Microbial Desulphurization of Turkish Lignites by White Rot Fungi

Pınar Aytar,[†] Mesut Şam,*[,†] and Ahmet Çabuk[‡]

Department of Biology, Faculty of Arts and Science, Balıkesir University, Balıkesir, Turkey, and Department of Biology, Faculty of Arts and Science, Eskişehir Osmangazi University, 26480 Eskişehir, Turkey

Received November 6, 2007. Revised Manuscript Received December 25, 2007

Biodesulphurization experiments were carried out with Tunçbilek lignite, characterized by high sulfur content (2.59%) by using *Trametes versicolor* ATCC 200801 and *Phanerochaete chrysosporium* ME 446. At fungal biomass studies, the effects of various parameters on fungal desulphurization of coals such as pH, temperature, pulp density, incubation time, and sterilization were investigated for both microorganisms. The maximum desulphurization (40%) was observed after 6 days of incubation at 35 °C for *T. versicolor*. The optimum pH was measured at 6, and the agitation rate was fixed at 125 rpm. The pulp density was found as 5% (w/v) for the high extent of desulphurization. Also, calorific value did not change during this experiment. However, the ash and metal contents of coal were eliminated.

1. Introduction

Coal is the most important nonrenewable energy source of fossil fuels. Among energy sources, coal has been accepted as a major fuel for centuries owing to the fact that it is widespread, cheap, and reliable. While coal is burnt, its sulfur content combines with oxygen to form sulfur dioxide, which causes both acid rain and pollution. The concentration of this gas needs to be reduce for protection of the environment throughout the world. Many countries have noticed the problems and started to reduce the amount of SO_2 emission through legislation.[1] Microbial metabolism of sulfur compounds is interesting in the coal industry for desulphurization. Biodesulphurization may be taken into account as a biochemical reaction catalyzed by microorganisms concluding in the oxidation and sulfur content being transfered into water soluble compounds such as sulfates.[2] This is one of the other desulphurization methods arousing the most interest. Bioprocesses might have important advantages over the conventional technologies of breakdown or cleaning currently in use, and they may open up the possibility of the economic production of new products. Furthermore, biological methods have simple installations would be much milder than equivalent chemical transformations and low energy consumption. In addition to removing pyritic sulfur, this method eliminates organic sulfur which is bound to a matrix with covalent bonds making separation from coal difficult.[3]

Low-rank coals which are lignites of Turkey mainly occur in a number of fault-bounded Miocene and Pliocene lacustrine basins in intermontane regions. The Tunçbilek-Domanic basin, which is one of the most productive coal basins of western Anatolia-Turkey, contains a thick and lateral extensive coal bed at the base of the Miocene Tunçbilek formation.[4]

Lignite was found to be most convenient coal for microbial desulphurization because of the fact that it is a younger coal than other coal types. The pyrite in lignite is poorly attached to the coal making the bond easy to remove.[5]

Biodesulphurization studies of coal have been reported with pure cultures of *Thiobacillus ferrooxidans, Thiobacillus thiooxidans*,[6] *Sulfolobus acidocaldarius*,[7] and *Acidianus brierleyi*.[8] Many bacterial organisms including *Pseudomonas* and *Sulfolobus* species were of great interest in the early success of organic sulfur removal. A new isolated bacterium, *Gordonia alkanivorans* RIPI90A, has been shown to desulphurize both dibenzothiophene (DBT) and DBT-containing hexadecane.[9] The ability to remove both organic and inorganic sulfur has been found in *Rhodococcus* species, and for this reason, biodesulphurization processes in a new era have been mostly carried out with these species. Desulphurizing *Rhodococcus* species[10] include *Rhodococcus erytropolis* IGTS8,[11] *R. erytropolis* D-1,[12] *R. erytropolis* H-2,[13] and *Rhodococccus* sp. ERCD-1.[14]

In spite of the fact that much of sulfur removal from coal has been attempted with various bacteria and little work has

* Corresponding author: Tel.: +90 (0266) 612 1000. Fax: +90 (0266) 612 1215. E-mail: msam@balikesir.edu.tr.
† Balıkesir University.
‡ Eskişehir Osmangazi University.

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10.1021/ef700664t CCC: $40.75
Published on Web 02/14/2008

Table 1. Immediate Analysis: Total Sulfur, Ash Content, Calorific Value, and Metal Content of the Lignite, before Subjection to Biodesulphurization

analyses	content
total sulfur (%)	2.59
ash (%)	20.47
GCV (cal/g)	5182
iron (mg/L)	5.4 ± 0.4
zinc (mg/L)	740 ± 2.4
manganese (mg/L)	30.5 ± 5.1
copper (mg/L)	1.8 ± 0.2
nickel (mg/L)	27 ± 0.4
chromium (μg/L)	286 ± 53.4
cobalt (μg/L)	661 ± 37.9
cadmium (mg/L)	38.1 ± 0.1
lead (μg/L)	822 ± 1.3
magnesium (mg/L)	1520 ± 3.4
aluminum (mg/L)	3.8 ± 0.4

been carried out with fungi, Fungi can metabolize a wide range of recalcitrant compounds via extracellular enzymes and the system of cytochrome p-450.[15] It is known that white rot fungi are also involved in degrading sulfur components. Actually, the lignin polymer is structurally similar to lignite.[16] For these reasons, it seemed possible that ligninolytic systems of white rot fungi may be used for the biodesulphurization of coal and petroleum. A lignin-degrading fungus *Phanerochaete chrysosporium* was studied for the mechanism of the ligninase-catalyzed oxidation of thiantrane, the chosen model sulfur compound.[17] The fungal conversions of sulfur-containing heterocyclic compounds were investigated using the lignin-degrading basidiomycetes *Trametes versicolor*. The fungus metabolized a series of sulfur compounds, thiophene derivatives, via different pathways.[18] In the action of biodesulphurization, coal is utilized as a carbon and energy source by microorganisms.[19]

