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Desulfurization of coal using heterogeneous microbial species isolated from acid mine drainage, Talabira Coal Mines, Sambalpur, Odisha

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Abstract

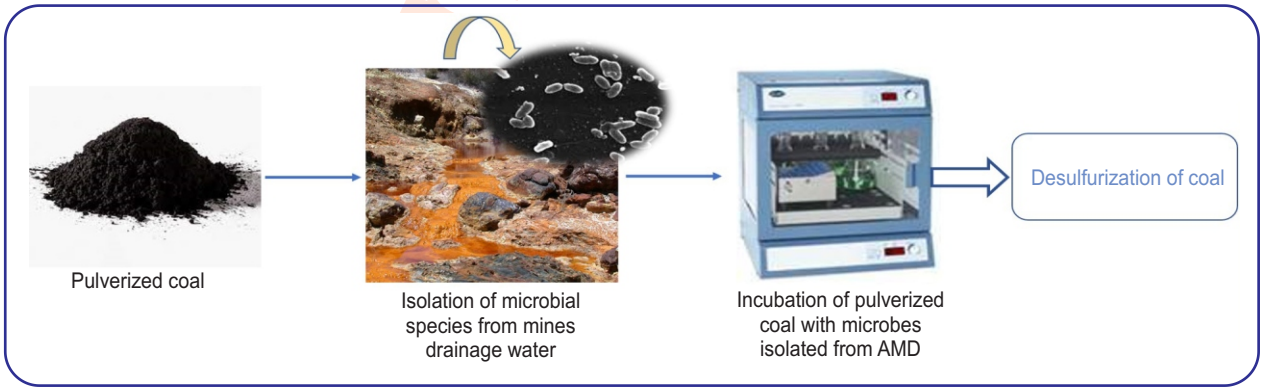
Aim: The present study aims to assess the coal desulfurizing efficiency of heterogenous microbial consortia isolated from drainage water of Talabira Coal Mines, Sambalpur, Odisha.

Methodology: Microbial species in the mine drainage water were isolated and cultured using 9K+ medium. Identification of microbial species was performed by the amplification of 16S rDNA. The heterogeneous microbial consortia were incubated with pulverized coal to assess their desulphurizing efficiency.

Results: The proximate analysis of the coal sample revealed 34.92% fixed carbon, 9.01% moisture, 33.38% ash and 25.44% volatile matter. After incubation of the pulverized coal with isolated and cultured heterogeneous microbial species from MDW water of Talabira coal mines area, Odisha for 24 days in aerobic condition, the total sulphur content was reduced from 2.86% to 1.61%, while significant removal of pyritic Sulphur (48%) was obtained from 1.72% to 0.91%. However, no remarkable reduction was seen in the case of organic Sulphur.

Interpretation: The results showed that drainage water of Talabira Mines inhabits numerous potential desulfurizing bacterial species that need further isolation, characterization, and process optimization to achieve optimal sulphur removal efficiency.

Key words: Autotrophic bacteria, Coal, Microbial desulfurization



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Introduction

Coal provides approximately 66.8% of the total energy generated, contributing as the largest source of energy across the globe (Zhang *et al.,* 2017). Coal is used as a raw material for producing energy in industries like iron, steel, aluminium, plastic, and paint (Prayuenyong, 2002; Cao *et al.,* 2016). The coal reserve in India (98% Gondwana + 2% Tertiary) are of different grades ranging from sub-bituminous to bituminous and lignite type. Depending on the grade of Indian coal, sulphur content in Indian coal varies widely, from less than 0.5% to over 11% (Chandra *et al.,* 2004). Sulphur compounds play a key role in forming sulphur oxides that are released into the environment during combustion. Therefore, the development of novel and sustainable clean coal technologies in the energy sector is one of the major thrust areas for researchers. In coal, sulphur is found in three forms, *i.e.,* organic, pyritic, and sulfate sulphur (Mishra *et al.,* 2016, Etemadzadeh *et al.,* 2016). Sulphur can be removed from the coal matrix by chemical, physical, and biological processes for obtaining cleaner coal. Although physical methods effectively remove pyritic sulphur, they are not suitable for the removal of organic sulphur from coal. Chemical methods need specific operating conditions such as high temperature and pressure to effectively remove both organic and inorganic sulphur from the coal (Mishra *et al.,* 2016) without additional loss of partial combustible matters. The chemical methods also release huge amounts of CO_2, a potent greenhouse gas, and secondary hazardous by-products.

The biological method is emerging as an effective way to remove sulphur from coal without releasing any toxicants into the environment. Biological methods of desulfurization have several advantages over conventional physical and chemical methods such as mild operational conditions and the least generation of pollutants. Sulphur is one of the major elements required for the growth and metabolism of microbes. Various enzyme cofactors and other important chemical compounds such as amino acids, proteins, biotin, thiamine, and cofactor-A have sulphur as their principal structural component. Hence, several microorganisms can uptake sulphur from the coal matrix to fulfill their metabolic requirement for sulphur via various mechanisms such as oxidation of carbon-carbon bond cleavage or carbon-sulphur bond cleavage (Gupta *et al.*, 2005; Rout *et al.,* 2021).

