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Microorganisms in Coal Desulfurization (Review)

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Abstract—The information on the use of microorganisms and mixed consortia in the biological desulfurization of coal is summarized. The ecological problems that accompany the burning of high-sulfur coals are shown, and the prospects of environmentally friendly and resource-saving biotechnological approaches to coal desulfurization are considered. Analysis of available literature indicates the enormous role of microorganisms of various taxonomic groups in the removal of inorganic and organic sulfur in coals. Mesophilic and moderately thermophilic acidophilic chemolithotrophic bacteria (ACB) of the genus *Acidithiobacillus—A. ferrooxidans*, *A. thiooxidans*, *A. caldus*, as well as some heterotrophic bacteria *Bacillus subtilis and paenibacillus polymyxa*—play the dominant role in the removal of inorganic sulfur. Mixed cultures and associations of mesophilic and thermophilic bacteria isolated from coal mines or from the coal surface structure are considered an effective tool in the biosulfurization of pyrite sulfur. The prospects of microbial desulfurization of organic coal sulfur with the use of heterotrophic microorganisms of the genera *Pseudomonas, Sulfolobus, Rhodococcus,* the fungi *Agrocybe aegerita* and *Alterneria* sp., the bacterial–fungal consortia *Sulfolobus solfataricus* and *Phanerochaeta chrysosporium* ME446, and the laccase enzyme of the basidiomycetes *Trametes versicolor* ATCC 20080 are examined.

Keywords: coal, sulfur, desulfurization, mesophilic and thermophilic acidophilic chemolithotrophic bacteria, heterotrophic microorganisms, bacteria, fungi, dibenzothiophene

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INTRODUCTION

The most important environmental characteristic affecting the quality of coal is the presence of sulfur in it. The sulfur content in the coals of various basins and deposits varies widely. The sulfur content in ordinary coals is 0.4–8% in Russia and 0.7–5.4% in the United States. The average for this indicator is 1.8–2.2%. The range of fluctuations in the total sulfur content in Donetsk coals is extremely large: 0.46–9.28% [1].

When coal is burned, sulfur compounds turn into sulfur dioxide gases. When released into the atmosphere, these gases lead to the formation of acid rain, which has a harmful effect on the environment and the vital activity of living organisms. Moreover, high-sulfur coals are poorly coked and therefore cannot be used in nonferrous metallurgy [2, 3].

The purification of sulfur compounds from coal is an important problem for the fuel and energy industry. Despite the large number of mechanical, thermal, and physicochemical methods proposed and tested under production conditions, it remains unresolved [4]. The separation of sulfur from coal based on mechanical methods can reduce the sulfur content in them only by 15–20%, while the use of thermochemical processing methods requires a high temperature and pressure, which are associated with high operating costs, a partial loss of combustible substances, and the release of a large amount of carbon dioxide [5–7]. The most promising and effective methods involve the removal of sulfur from coal with biotechnological processes based on the decomposition of sulfur compounds by microorganisms. The advantages of these processes are their low energy costs and environmental friendliness with maintenance of the energy value of the coal [8–10].

Over the past decades, there have been a sufficient number of publications indicating the ability of a wide range of taxonomic groups of bacteria to reduce the sulfur content in coal [11–13].

This review summarizes the available literature on coal biodesulfurization and the ability of various microorganisms to effectively desulfurize coal (the literature search was conducted in 2019, and the search years were 2009–2019).

Sulfur compounds in coal are present mainly in the form of inorganic or pyrite (S_{pyr}), organic (S_{org}), and sulfate (S_{sul}) sulfur [14, 15].

Th pyrite sulfur in coal is in the form of a mineral substance. It is weakly bound to the structure of coal, while organic sulfur is present as an integral part of the coal matrix. It is uniformly distributed throughout the seam and is covalently bonded to the carbon skeleton of coal [16, 17]. The process of the biotechnological

removal of sulfur occurs through the thiosulfate and polysulfate pathways. It proceeds as a biochemical reaction catalyzed by microorganisms in a liquid medium. This leads to the oxidation of sulfur to sulfites and sulfates, which are water soluble [18].

