acidophilic and non-acidophilic thiobacilli, 9K S and 9K Fe of pH 2.5, 9K S+ST of pH 4.5 and 7.5 were tried. The coal mine samples were found devoid of such bacteria (even after 28 days of incubation) indicating that this coal mine in its surrounding does not have sulphur source of energy for such bacteria.

### Isolation of Thiophene Oxidizing Bacteria:

The results revealed that two samples out of 12 tested, sulphate ions were formed to a level significantly higher than in control flasks as well as other tested flasks. Purification of these bacteria is underway.

### Analysis of Coal Samples:

Sulphur contents (information provided with the samples) of these coal samples is given in Table 1. Total ash contents (%) and mineral analysis of three coal samples is given in Table 2. The most dominant part is iron. It may be assumed that some of these metals are sulphur bound i.e., sulphidic in nature whereas, a part of these metals may be in oxide or calcite form.

Table 1: Content of sulphur in coal samples.

Sample	S (ppm)		
	Free	Inorganic	Organic
No. 1	0.2	400	2.56
No. 2	.1	141	0.33
No. 3	0.1	100	1.20

Table 2: Oxidation of element sulphur 1% w/v by two *Thiobacillus* spp. inoculated in chloride mineral medium and incubated at 100 rpm and 30°C.

	Days of incubation					
	1		2		3	
	pH	SO <sub>4</sub> <sup>-2</sup> g/l	pH	SO <sub>4</sub> <sup>-2</sup> g/l	pH	SO <sub>4</sub> <sup>-2</sup> g/l
Control	2.5	0.05	2.5	0.06	2.5	0.06
<i>T. thiooxidans</i>	2.5	0.05	1.41	12.92	1.11	21.80
<i>T. ferrooxidans</i>	2.5	0.06	1.48	10.83	1.15	16.80

### Adaptation of Bacterial Cultures:

Since these strains of thiobacilli were enriched and being grown on sulphate media (Khalid and Malik 1987, 1988) for a long time and now these were to be grown on chloride media they were subjected to adaptation process. Sulphate media grown cells of *T. thiooxidans* and *T. ferrooxidans* were separated by centrifugation and washed with normal saline (pH 2.5) and inoculated to modified 9K media (Chloride medium) with elemental sulphur as energy source. The cells were separated and reinoculated to fresh medium. After adaptation for 3 consecutive transfers, the cells separated by centrifugation were used as in-

Table 3: Chemical analysis of Japanese coal samples by Atomic Absorption Spectrophotometric Method.

Constituents	Concentration ug/g (ppm)		
	Coal Sample 1	Coal Sample 2	Coal Sample 3
Ash content (%)	20.62	11.70	10.70
Fe (%)	1.46	0.36	0.47
Mn	97	98	149
Zn	29	ND*	ND*
Cu	34	14	ND*
Co	21	15	15
Nl	28	12	25

\* ND = Not Detectable

Note: SiO<sub>2</sub>, SO<sub>4</sub> and CO<sub>3</sub> compounds also present in all three samples.

oculum for studying sulphate production. The results are presented in Table 3. *T. thiooxidans* was found to be relatively more efficient in sulphur oxidation than *T. ferrooxidans*.

Table 4: Production of sulphate by bacteria inoculated to chloride medium containing 3 coal samples 10% w/v and incubated at 30°C, 100 rpm.

	Days of incubation					
	0		5		10	
	pH	SO <sub>4</sub> <sup>-2</sup> (g/l)	pH	SO <sub>4</sub> <sup>-2</sup> (g/l)	pH	SO <sub>4</sub> <sup>-2</sup> (g/l)
<u>Coal Sample No. 1</u>						
T1	5.77	0.02	5.77	0.33	5.66	0.40
T2	5.80	0.01	5.89	0.53	5.82	0.76
T3	5.81	0.01	5.88	0.65	5.78	0.80
<u>Coal Sample No. 2</u>						
T1	5.85	0.01	5.81	0.02	5.81	0.03
T2	5.80	0.03	5.74	0.33	5.74	0.38
T3	5.80	0.00	5.48	0.38	5.55	0.40
<u>Coal Sample No. 3</u>						
T1	5.81	0.02	5.85	0.05	5.83	0.09
T2	5.82	0.03	5.79	0.44	5.78	0.62
T3	5.85	0.02	5.79	0.26	5.76	0.27

T1 = uninoculated control; T2 = Inoculated with *T. thiooxidans*  
T3 = Inoculated with *T. ferrooxidans*

Cells grown for 10 days were separated and used as inoculum to media containing coal (10% w/v) samples. Periodically the changes in pH and sulphate contents produced by bacteria were compared with uninoculated (control) and are reported in Table 4. The increases in sulphate contents with incubation is indicative of bacterial utilization of inorganic sulphur



present in coal. *T. ferrooxidans* were found to be more effective with coal samples No. 1 and No. 2. Whereas for coal sample No. 3 *T. thiooxidans* were better choice.

### CONCLUSIONS

The coal mine samples were devoid of acidophillic and non acidophillic sulphur, sodium thiosulphate or ferrous iron oxidizing bacteria.

The inorganic sulphur in these coal samples was found to be amenable to attack by both *T. thiooxidans* and *T. ferrooxidans*. Coal samples no 1 & 2 were better oxidized by *T. ferrooxidans* whereas for sample 3 *T. thiooxidans* were found better. These may be because of higher ash percentage in first two samples; more metals present are directly attached by *T. ferrooxidans* where *T. thiooxidans* lack the ability to attack the metals directly.

Further experiments are required to optimize the conditions for sulphur removal.

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