These bacterial strains can break the carbon-sulphur bond and release the sulphur atoms present in the aromatic ring of coal structure (Soleimani *et al.,* 2007; Akhtar *et al.,* 2016). Over the past few years, several species of microorganisms such as *Pseudomonas* and *Sulfolobus* have gained remarkable attention as these are effective in removing inorganic sulphur, while *Rhodococcus* species effectively remove both organic and inorganic Sulphur from coal (Kilbane *et al.,* 1992). Several bacterial species such as *Acidithiobacillus thiooxidans* (Silverman 1967), *Leptospirillum*-like bacterium (Merrettig *et al.,* 1989), *Acidianus brierleyi* (Larsson *et al.,* 1990; Olsson et al.1993), *Sulfolobus acidocaldarius* (Kargi and Robinson 1985; Durusoy *et al.,* 1992), *Pseudomonas putida* and *Pseudomonas aeruginosa* (Rai and Reyniers 1988) have been used for the biological removal of sulphur in coal. Among these bacterial species, the most widely studied chemolithotrophic bacterium is *Acidithiobacillus ferrooxidans*, that utilizes energy from the oxidation of pyrite for its metabolism (Bhanjadeo *et al.,* 2018).

About 56.26% of the total energy generated in India comes from coal-dependent thermal power plants. However, coal extracted in this region is of sub-bituminous type and contains 3-6% organic and inorganic sulphur. Sulphur removal from coal before combustion is of considerable interest to avoid the emission of sulphur oxides. Chemical and physical methods have been developed for removing inorganic sulphur component in coal which are expensive and energy intensive, and destroy the caking properties of coal. On the other hand, microbiological processes are known to remove most of the pyritic sulphur, as well as some of the organic sulphur, without affecting the caking properties of caking coal. Water discharge mostly from draining mineral spoil heaps and tailings as well as abandoned coal and metal mines are rich in components released through the oxidative dissolution of pyrites such as soluble iron, heavy metals and sulfate (Sun *et al.,* 2019).

Acid mine drainage water is reported to house numerous microbial species such as *Acidithiobacillus ferrooxidans, Acidithiobacillus ferrivorans* and *Acidiphilium multivorum,* which are well adapted to acidic water and possess the ability to remove sulphur (Aytar *et al.,* 2013; Singh *et al.*, 2018). Several microbial species with promising desulfurization capacity have been isolated from the drainage water of different mines across the globe. Talabira Coal Mine, a subsidiary of Mahanadi Coalfield Limited is situated within the Jharsuguda and Sambalpur districts of Odisha state. Preliminary studies carried out in our laboratory (Rao *et al*., 2023) showed the presence of numerous microbial species and high sulfur content in mine drainage water. However, no systematic scientific investigation has been carried out to asssess the desulfurization capacity of these microbial species. In the present study, a few microbial species inhabiting Talabira Coal Mines drainage water were isolated, and evaluated for their desulfurization efficiency of coal under aerobic conditions.

Materials and Methods

Collection and characterization of coal samples: Pulverized coal samples were collected from the captive thermal power plant of Vedanta Aluminium Limited, Jharsuguda, Odisha. For the characterization of coal samples, ultimate analyses followed by the proximate analysis were carried out.

Proximate analysis: Proximate analysis of coal sample included determining moisture content, volatile matter, carbon, and ash content. Pre-weighed sample of powdered raw coal of 200 µm size in an uncovered crucible was placed in the oven at 108±2°C along with the lid. The sample was cooled to room temperature and weighed again. The loss in weight gave moisture content. A fresh sample of crushed coal was weighed, placed in a covered crucible, and heated in a furnace at 900 ±15°C. The sample was

cooled and weighed. Loss in weight represents moisture and volatile matter. The cover of the crucible used in the last test was removed and the crucible was heated over the Bunsen burner until all the carbon is burned. The residue was weighed, which gave the incombustible ash. The difference in the weight from the previous reading was taken as the fixed carbon.

Ultimate analysis: Total sulphur, organic and inorganic sulphur was estimated by the Eschka method to determine the ultimate analysis of coal samples, approximately 1g of coal sample and 3 g of Eschka mixture were taken in a porcelain crucible (Eschka *et al.,* 1874; Mott *et al.*, 1953). The crucible was placed in a cold vented muffle and the temperature was gradually increased up to 800 ± 25°C for one hour till the disappearance of black particles. The ignited mixture was digested with 100 ml of hot water for 30 to 45 min. After filtering the solution thoroughly, it was neutralized methyl orange followed by slow addition of 1 ml Hcl and 10 ml of $BaCl_2$ solution with constant stirring after boiling and was allowed to stand just below boiling temperature. Filter paper without any ash residue was used to filter the solution. In a porcelain crucible, the wet filter was put and heated at 800 ± 25°C for 15 min in a muffle furnace and weighed after cooling the filter. A blank analysis without coal was carried out to avoid possible background contribution.

Characterization of acid mines drainage water collected from the Talabira Coal Mines area: Grab water samples were collected from the waste dumps at the Talabira Cool Mine. Samples for bacteriological analyses were collected in sterile glass bottles the samples were collected for physico-chemical analyses whereas in polyethylene bottles. Except for pH and conductivity, all the elemental and analyses heavy metals was carried out by Inductively Coupled Plasma-atomic Emission Spectrometry (ICP-AES).