Removal of inorganic sulfur. According to the literature, a wide range of microorganisms possess the ability to reduce the inorganic sulfur content. Of these mesophilic and moderately thermophilic acidophilic chemolithotrophic bacteria (ACB) and archaea predominate [19–22].

The most common microorganisms used to remove pyrite sulfur are mesophilic acidophilic bacteria: *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans.* These are non-spore-forming, gram-negative rod bacteria that are autotrophs with low power-source requirements. They are demanding with respect to oxygen: a 5% decrease in oxygen in the ambient air leads to a decrease in their activity. The pH range of bacteria activity is 1.5–3.5, and the optimum temperature is 25–40°C. Mesophilic bacteria are found in large numbers in natural and ore waters, as well as in coal mines [23].

The effectiveness of the biodesulfurization process depends on many factors, primarily, the pH of the medium, redox potential (Eh), temperature, pulp density, size of coal particles, the content and distribution of pyrite in coal, the type of microorganisms, etc. Studies [24] have shown the effect of particle size and pulp density on the biodesulfurization of coal from the Tabas mine (Iran) with the participation of *A. ferrooxidans*. It is noted that a decrease in particle size from 0.5–1.0 to 0–0.5 mm increased the desulfurization level by more than two times; the maximum sulfur removal was observed at a pulp density of 10%.

The results of coal desulfurization from a coal mine in Guizhou province (southwestern part of China) with the aerobic chemoautotrophic bacterium *A. ferrooxidans* YY2 isolated from acid-mine drainage are presented in [25]. In this case, the percentage of total sulfur removal with *A. ferrooxidans* YY2 in a sequencing batch reactor for 20 days amounted to 75%, including 86% of the pyrite sulfur.

He H. et al. [26] report Indonesian coal biodesulfurization with the thermophilic *A. caldus* strains isolated from the hot springs of Yun Nan Province in southeast China. A nutrient medium known as Starkey's basal-salt medium with the addition of sulfur pyrite and thiosulfate powder was used to cultivate *A. caldus*; the bacteria were cultured at a temperature of 40°C. The results showed that bacteria were able to remove 47% of the pyrite and 19% of the total sulfur from coal. The use of thermophilic bacteria in biodesulfurization made it possible to increase the rate of the process in bioreactors and reduced the likelihood of pollution of the nutrient medium [13].

In [27], the removal of total sulfur from Turkish coal by a pure culture of *A. ferrivorans* isolated from acidic drainage of the Bal mine (Turkey) was reported. Biodesulfurization proceeded at a pH of 2.5, an inoculum amount of 2%, a pulp density of 1%, and a coal particle size of 500–250 μm. In 14 days of *A. ferrivorans* incubation, it was possible to reduce the total sulfur content in coal by 33%.

An effective tool in coal biodesulfurization is the use of mixed cultures, associations, and consortia isolated from coal mines or from coal-surface structures. A native mixture of *A. ferrooxidans and A. thiooxidans,* which were isolated during acid drainage of coal mines and adapted for 6 months, was used to desulfurize two samples of Colombian coals from the southwest (Colombia) [28]. Within 30 days, it was possible to reduce the pyrite sulfur content in coal samples by 85–95% and the total sulfur content by 31–51%. The microorganisms were cultured on a solid nutrient medium, the process of coal desulfurization proceeded at a temperature of 30°C, the pulp density was 10%, and the coal particle size was 74 μm. The highest pyrite oxidation rate was characteristic of a high-sulfur coal sample. This is apparently due to its spheroidal shape, which facilitates the oxidation of minerals with a significant increase in the area of interaction with microorganisms.

