Bioelimination of sulfur from high-sulfur coal by selected strains of microorganisms

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Abstract. In this study, low-rank lignite coal sample collected from Lenger coal deposit (Turkestan province) in Kazakhstan was subjected to desulfurization by using three bacterial strains isolated from soil with silt and coal itself. The molecular identification of the 16S rRNA gene revealed that the isolated bacteria were *Atlantibacter* sp., *Pseudomonas* sp., *Bacillus* sp. denoted as S1, S2, and T1, respectively. *Pseudomonas* sp. showed the best result in removing organic sulfur (93%) and total sulfur (52%), while *Bacillus* sp. was effective in removing pyritic sulfur (19%) compared to other strains. However, *Atlantibacter* sp. had no significant influence on sulfur content after treatment, thereby reducing its chances to be used in decreasing sulfur content in lignite in future investigations. Additionally, this research would be valuable to develop an innovative biotechnological method for producing an environmentally friendly briquetted smokeless fuel from lignite.

1 Introduction

The coal industry is one of the main sectors of the economy in many countries of the world. Thus, coal is used as a fuel, for the manufacture of building materials, in medicine and the chemical industry. However, its extraction, processing and use lead to environmental pollution.

Therefore, the development of high-tech coal business and the implementation of new growth points, such as coal enrichment, coal gasification, coal hydrogenation, methane extraction from coal beds, catalytic processing of methane, etc. are becoming important for the future of the coal industry.

For example, the enrichment of coal is always aimed at not only removing ash but also reducing sulfur in coal, since sulfur is considered a harmful component of coal for many reasons.

With an increase in sulfur content, the heat of combustion of coal decreases, the amount of sulfur dioxide in flue products released into the surrounding atmosphere increases. Emissions of sulfur compounds cause morbidity and mortality in humans; the death of coniferous trees; acid rain; decrease in crop yields in agriculture, etc.

In order to improve the marketability of the fine coal produced during mining, it is desirable to remove as much sulfur and ash as possible [1].

Sulfur in coal is present in both inorganic and organic forms. The inorganic sulfur in coal consists predominantly of sulfides and sulfates. Sulfide minerals include pyrite, sphalerite, galena, arsenopyrite and others. The sulfate minerals include barite, gypsum, anhydrite, and a number of iron sulfates and others. The organic sulfur in coal is covalently bound into its large complex structure and is difficult to remove physically or chemically, in contrast to pyritic or inorganic sulfur [2].

In this regard, research is being conducted on new physical methods for cleaning coal, such as multistage flotation, electrostatic separation, and oil agglomeration. According to preliminary data, these methods can remove up to 90% of all pyritic sulfur and up to 65% of the total amount of sulfur from coal. However, complete desulfurization is possible as a result of the removal of bound organic sulfur. Therefore, at present, methods of chemical and microbiological purification of coal from organic sulfur are being developed.

The method of chemical cleaning involves the treatment of coal with special chemicals or solvents under pressure. It is possible to remove up to 95% of all sulfur. However, the method is much more expensive compared to the physical, as a result, the cost of coal increases.

Methods of microbiological cleaning, green-based approaches are based on the fact that certain bacteria and fungi absorb sulfur, while coal liquefaction is also possible. Microbial coal desulfurization before combustion has low capital and operating costs and is more energy efficient compared with high-temperature chemical processes [3]. There are diverse representative microbial groups responsible for sulfur oxidation and reduction in different ecosystems [4].

Kazakhstan is among the top ten countries with the largest coal deposits in the world. Brown coal which has

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low calorific value and high ash content is piled up as rubbish causing a serious waste of resources as well as environmental pollution. Kazakhstan needs to acquire new technologies for coal processing, especially green-based approaches [5].

Relatively little studies have been conducted to evaluate properties of Kazakhstan brown coal, specifically reduction of sulfur content of coal by the bacterial means. The present work is intended to show comparative analysis before and after desulfurization of lignite by microorganisms.

2 Materials and methods

2.1 Coal samples

Brown coals of the Lenger coal deposit (South Kazakhstan region) (42°10'51.7"N 69°52'58.8"E) (Fig.1.), which are characterized by medium ash content and significant sulfur content, were used. Coal sampling was carried out in accordance with ISO 18283: 2006 “Hard coal and coke - Manual sampling and ISO 13909–4: 2016 Preview Hard coal and coke - Mechanical sampling - Part 4: Coal - Preparation of test samples”. The top layer of 1,5-2,0 cm coal removed with a sterile knife and 500-600 grams of lignite samples gathered with a sterile spatula to the depths of 30 cm. The samples were placed in a sterile container and transported to the laboratory. Each sample was labeled indicating the date and sample number. During transportation and storage of coal samples the rules have been followed in order to prevent the possibility of secondary pollution [5].