Mixed microbial consortia and growth culture media: In the present study, mixed cultures were used as these are reported to have more efficiency in the desulfurization of coal than pure culture (Panda *et al.,* 2013, Marhual *et al.,* 2008). The strains were enriched from the drainage water of Talabira coal mines for bio-desulfurization experiments. The microorganisms were grown in the standard 9K+ media. comparasing $(NH_4)_2SO_4$ - 3 g l^{-1}, KH_2PO_4 - 0.5 g l^{-1}, $MgSO_4.7H_2O$- 0.5 g l^{-1}, KCl - 0.1 g l^{-1} and $FeSO_4$ - 44.2 g l^{-1} (Silverman and Lundgren, 1959). The strains were repeatedly sub-cultured in the media at 30°C within an interval of 4 days. The strains were adapted to a pulp density of 5% (w/v). The adapted strains with a steady iron oxidation rate were further used for bio-desulfurization studies.

Optimization of desulfurization process: Previous studies indicate that desulfurization is more effective in an iron-free 9K media, hence, optimum conditions were evaluated using a mixed acidophilic consortium (Merrettig *et al.*, 1989). Shake-flask experiments were performed using 9K- medium at pH 1.8. Several factors were optimized, particularly incubation duration, pulp density, and size fraction. Pulp density (5%) of the coal sample was taken and subjected to bio-desulfurization testing utilizing 10% inoculum at 30°C under shaking conditions (150 rpm) for 24 days in five different Erlenmeyer flasks to optimize time duration. Each solid coal sample from each experimental flask was tested for desulfurization efficiency at an interval of 3 days.

Desulfurization of coal samples by the microbial isolates from the AMD sample: The microbial desulfurization of coal sample was performed as per the procedure laid by IS 1350-3 (1969). Prior to bio-desulfurization experiments, the coal samples were ground and sieved to get a size fraction of 45µm. In order to perform the preliminary shake-flask experiments, a pulp density of 5% and an inoculum size of 10% were taken in a 250 ml Erlenmeyer flask. For the microbial growth, both 9K+ media (presence of ferrous iron) and 9K- media (devoid of ferrous iron) were used with pH 1.8 and a temperature of 30°C and an incubation period of 15 days under shaking conditions (150 rpm). Desulfurization efficiency was determined by analysis of total sulphur in the coal samples after 24 days of treatment, and calculated as the difference in the Total sulphur content of coal before and after treatment divided by the initial content.

Phylogenetic analysis of acidophilic isolates: 16S rDNA gene sequences of the selected isolates were compared with those available in the data banks for phylogenetic analysis. Bacterias grown on solid media were purified by serial streaking method (Caicedo *et al*., 2012). After ascertaining the purity of the colony, selected one or two colonies were suspended with polymerase chain reaction lysis buffer and heated at 95°C for 10 min. The suspended lysis buffer was then mixed with dH_2O (180 µl) and 1 µl of this crude lysate that served as a template for the amplification of 16S rDNA by PCR (Silverman and Lundergren, 1959) using primers complementary to positions 8 ±27 and 1510 ±1492 of *Escherichia coli* 16S rDNA. After amplification, the product was cloned into the pGEM-T Easy vector (Promega) and sequenced commercially (MWG Biotech). The 16S rDNA sequences were compared with others in the GenBank database using the BLAST program. 16SrDNA sequences of selected microorganisms were obtained from the GenBank and aligned with the sequences obtained in this study using CLUSTALW (Thompson *et al.,* 1994). Accession numbers of the partial 16S rDNA gene sequences for the isolates described in this study are given in Table 3, along with the closest matching sequences already in databases.

Statistical Analysis: The data obtained for assessment of the coal desulfurization capacity of microbial consortia were analyzed using student' s t-test . The statistical significance was assessed at p-value less than 0.05. The statistical analysis was performed using MS Excel and presented as Mean ±SD.

Results and Discussion

Acid Mine Drainage Water is one of the crucial environmental challenges and represents an extreme

Table 1: Physico-chemical characteristics of mine drainage samples

Parameters	Talabira Outlet
pH	3.09 ± 0.32
Conductivity (μs cm^{-1})	56.3 ± 11.2
Chemical parameters (mg l^{-1})	
Ca	3.78 ± 0.51
Mg	5.38 ± 0.73
Fe	18.8 ± 1.06
Al	6.50 ± 0.84
Cu	3.98 ± 0.29
Mn	0.19 ± 0.08
Ni	0.03 ± 0.002
Co	0.05 ± 0.008
Pb	0.05 ± 0.006
Cd	0.01 ± 0.003
Zn	5.78 ± 1.13
Si	3.22 ± 0.93

Table 2: Name of microorganisms in acid mine drainage water isolates based on 16S rDNA gene sequence

	Isolates nearest relative	Identity (%)
1	*Leptospirillum ferrooxidans* DSM 9468 (X72852)	98.9
2	*Acidithiobacillus caldus* DSM 8584T (Z29975)	97.9
3	*At. ferrooxidans* ATCC 33020 (AFE278719)	98.0
4	*Acidocella facilis* WJB-3 (AF253412)	96.1
5	*At. Thiooxidans* ATCC 19377T (Y11596)	99.6
6	*Acidiphilium acidophilum* ATCC 27807T (D86511)	99.8
7	*Thermithiobacillus tepidarius* DSM 3134T (M79424)	94.5
8	*Thermodesulfovibrio islandicus* (X96726)	95.7