A mixed culture of mesophilic microorganisms *A. ferrooxidans, A. thiooxidans,* and *Leptospirilium ferrooxidans* was used to remove pyrite sulfur from high-sulfur coal of the Mehr-Azin section (Tabas, Iran). It was possible to reduce the total sulfur content from 3.87 to 1.92%, with a total efficiency of 50.3%, at the following initial contents: S_{total}, 3.87%; S_{org}, 1.53%; S_{pyr}, 2.31%; S_{sul}, 0.03% [29].

The authors of [30] reported the ability to desulfurize low-grade lignite with a mixed culture of *A. ferrooxidans* and *Pseudomonas* sp. NP22. The studies used a sample of brown coal from the Jining deposit (Shandong, China). The sulfur content decreased by 46% as a result of desulfurization with *Pseudomonas* sp. NP22 and by 37% with *A. ferrooxidans*. The desulfurization process took place at a pH of 3–5, the coal-particle size was 75–45 μm, the pulp density was 5%, the temperature was 35°C, and the incubation time was 8 h. Chemical studies also revealed a decrease in the ash content and an increase in the calorific value of coal from 6219 cal/g to 6406 and 6315 cal/g, which indicated a positive effect of biodesulfurization on the energy value of the coal.

The biodesulfurization data for high-sulfur Colombian coal (Cordoba, Colombia) with initial pyrite and organic sulfur contents of 1.03 and 0.9%, respectively, are presented in [31]. As a result of the study, it was possible to reduce the pyrite sulfur content by 59.22% within 4 days without preliminary grinding of the coal to fine fractions. Coal desulfurization was carried out with a mixed culture of *A. ferrooxidans* and *A. thiooxidans* bacteria (National University of Colombia, Sede Medellín). The process was carried out in two-phase

mode at room temperature with an acidic reaction of the medium, a coal-particle size of 3/4 (<19.05 mm), and a duration of 4–8 days in a stirred reactor with a stirrer having a capacity of 4000 L.

Studies [32] on the desulfurization of Indian coal (Nagaland Northeast India) have shown the effectiveness the strain *Pseudoxanthomonas sp*. in the removal of total sulfur. As the results showed, a high pyrite content was noted in the coals of these deposits; therefore, grinding to a size of 210 μm was used to process the coal samples. Of the nine studied coal samples, four samples were taken for desulfurization. As a result of the studies, the maximum sulfur removal (28.8%) was achieved for samples with a significant number of cavities and cracks in their structure, which indicated the dependence of desulfurization on coal structure.

The ability of heterotrophic bacteria *Bacillus subtilis* and *Paenibacillus polymyxa* to reduce the amount of pyrite sulfur and ash in coal was shown in [33]. Bacteria were isolated from the water of the mine of Al-Mahar (Egypt). As a result of the desulfurization of coal with an initial content of total sulfur of 3.3%, the best result as compared to *P. polymyxa* was noted with *B. subtilis*. Bioflotation tests based on the natural buoyancy of coal and the hydrophilicity of bacteria have shown good potential. *B. subtilis* remove more than 70% of pyrite sulfur and ash from coal.

This literature analysis indicates the ability of mesophilic and moderately thermophilic ACB and archaea to significantly reduce the content of inorganic sulfur in coal. An effective tool in coal biodesulfurization is the use of mixed cultures, associations, and consortia of bacteria isolated from coal mines or from the surface structure of the coal.

Removal of organic sulfur. Organic sulfur is covalently bonded to the atoms of the carbon matrix of coal in the form of sulfur compounds, complex thiophene ring systems, and dibenzothiophene with a C–S bond. The complex molecular structure and low solubility in water limit the use of aerobic chemolithotrophic bacteria to remove organic sulfur. The cleavage of organic sulfur-containing compounds, such as dibenzothiophenes (DBTs), requires the participation of microorganisms capable of destroying C–S bonds with the release of sulfur atoms present in the aromatic ring. Most often, DBT is considered a model compound for the removal of organic sulfur from fossil fuels (oil, coal), since thiophene sulfur most likely constitutes the bulk of the organic sulfur of coal [34].