Fig.1. The coal (1 – “Lenger” coal deposit) and microbial (2 – “Oikaragai”coal deposit) sampling points

2.2 Isolation and identification of native microorganisms

Strains were isolated from different ecological niches like local rivers, flowing water, stagnant soil with silt, rusty pipe, soil near the road, sewer cover and coal itself (Oikaragai coal deposit, Kazakhstan) (42°10'51.7"N 69°52'58.8"E) (Fig.1.). Identification of the isolated bacteria was carried out by determining the direct nucleotide sequence of the 16S rRNA gene fragment, followed by determining the nucleotide identity with the sequences deposited in the international GeneBank database, and also constructing phylogenetic trees.

2.3 Nutrient media composition

The following nutrient media for the growth of microorganisms were used:

1) The SOB medium of the following composition: bacto-peptone - 10 g, K_2HPO_4 - 1.5 g, iron-ammonium citrate - 0.75 g, $Na_2S_2O_3$*$5H_20$ - 1 g per 1000 ml of distilled water. Agar with a concentration of 15 g/l was added to the SOB agar medium. The inoculums were incubated for 24 hours at a temperature of 30°C.

2) Thiobacillus Broth of the following composition: $(NH_4)_2SO_4$ - 0.400 g/l, KH_2PO_4 - 4.000 g/l, $CaCl_2$ - 0.250 g/l, $FeSO_4$ - 0.010 g/l, $MgSO_4$ - 0.500 g/l, $Na_2S_2O_3$ - 5.000 g/l. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Samples are inoculated into Thiobacillus Broth. After incubation at 25-30°C for about 7 days or more, turbidity or sulfur precipitation on the surface of the liquid or against the walls of the flasks indicates growth of bacteria. Isolation is subsequently done on Thiobacillus Agar (Agar - 12.500 g/l).

2.4 Treatment conditions

The ability of pure cultures of bacteria to grow on brown coal was studied. Conditions for treatment: pH – 3; Incubation time – 12 days; Pulp density – 5%; Particle size – 2 mm; Temperature – 30°C. Reactions were conducted in a shaking incubator with a rotational speed of 180 rpm. After optimizing the pH, incubation time, pulp density, particle size, and incubation temperature for the microorganisms, the desulphurization of the lignite coal sample was further analyzed. At the end of the incubation time, the coal samples were separated by centrifugation and then washed with distilled water. The coal samples were dried at 45 °C overnight for further analysis [6].

2.5 Determination of sulfur forms

Methods for determining the forms of sulfur are based on the different solubilities of sulfur compounds in hydrochloric and nitric acid solutions according to ISO 157-75 Solid mineral fuel. Determination of sulfur forms (Fig.2.).

The essence of the method: pyrites do not dissolve in hydrochloric acid, but dissolve in nitric acid; sulfates are dissolved in hydrochloric and nitric acids, and organic sulfur compounds do not dissolve in hydrochloric or nitric acids.

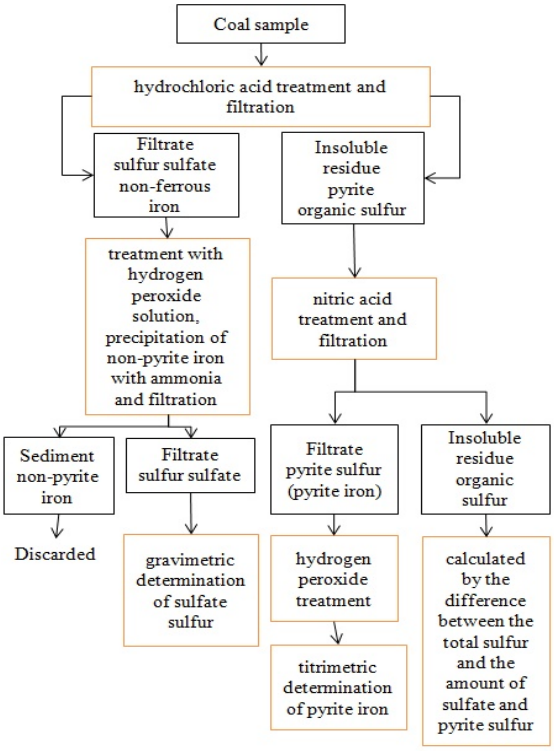

Fig. 2. The general scheme for determining the forms of sulfur in coal.

3 Results and discussion

As a result of the research, 3 cultures of microorganisms were isolated (S1, S2, T1) for further identification.

The isolates were genotyped by the method of determining the direct nucleotide sequence of the fragment of the 16S rRNA gene and the phylogenetic trees were constructed with the nucleotide sequences of the 16S rRNA gene of the reference strains of these species (Fig.3-5).

S1 is identified as *Atlantibacter* sp. and this sample was isolated from soil with silt.