environment to the ecosystem. It harbors comprehensively studied taxa such as *Leptospirillum* spp. and *Acidithiobacillus* spp. *Leptospirillum* spp. oxidizing Iron while *Acidithiobacillus* spp. are Iron, and/or sulphur-oxidizing. Most members of these genera can fix carbon and hence, are considered as autotrophs. With the autotrophic species co-occur many heterotrophic acidophiles. Members of *Acidicaldus, Acidocella, Acidiphilium, Acidomonas* are heterotrophic acidophiles. Along with autotrophs and acidophile heterotrophs, mixotrophic species such as *Sulfobacillus* spp. (Firmicutes) are found in the acid mine drainage water. Incubation of coal sample with microbial culture for 24 days significantly reduced the pyritic sulphur content of coal sample. It was found that drainage water from coal washeries is enriched with acidophilic bacteria and mixed culture of the acidophiles effectively bioleach pyrite from coal (Bressler *et al.*, 2001; Dutta *et al.,* 2020). Therefore, for exploring the potential of desulfurization of coal, cultured microbes from acid drainage were used as inoculum. rDNA sequence analysis of various microbial organisms isolated from the acid mine drainage of Talabira Coal Mines identified eight microbial species with potential desulfurization capacity with high conformity. Dominant population of some known desulfurizing bacterial populations such as *Acidithiobacillus caldus*, *At. Thiooxidans* and *Leptospirillum ferrooxidans* was observed in the acid mine drainage water (Table 2).

Acid Mine Drainage samples collected from Talabira Coal Mines were acidic in nature due to mineral acidity (presence of soluble iron and aluminium) and proton acidity (pH) (Table 1). Factors such as variation in pH , temperature, mineral content and heavy metal concentration in drainage water of coal mines directly influence the community structure of the inhabiting microbial species. Microbial species capable of carrying out biochemical processes such as oxidation of iron, reduction of sulphur as well as iron, production of extracellular slime mostly dominate in most of the acid mine drainage environments. Though acid mine drainage water from Talabira Coal mines inhabit a few taxa of the microbial population but these bacterial species were observed to be phylogenetically diverse. The heterogeneous population of bacterial species in the present study caused higher desulfurization of coal which could be attributed to the existence of microbial species interaction and metabolic relationship accelerating the sulfur removal process. Supporting our findings, several studies have indicated that the microbial consortia with diverse microbial species more efficiently remove sulfur from coal compared to the desulfurization using individual microbial species. Heterotrophic acidophiles remove toxic organic compounds which may be lethal to autotrophs, while autotrophs provide organic substances for grow and maintain once of heterotrophic population (Das *et al.,* 1996; Liu *et al.,* 2020). Norris *et al.* (1980) demonstrated that organic matter obtained from the culture filtrate was sufficient to sustain the heterotrophic growth of *S. thermosul¢dooxidans* from the autotroph *Acidithiobacillus ferrooxidans* consortia. In another experiment conducted by Clark and Norris (1996) with *Acidimicrobium ferrooxidans* and both *S. acidophilus* and *S. thermosulfodooxidans* efficiency of iron oxidation by mixed cultures of *Sulfobacillus* spp. and *Acidithiobacillus ferrooxidans* was found to be more extensive than pure cultures. Mixotrophic growth of *Sulfobacillus* removed organic by-products of *A. ferrooxidans* resulting in greater leaching.

Table 3: Proximate and ultimate analyses of raw coal and residual coal sample after treatment

	Proximate analysis (Dry basis %)				Sulphur component (%)			
Samples	Moisture	Ash content	Volatile matter	Fixed carbon	Total sulphur	Pyritic	Sulfate	Organic
Raw coal	9.01	33.38	25.44	34.92	2.86	1.72	0.71	0.43
Residual coal	7.15	28.93	19.82	34.93	1.61	0.91	0.56	0.428

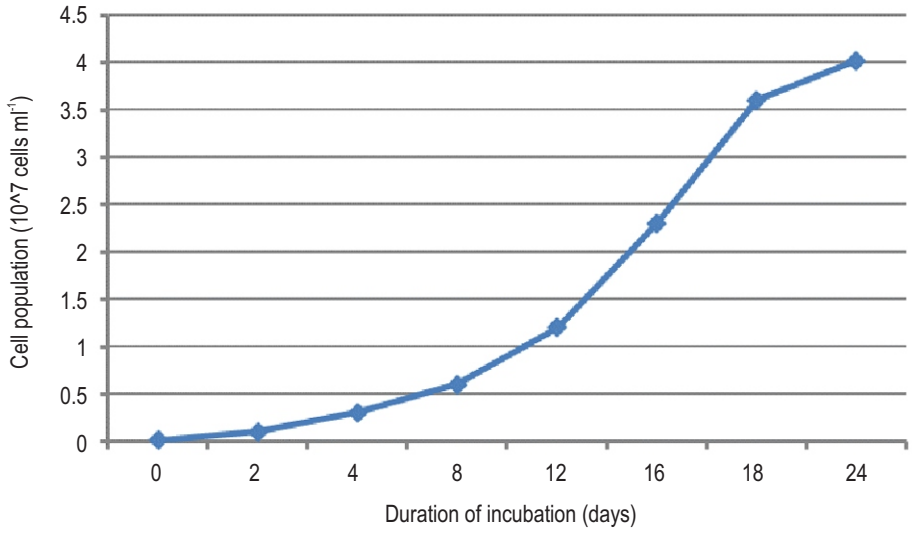

Fig. 1: Growth curve of bacterial consortia isolated from mines drainage water in $FeSO_4$ media