Three major pathways for the destruction of DBT by microorganisms have been reported [35]. The first is known as the Kodama pathway (the oxidizing pathway), in which DBT is partially oxidized to water-soluble intermediates. The second pathway, the sulfur-specific pathway, degrades the compound. It undergoes desulfurization with cleavage of the C–S bond, which leads to the accumulation of hydroxybiphenyl. The third pathway is completely destructive; DBT is mineralized to CO_2 sulfite and water.

The ability to split aromatic rings of organic sulfur in coals is inherent only to some strains of the bacteria of the genera *Pseudomonas*, *Sulfolobus*, and *Rhodococcus,* as well as bacterial and fungal consortia and enzymes. Aerobic representatives of microorganisms, such as bacteria of the genus *Rhodococcus,* are capable of sequential, selective oxidation of the sulfur atom in the DBT molecule, followed by breakage the C–/S bond and the formation of sulfite/sulfate and the organic component of 2-hydroxybiphenyl (2-HBP) [36].

The results of studies on DBT biodegradation by the native strain *Rhodococcus ruber* are described in [37]. Two coal samples were used for analysis: NE coal, which has a high organic-sulfur content, and lignite and calcined coke (CC). Within 7 days, native *R. ruber* strains reduced the total sulfur content in the NE coal sample by 36%, of which organic sulfur accounted for 53%. The decrease in the sulfur content in Indian lignite and CC was 15.87 and 14.83%, respectively. At the same time, the energy value of NE coal increased from 6698 to 6812 k/cal, which indicated promise for its use in coke production.

The effectiveness of the mixed consortium *Sinomonas flava* 1C and *A. ferrooxidans* for the removal of organic sulfur from Magalai coal of India was demonstrated in [38]. The desulfurization process was carried out in two stages: *S. flava* 1C was used to remove organic sulfur, and *A. ferrooxidans* was used to remove the pyrite. The research results showed that the sequential treatment of coal with particle sizes of 500–300 μm with mixed bacterial cultures reduced the total sulfur content by 3.09%, including organic sulfur by 2.5% and pyrite from 0.1 to 0.8%, with an increase in the calorific value of coal from 26208 to 29481 J/g

The authors of [39] conducted a very interesting work in which they described the biodesulfurization of two high-sulfur Bulgarian coals (coal and lignite) and one Turkish lignite with the participation of a consortium consisting of the fungi *Phanerochaeta chrysosporium* ME446 and the thermophilic, acidophilus bacteria *Sulfolobus solfataricus* ATCC 35091. Before the process, the coal samples were subjected to chemical demineralization and depyritization with the removal of 25.3–54.2% sulfur. A higher degree of desulfurization was achieved with. *P. chrysosporium* ME446; in 6 days, it was possible to reduce the amount of total sulfur by 24.2% and organic sulfur by 23.8%. *S. solfataricus* ATCC 35091 reduced the total sulfur in coal by 16.9% and organic sulfur by 18.3%.

The effective use of the bacterium *Pseudoclavibacter* sp. SKC/XLW-1 and its metabolic products for the oxidation of organic coal compounds of Dongdongur (Indonesia) with multistage biological treatment was reported in [40]. The multistage biological treatment consisted of biooxidation and subsequent bioflotation. In the removal of organic sulfur, accord-

ing to the authors, biooxidation is of great importance, which accounts for 52–100% of the multistage process. As a result of processing, 27 to 31.6% of the organic sulfur of coal was removed. It should also be emphasized that the processes of the removal of pyrite and total sulfur had the same regularities as the removal of organic sulfur. The results indicate the effectiveness of *Pseudoclavibacter* sp. SKC/XLW-1 in the desulfurization of organic sulfur in coal.