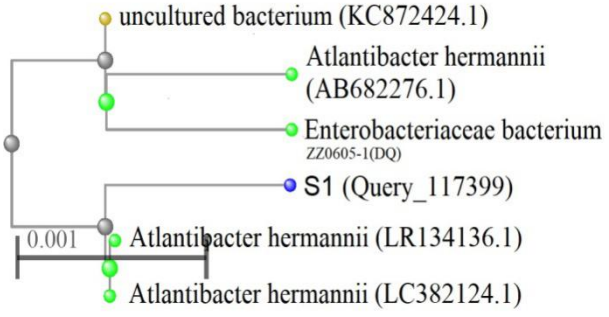

Fig.3. Phylogenetic tree, based on the analysis of a fragment of the 16SrRNA gene of the group *Atlantibacter* sp.

S2 is identified as *Pseudomonas* sp. and this sample was taken from soil with silt.

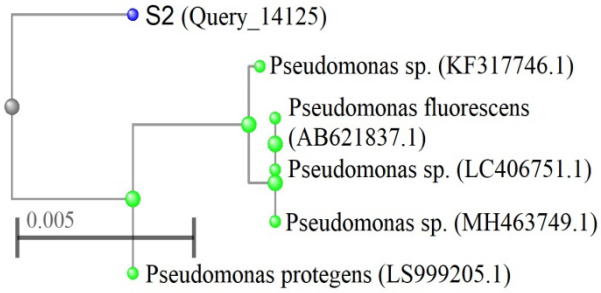

Fig.4. Phylogenetic tree, based on the analysis of a fragment of the 16SrRNA gene of the group *Pseudomonas* sp.

T1 is identified as *Bacillus* sp. and this sample was isolated from coal itself (Oikaragai coal deposit).

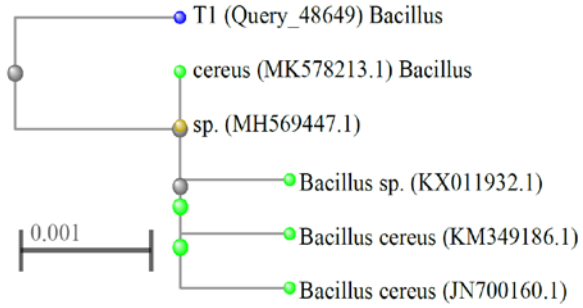

Fig.5. Phylogenetic tree, based on the analysis of a fragment of the 16SrRNA gene of the group *Bacillus* sp.

The results show that the strain S1 is located on the same clade with the nucleotide sequences from the reference strain *Atlantibacter hermannii*, while S2 is located on the same clade with the nucleotide sequences from the reference strain *Pseudomonas* sp. As shown in Fig.5, the T1 strain is located on the same clade with *Bacillus cereus.*

The data obtained as a result of a comparison of genetic characteristics made it possible to determine the species of the strains S1, S2, and T1 in the genera *Atlantibacter, Pseudomonas and Bacillus*, respectively.

According to the total sulfur content, the coal is divided into low-sulfur (up to 1.5% sulfur), medium-sulfur (1.5-2.5%), sulfur (2.5-4%) and high-sulfur (more than 4%). Pyrite, organic, sulfate and elemental sulfur are distinguished in coal [7].

Sulfur compounds in coal are present mainly as organic sulfur, pyritic sulfur, and sulfate sulfur. Pyritic sulfur occurs in coal as mineral matter whereas the organic sulfur is present as an integral part of the coal matrix covalently bounded to its complex structure [6].

One of the indicators characterizing the technical nature of coal - total sulfur (S,%) indicates the total content of this element in all compounds, converted conditionally to elemental sulfur (%) in relation to the analyzed sample.

According to the results obtained (Table 1), sulfur content in the coal of the Lenger coal deposit is about 3.14%.

Table 1. Sum of various forms of sulfur in a sample of brown coal from the Lenger coal deposit

Sulfur forms	Content, %
Total sulfur –S_{total}	3,14
Sulphate sulfur – S_S	<0,01
Pyritic sulfur – S_P	1,61
Organic sulfur – S_O	1,53

The lignite coal was desulfurized using the newly isolated bacteria *Atlantibacter* sp., *Pseudomonas* sp., *Bacillus* sp.

Conditions for treatment:

- pH – 3;
- Incubation time – 12 days;
- Pulp density – 5%;
- Particle size – 2 mm;
- Temperature – 30°C.

Our team conducted an experiment to study the process of desulfurization, and the results of the analysis of the effects of microorganisms on total sulfur and sulfur forms of brown coal were analyzed and demonstrated on Table 2.

Table 2. Sulfur concentration before and after microbial treatment of coal sample from Lenger coal deposit.