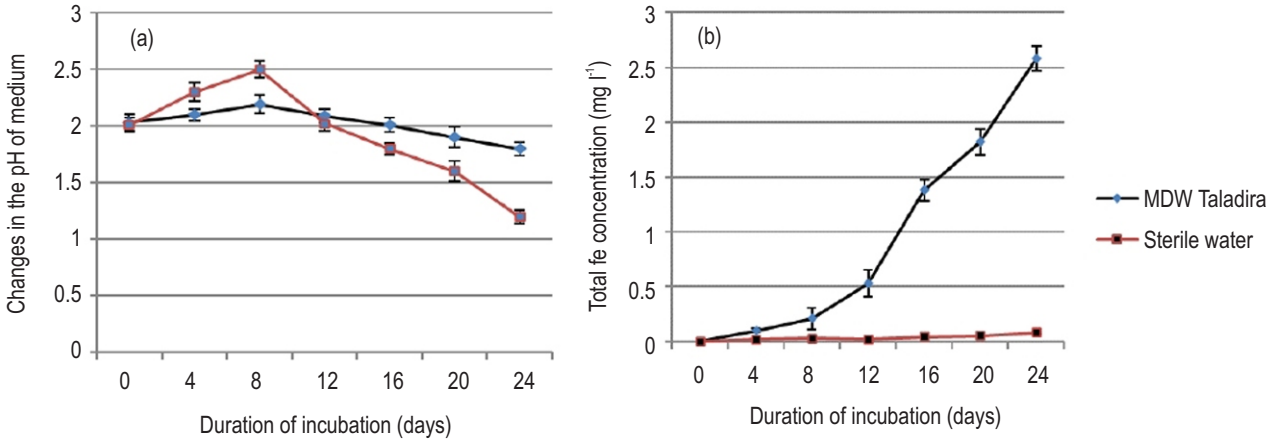

Fig. 2: (a) Decrease in pH of the media following 24 days of incubation and (b) Showing changes in Fe content of coal.

This accounts for close correlation in the populations of *A. ferrooxidans/T. thiooxidans* and *Acidiphilum* in environments studied by Peccia *et al.* (2000). The proximate and chemical analysis of coal sample used in the study disclosed typical composition (Table 3). The total sulphur in the coal sample was 2.86%, comprising 0.43% organic sulphur 0.71%, sulphate sulphur and 1.72% pyritic sulphur 1.72 with a fixed carbon of 34.92%. The proximate analysis of raw coal indicates that coal samples collected for the study contain low to medium quantities of moisture, medium to high amount of volatile matter, and high amount of ash. The residual coal sample was analyzed after undergoing desulfurization for 24 days in a culture flask. The ash content of the coal sample reduced from 33.38 to 28.93%, volatile matter reduced from 25.44 to 19.82%, and moisture content reduced from 9.01 to 7.15%., respectively. On the incubating the culture media at room temperature, the bacterial population in the mine drainage water increased and reached to stationary phase after 24 days of incubation with a population size of approximately 4 million cells (Fig. 1).

The rate of bacterial growth and metabolism depends on the amount of dissolved oxygen available. The liquid must be aerated to provide dissolved oxygen (Mishra *et al.,* 2016) because partial solubility of oxygen takes place in liquids when propagating bacteria in liquid culture. Previous studies have reported that initial pH highly influences the desulfurization process. There was a reduction in the pH of the medium from 2.03 to 1.2 (Fig. 3a). The initial pH ranged between 1.5 and 2.5 not only reduced the lag phase of the bacteria but also prevents precipitation of jarosite and another mineral on the coal surface,

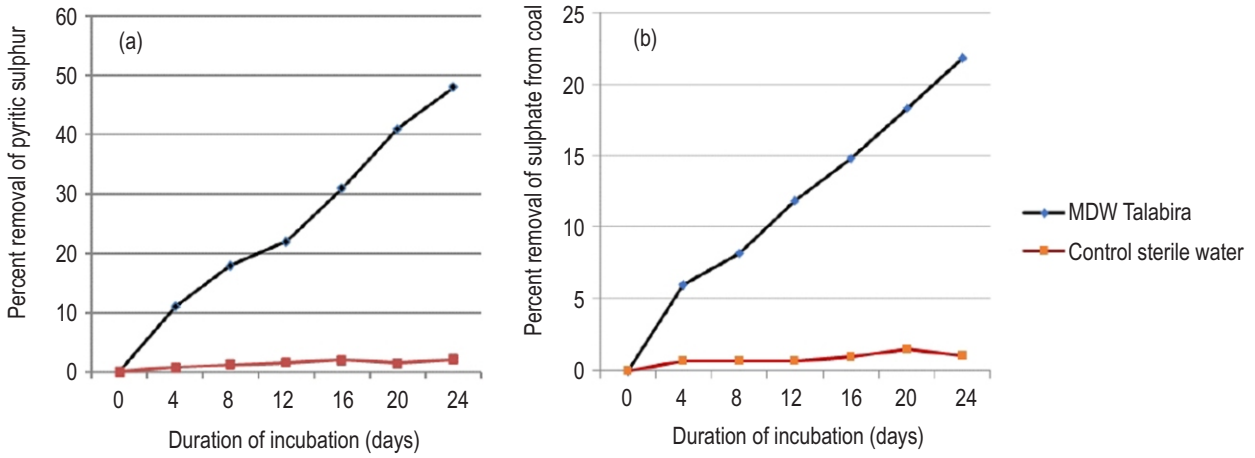

Fig. 3: (a) Removal rate of pyritic sulphur and (b) Removal of sulphate sulphur from coal following incubation with microbial consortia for different duration under aerobic condition.