Data are presented in [13] on the efficiency of the use of certain fungal classes in the removal organic sulfur from coal, which is probably associated with the production of enzymes, in particular, sulfatases, which catalyze the oxidation of sulfonated phenolic compounds. The effectiveness of basidiomycetes fungi *Agrocybe aegerita* for DBT degradation in vivo and in vitro are reported in [41]. It is noted that *A. aegerita* produces about eight different metabolic products, in particular, DBT sulfoxide, DBT sulfane, etc., which can oxidize up to 100% DBT in 16 days of incubation.

Isolates of six different bacteria, five types of mold fungi, and seven varieties of yeast isolated from different locations (open, closed, and underground quarries, feed, plants, and food products) were used for the desulfurization of lignite from the Michalikik region (Eskisehir, Turkey) with low and high sulfur and ash contents [42]. The obtained isolates were used to study the possibility of coal biodesulfurization. In the studies, *Alterneria* sp. CF1, an effective isolate of endophytic fungi, was isolated. The optimal conditions for sulfur removal were a pH of 4, a particle size of 0.106–0.038 mm, a pulp density of 1%, and an inoculum concentration of 2%. In 12 days of incubation, it was possible to reduce the organic sulfur in the studied coal samples by 38% and the sulfide by 51%.

Upon the desulfurization [43] of low-grade Turkish lignites with a crude laccase enzyme isolated from lignin destructive of basidiomycete *Trametes versicolor* ATCC 200801, the content of both pyrite and organic sulfur was reduced by 35.13 and 25%, respectively. In this case, the optimal coal-particle size was 200 μm, and the pH was 4. The process proceeded at a temperature of 35°C. The complex molecular structure of organic sulfur in the form of sulfur-containing compounds, complex thiophene ring systems, and DBT with the C–S bond, limited the use of ACB in the removal of organic sulfur. Heterotrophic microorganisms, bacteria of genera *Pseudomonas*, *Sulfolobus*, *Rhodococcus* and bacterial–fungal consortia and enzymes, can be used to remove organic sulfur in coals.

CONCLUSIONS

Thus, the literature data collected in this review indicate significant success with various microorganisms in studies on the removal of sulfur from coal. Inorganic sulfur can be removed mainly by mesophilic and moderately thermophilic ACB of the genera *Acidithiobacillus* and *Leptospirilium* and some heterotrophic bacteria of the genera *Bacillus*, *Paenibacillus*, *and Pseudomonas,* and mixed cultures and microbial associations. Heterotrophic microorganisms of the genera *Pseudomonas*, *Sulfolobus*, *Rhodococcus*, *Brevibacterium,* etc., can reduce organic sulfur. In addition, organic sulfur can also be removed by the fungal microflora *Agrocybe aegerita* and *Alterneria* sp.*,* the bacterial–fungal consortium *Sulfolobus solfataricus* and *Phanerochaeta chrysosporium* ME446, and fungal metabolism products.

The biological desulfurization of coal is undoubtedly a complex biological process that is apparently due to the potential of microbial enzymes and cyclic complex compounds secreted by various microorganisms living on coal. The studies conducted to date indicate that the biotechnological methods of coal biodesulfurization are currently carried out mainly on a laboratory scale, and the large-scale commercialization of these technologies still remains insufficiently implemented. The potential commercial potential for the use of the biooxidation of pyrite from coal has been studied in the United States, Italy, and Germany [44–46]. Their very promising results contributed to the design and construction of semicommercial, pilot plants for coal biodepairization in several European countries [11].

In summary of the review of coal biodesulfurization, it should be noted that the biotechnological approach is promising for the processes of their desulfurization, which will solve the environmental problems associated with coal burning. The use of the potential ability of microorganisms to oxidize sulfur to sulfites and sulfates during the biodesulfurization of high-sulfur coals will allow the creation of bioreactors with the required capacities on an industrial scale.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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