Sulfur, in %	Initial coal	Biodesulfurized coal with		
		S1	S2	T1
S_{Total}	3,14	2,65	1,52	1,94
$S_{Sulphate}$	<0,01	<0,01	<0,01	<0,01
S_{Pyrite}	1,61	1,56	1,41	1,3
$S_{Organic}$	1,53	1,09	0,11	0,64

As can be seen from Table 2, the total sulfur content of the initial coal sample was 3,14%, where 1,53% corresponded to the organic sulfur and less than 1,62% to the inorganic sulfur (1,61% was pyritic sulfur and less than 0,01% was sulfate sulfur).

It was found that 16% of the total sulfur was removed from the lignite coal after *Atlantibacter* sp. (S1) treatment under optimal conditions, the total sulfur content was reduced to 2,65%, organic sulfur was reduced to 1,09% and inorganic sulfur to less than 1.57% (where 1.56% was pyritic sulfur and less than 0.01% was sulfate sulfur), which was a reduction of 29% in organic sulfur and 3% in pyritic sulfur, while sulfate sulfur had no significant changes.

After the treatment with *Bacillus* sp. (T1), 38% of the total sulfur was removed from the lignite coal, the total sulfur content was reduced to 1,94%. More than half of the organic sulfur (58%) was extracted, reaching 0,64%. Inorganic sulfur in the form of pyrite decreased till 1,3%, which is the 19% from initial coal, however, sulfate sulfur remained stable (<0,01%).

For *Pseudomonas* sp. (S2), the increase of the percentage of the removed total sulfur showed the best results, thus 52% sulfur removal was achieved. The total sulfur content was reduced to 1,52%. Organic sulfur was almost fully removed, reducing to 0,11%, and inorganic sulfur to 1,42% (where 1,41% was pyritic sulfur and less than 0,01% was sulfate sulfur). The reduction was of 93% for organic sulfur and 12% for pyritic sulfur.

Based on these studies and results, a literature review was conducted on these strains. Thus, there are relatively few studies on *Pseudomonas* sp. and *Bacillus* sp. used in desulfurization. For example, Charanjit Rai and Jon P. Reyniers stated that the results from the preliminary microbial desulfurization of Illinois and Texas lignite by the organisms of the genus *Pseudomonas* show that *P. putida* was much more effective than *P. aeruginosa*. The *P. putida* reduced the pyritic sulfur content by 76% in 5 to 7 days. Whereas, *P.aeruginosa* was hardly effective (26 to 32.5%) in reducing the pyritic sulfur content [8].

The other study demonstrated the mutant strain *Pseudomonas stutzeri* LH42 (Mutant ZW-15) which was used for the biodesulfurization of coal. After 15 days' processing, it degraded 93.25% of organic sulfur and 41% of total sulfur. Thus, we can conclude that Mutant ZW-15 can be used as an efficient strain in the coal biodesulfurization [9].

A.A. El-Midany, M.A. Abdel-Khalek stated that *B. subtilis* can remove more than 70% of sulfur and ash content in the feed. The final concentrate, using *B. subtilis*, contains 0.9% sulfur and 1.95% ash with a recovery of 90–92% [10].

4 Conclusion

Recently, a number of entrepreneurs have become interested in the problem of reducing the sulfur content in coal. This is due to the sale of coal for export to other countries, where there are rather strict requirements for the sulfur content of concentrates [11].

Also, the current environmental situation in urbanized regions tightens the requirements for the quality of municipal solid fuel that is burned, along with that, the municipal fuel that is burned must have a low sulfur content, minimal opacity and the required particle size distribution.

To date, the Association of Mining and Metallurgical Enterprises is the largest industry association in Kazakhstan, which is composed of more than 100 companies of ferrous and non-ferrous metals, uranium and coal mining. Experts of this Association predict that coal will remain the main most reliable strategic type of fuel, ensuring the development of electric power. Therefore Kazakhstan needs to acquire new technologies for coal processing, especially green-based approaches.

As a result of the studies, three cultures of microorganisms were identified and comparisons of genetic characteristics made it possible to determine the species of these strains in the genera *Atlantibacter, Pseudomonas,* and *Bacillus*, respectively. The experiment to study the desulfurization process was conducted and the results of the effect of the

microorganism treatment on total sulfur and sulfur forms of lignite coal were analyzed

Thus, *Pseudomonas* sp. showed the best result in removing organic sulfur (93%) and total sulfur (52%), while *Bacillus* sp. was effective in removing pyritic sulfur (19%) compared to other strains. However, *Atlantibacter* sp. had no significant influence on sulfur content after treatment, thereby reducing its chances to be used in decreasing sulfur content in lignite in future investigations. Additionally, this would be valuable information to consider while conducting the development of a biotechnological method of producing an environmentally friendly briquetted smokeless fuel from brown coal.

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