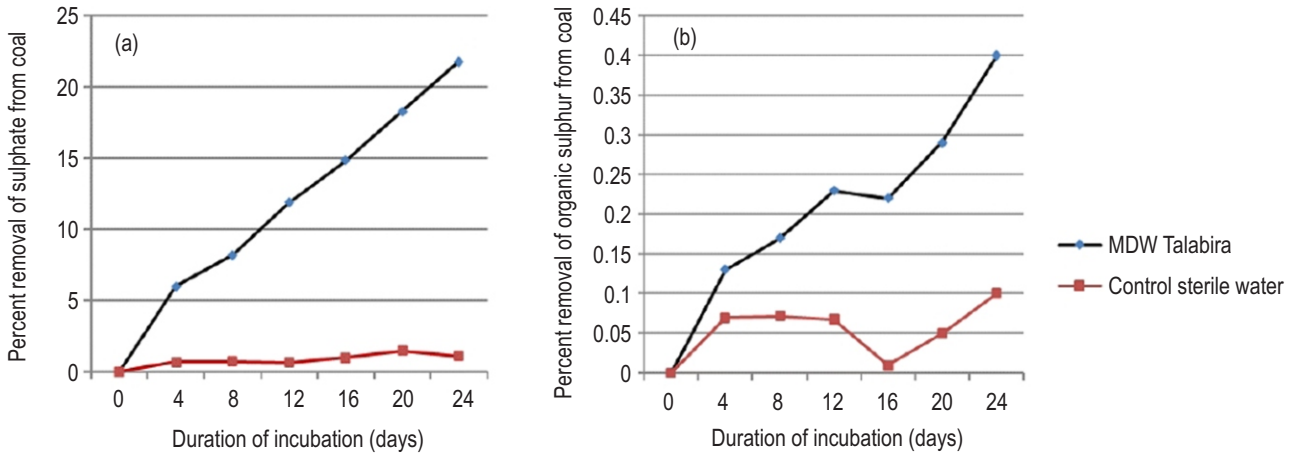

Fig. 4: (a) Percent organic sulphur removal rate from coal and (b) showing removal of organic sulphur from coal following incubation with heterogenous microbial isolates at different durations.

and the acidophilic bacteria thrive best at pH below 2.0 (Acharya *et al.*, 2004; Falagan and Johnson, 2018), however, initially, there was a slight increase in the pH of the medium from 2.01 to 2.5 till 8th day of incubation.

The present study showed that there was a significant increase in the leached iron content in the medium which indicates the bio-oxidation of pyritic sulphur present in the coal to iron (Fig. 3b). Incubation of coal with microbes isolated from the Mines drainage water, Talabira mines significantly increased (t= 15.27, p≥0.05) the Total iron content in the medium compared to control sterile water without any microbial consortia from 0.1mg l^{-1} on day 1 to 2.58 mg l^{-1} on day 24. The findings of this study corroborate with the previous reports (He *et al.*, 2013; Ye *et al.*, 2016; Li *et al.,* 2023) where similar leaching of iron, following incubation of coal sample with a pure culture of *Acidithiobacillus ferrooxidans* was observed. When investigated for the effect of different shaking speeds (experimental conditions 10% coal pulp density, particle size 180µm, and pH=1.5), the maximum desulfurization was achieved at 180 and 210 rpm with 20% sulphur removal in 5 days.

The microbial consortia significantly (t= 32.11, p≥ 0.05) removed the pyritic sulphur from coal compared to coal incubated with control sterile water. There was duration dependent increase in the removal of pyritic sulphur from 11 % on day 1 to 48% on day 24 (Fig. 5a). The microbial consortia significantly (t= 12.11, p≥ 0.05) removed the sulfate sulphur from coal compared to coal incubated with control sterile water (Fig. 5b). The removal efficiency of sulfate sulphur increased with an increase in the incubation duration.

On the contrary, microbial consortia hardly removed any organic sulphur from coal and were comparable to coal incubated with control sterile water (Fig. 6b). There was no change in the organic sulphur content of the coal incubated with an increase in the duration of incubation for 24 days with microbial consortia in mine drainage water. Moreover, the non-uniform distribution of sulphur in coal might have been caused due to reduction in size (Shang *et al.,* 2016; Tang *et al*., 2020). The sulphur-free coal particles may have created on extra absorption sites for microorganisms resulting in a low concentration of microbial cells on sulphur-containing coal particles and therefore, a reduction in the removal of sulphur. After optimization of the parameters that affect the process, a total reduction of 48% in pyritic sulphur and 21% in sulfate sulphur) was achieved using a coal particle size of 850 µm with 10% pulp density, at room temperature and pH 2.0 after incubation for a period of 24 days.

Incubation of the pulverized coal with isolated and cultured heterogeneous microbial species from Mines drainage water water of Talabira coal mines drainage for 24 days in aerobic condition led to significant reduction in pyritic sulphur content, while no remarkable reduction was seen in the case of organic sulphur. The present study demonstrates that Mines drainage water from Talabira mines inhabits numerous potential desulfurizing bacterial species that need further isolation, characterization, and process optimization to achieve optimal sulphur removal efficiency.

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Authors' contribution: S.N. Ahmed: Experimental designing, data curation, original draft; **N.J. Ekka:** Writing and editing; **Baitharu:** Guidance, supervision.

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References

Acharya, C., R.N. Kar and L.B. Sukla: Microbial desulfurization of different coals. *Appl. Biochem. Biotechnol.,* **118**, 47-63 (2004).

Aytar, P., C.M. Kay, M.B. Mutlu and A. Cabuk: Coal desulfurization with *Acidithiobacillus ferrivorans* from Balya Acidic Mine drainage. *Ene. Fuels*, **27**, 3090–3098 (2013).

Akhtar, N., M.A. Ghauri and K. Akhtar: Dibenzothiophene desulfurization capability and evolutionary divergence of newly isolated bacteria. *Archi. Microbiol.,* **198**, 509–519 (2016).

Bhanjadeo, M.M., K. Rath, D. Gupta, N. Pradhan, S.K. Biswal, B.K. Mishra and U. Subudhi: Differential desulfurization of dibenzothiophene by newly identified MTCC strains: influence of Operon Array. *PLoS ONE,* **13**, 0192536 (2018).

Bressler, D.C. and P.M. Fedorak: Purification, stability, and mineralization of 3-hydroxy-2- formylbenzothiophene, a metabolite of dibenzothiophene. *Appl. Environ. Microbiol.,* **67**, 821-826 (2001).

Clark, D.A. and P.R. Norris: *Acidimicrobium ferrooxidans* gen. nov., sp. nov. mixed-culture ferrous iron oxidation with *Sulfobacillus* species. *Microbiology*, **142**, 785-790 (1996).

Chandra, D., P. Roy, A.K. Mishra, J.N. Chakrabarti, N.K. Prasad and S.G. Chaudhuri: Removal of sulphur from coal by *Thiobacillus ferroxidans* and by mixed acidophilic bacteria present in coal. *Fuel*, **59**, 249-252 (2004).

Caicedo, G., M. Prada and H. Pelaez: Evaluation of a coal biodesulfurization process (semi-continuous mode) on the pilot plant level. *Dyna.,* **79**, 114–118 (2012).

Cao, D., G. Qin, Y. Zhang, S. Ning, G. Wu, M. Chen, Y. Qin, S. Zhu and H. Zhu: Classification and combination relationship of mineral resources in coal measures (in Chinese). *J. China Coal Soc.,* **41**, 2150–2155 (2016).

Das, A. and A.K. Mishra: Role of *Thiobacillus ferrooxidans* and sulphur (sulphide)-dependent ferric-ion-reducing activity in the oxidation of sulphide minerals. *Appl. Microbiol. Biotechnol.,* **45**, 377- 382 (1996).

Durusoy, T., T. Ozbas, A. Tanyolac and Y.Yurum: Biodesulfurization of some Turkish lignites by *Sulfolobus solfataricus. Energy Fuels*, **6**, 804–808 (1992).

Dutta, M., N. Islam, S. Rabha, B. Narzary, M. Bordoloi, D. Saikia, L.F. Silva and B.K. Saikia: Acid mine drainage in an Indian high-sulphur coal mining area: Cytotoxicity assay and remediation study. *J. Hazard. Mater.,* **389**, 121851 (2020).

Eschka, A.: *Chem. Zbl.,* 301(1874).

Etemadzadeh, S.S., G. Emtiazi and Z. Etemadifar: Heterotrophic bioleaching of sulphur, iron, and silicon impurities from coal by *Fusarium oxysporum* FE and Exo-phialaspinifera FM with growing and resting cells. *Curr. Microbiol.,* **72**, 707–715 (2016).

Falagan, C. and D.B. Johnson: The significance of pH in dictating the relative toxicities of chloride and copper to acidophilic bacteria. *Res. Microbiol.,* **169**, 552-557 (2018).

Gupta, N., P.K. Roychoudhury and J.K. Deb: Biotechnology of desulfurization of diesel: Prospects and challenges. *Appl. Microbiol. Biotechnol.,* **66**, 356–366 (2005).

He, H., F.F. Hong, X.X. Tao, L. Li, C.Y. Ma and Y.D. Zhao: Biodesulfurization of coal with *Acidithiobacillus caldus* and analysis of interfacial interaction between cells and pyrite. *Fuel Proc. Technol.,* **101**, 73–77 (2012).

International Conference on Advanced Materials and Energy

Sustainability (AMES2016), Wuhan, Hubei, China, 27–29 May 2016, pp. 521-527. https:// doi.org/ 10.1142/ 978981 3220393_0064.

IS 1350-3: Methods of Test for Coal, Coke, Part III: Determination of Sulfur [PCD7: Solid Mineral Fuel] (1969).

Kargi, F. and J.M. Robinson: Removal of sulphur compounds from coal by the thermophilic organism *Sulfolobus acidocaldarius. Appl. Environ. Microbiol.,* **44**, 878–883 (1985).

Kilbane II, J.J.: Mutant microorganisms useful for cleavage of organic CS bonds. Institute of Gas Technology, Google Patents US5002888A (1992).

Larsson, L., G. Olsson, O. Holst and H.T. Karlsson: Pyrite oxidation by thermophilic archaebacteria. *Appl. Environ. Microbiol.,* **56**, 697–701 (1990).

Li, W., Q. Feng and Z. Li: Isolation and characterization of a novel iron-Sulphur oxidizing bacterium *Acidithiobacillus ferrooxidans* YQ-N3 and its applicability in coal biodesulfurization. *Mineral,* **13**, 95 pages (2023).

Liu, F.W., Y.S. Lei, J. Shi, L.X. Zhou, Z.H. Wu, Y. Dong and W.L. Bi: Effect of microbial nutrients supply on coal bio-desulfurization. *J. Hazard. Mater.,* **384**, 121324 (2020).

Marhual, N.P., N. Pradhan and R.N. Kar: Differential bioleaching of copper by mesophilic and moderately thermophilic acidophilic consortium enriched from same copper mine water sample. *Bioresour. Technol.,* **99**, 8331–8336 (2008).

Martanez, I., M.M. El-Said, V.E. Santos, J.L. Garcia., F. Garcia-Ochoa and E. Diaz: Metabolic and process engineering for biodesulfurization in Gram-negative bacteria. *J. Biotech.,* **262**, 47–55 (2017).

Merrettig, U., P. Wlotzka and U. Onken: The removal of pyritic sulphur from coal by Leptospirillum-like bacteria. *Appl. Microbiol. Biotechnol.,* **31**, 626–628 (1989).

Mishra, S., P.P. Panda, N. Pradhan, D. Satapathy, U.K. Subudhi and S. Biswal: Effect of native bacteria *Sinomonas flava 1C* and *Acidithiobacillus ferrooxidans* on desulfurization of Meghalaya coal and its combustion properties. *Fuel,* **117**, 415-421(2018).

Mishra, S., N. Pradhan, S. Panda and A. Akcil: Biodegradation of dibenzothiophene and its application in the production of clean coal. *Fuel Proce. Technol.,* **152**, 325–342 (2016).

Mott, R.A. and H.C. Wilkinson: The use of the Eschka method for the determination of high sulphur contents. *J. Appl. chem.*, **3**, 218-223 (1954).

Norris, P.R. and D.P. Kelly: Dissolution of pyrite (FeS_2) by pure and mixed cultures of some acidophilic bacteria. *FEMS Microbiol. Lett.,* **4**, 143-146 (1980)

Olsson, G., L. Larsson, O. Holst and H.T. Karlsson: Desulfurization of low-sulphur coal by *Acidianus brierleyi*. Effects of microbial treatment on the properties of coal. *Fuel Proc. Technol.,* **33**, 83–93 (1993).

Panda, S., N. Pradhan and U.B. Mohapatra: Bioleaching of copper from pre and post thermally activated low-grade chalcopyrite contained ball mill spillage. *Front Environ. Sci. Eng.,* **7**, 281–293 (2013).

Peccia, J., E.A. Marchand, J. Silverstein and M. Hernandez: Development and application of small-subunit rRNA probes for assessment of selected *Thiobacillus* species and members of the genus *Acidiphilum. Appl. Environ. Microbiol.,* **66**, 3065-3072 (2000).

Prayuenyong, P.: Coal biodesulfurization processes. *Songklanakarin J. Sci. Technol.,* **24**, 493–507 (2002).

Rai, C. and J.P. Reyniers: Microbial desulfurization of coals by organisms of the genus *Pseudomonas*. *Biotechnol. Progr.,* **4**, 225–230 (1988).

Rao, T.M., S.N. Ahmed, A.K. Sahu, S. Shroff, A.K. Behera and I. Baitharu: Impact of surface coal mining on soil properties of the region at Talabira mining area, Sambalpur, Odisha. *Eco. Env. Cons.*, **29**, 229-240 (2023).

Rout, P.G., A.K. Mohanty, N. Pradhan, S.K. Biswal and S.K. Behera: Study on the reaction mechanism of oxidative microbial desulfurization of organic sulphur-rich coal. *Geomicrobiol. J.,* **3**, 210-218 (2021).

Shang, H., J.K. Wen, B. Wu and B.W. Chen: The study of *Thiobacillus ferrooxidans* on desulfurization of high sulphur coal from Shanxi province. *Advan. Mater. Ener. Sustain.,* pp. 521-527 (2017)

Silverman, M.P.: Mechanism of bacterial pyrite oxidation. *J. Bacteriol.,* **94**, 1046–1051 (1967).

Silverman M.P. and D.G. Lundgren: Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. *J. Bacteriol.,* **77**, 642–647 (1959).

Singh, A.K., A. Kumar, P.K. Singh, A.L. Singh and A. Kumar: Bacterial desulfurization of low-rank coal: A case study of Eocene lignite of western Rajasthan, India. Energy Sources, Part A: Recovery, Utilization and Environmental Effects. **40**, 1199–1208 (2018).

Soleimani, M., A. Bassi and A. Margaritis: Biodesulfurization of refractory organic sulphur compounds in fossil fuels. *Biotechnol. Advan.,* **25**, 570–596 (2007).

Sun, W., E. Xiao, V. Krumins, Y. Dong, B. Li, J. Deng, Q. Wang, T. Xiao and J. Liu: Comparative analyses of the microbial communities inhabiting coal mining waste dump and an adjacent acid mine drainage creek. *Environ. Microbiol.,* **78**, 651–664 (2019).

Thompson, J.D., D.G. Higgins and T.J. Gibson: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions specific gap penalties and weight matrix choice. *Nucleic Acids Res.,* **22**, 4673- 4680 (1994).

Ye, J., P. Zhang, G. Zhang, S. Wang, M. Nabi, Q. Zhang and H. Zhang: Biodesulfurization of high sulphur fat coal with indigenous and exotic microorganisms. *J. Clea. Produc.,* **197**, 562-570 (2016).

Zhang, M., T. Hu and G. Ren: Research on the effect of surfactants on the biodesulfurization of coal. *Ener. Fuels,* **31**, 8116–8119 (2017).