This research aims to investigate the removal of sulfur from Turkish lignites by *T. versicolor* and *P. chrysosporium* cells as alternative biosorbents. The effects of various operating parameters including pH, temperature, pulp density, incubation time, particle size, and sterilization role on this process were evaluated.

2. Materials and Methods

2.1. Coal Samples and Characteristics. A coal sample obtained from Garp Lignite Management of the Tunçbilek (Kütahya-Turkey) was used in this study. In all tests, coal was ground at Retsch cross beater mill SK1, generating a grain of size 200 μm.

Proximate and ultimate analyses of the lignite were carried out with LECO equipment TGA701 for determining the ash amount. The heating value was obtained with a LECO AC-350 automatic calorimeter. Table 1 shows characteristics of the coal. Total sulfur analysis was done using a LECO SC-144DR sulfur analyzer with an infrared absorption detection procedure.[20] The immediate analysis, gross calorific value (GCV), ash content, total sulfur, and metal analyses of lignite are also shown (Table 1).

2.2. Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) Analyses. Metal analyses of coals were carried out using Perkin-Elmer 3100 model ICP-AES. To determine metal contents, the coal samples were digested by the aqua regia extraction method (HCl/HNO_3 3:1).[21] About 5 g (±0.01 g) of the coal samples were weighed into digestion vessels and burned at 600 °C. Then, 20 mL of 1 M HCl and 10 mL of 1 M HNO_3 were used to quantitatively transfer the digest solutions. These solutions were kept overnight. After digestion, coal samples were filtered. The resultant solutions were transferred into 50 mL volumetric flasks and filled to the desired volume using distilled and deionized water prior to metal analyses by ICP-AES. The aim of these dilutions was to reduce viscosities of the solution rendering them more suitable for ICP-AES analyses. Metal contents in this solution were analyzed by inductively coupled plasma atomic emission spectrometry. The elemental composition of the given samples was quantified relative to a reference standard.[21,22]

2.3. Microorganisms and Medium Composition. The two members of basidiomycetes, *Trametes versicolor* ATCC 200801 and *Phanerochaete chrysopsorium* ME 446, were used in this experiment. The microorganisms were first grown on agar slants using a malt extract−agar (Fluka) medium. The microorganisms were subcultured and incubated at 30 °C for 10 days. The inocula from the slope culture were suspended in distilled water and homogenized with 1 mL of inoculant prepared by suspending the stock culture which was added into the 250 mL Erlenmeyer flask containing 100 mL of malt broth (Fluka) medium.

2.4. Optimization of Conditions. Biomass assays of fungal cultures were made for desulphurization studies. We chose the former for optimizing pH, temperature, pulp density (percent of coal weight/100 mL of medium), and incubation time for desulphurization experiments. Desulphurization tests were carried out using biomasses of the fungal cultures mentioned above. Tests were made at pH values of 3.0, 4.0, 5.0, and 6.0, at temperatures of 25, 30, 35, and 40 °C, at pulp densities of 3, 4, 5, and 6% (w/v), and at incubation times of 2, 4, 6, 8, and 10 days. Working conditions for all the samples were 1 mL of inoculum to a 250 mL flask with a medium volume of 100 mL. Used coal was 200 μm except for particle size assays. The agitation rate was kept stable at 125 rpm. To investigate the influence of particle size on sulfur removal, fungal desulphurization was achieved at <5.0, <2.0, and <1.68 mm. Various size fractions were separated by using standard sieve plates. A sample of the same coal, sterilized, was also used to test the effect which any such natural microorganism could have on the desulphurization process. After optimizing the pH, temperature, pulp density, and incubation time for these fungi, the desulphurization of the sterilized lignite was tested.

For each optimization stage, at the end of incubation time, the coal samples were separated by filtration and then washed with distilled water. The coal samples were dried at 45 °C and analyzed for total sulfur content by using a LECO SC-144DR sulfur analyzer. The extent of desulphurization was followed by an analysis of the total sulfur in the coal samples after microbial treatments, and the calculated percentage was found as the difference in total sulfur content of coal before and after treatment.

3. Results and Discussions

3.1. pH Optimization. Extent of desulphurization of the cultures was investigated at pH values of 3, 4, 5, and 6. There was a maximum removal of total sulfur at initial pH values of 3 and 6 for the lignite (Figure 1). While the extent of biodesulphurization was 21.2% for *T. versicolor*, this percent change was 22.7% for *P. chrysosporium* at pH 3. Total sulfur removal with *T. versicolor* was 23.93%, while with *P. chrysosporium*, it was 20.4%. The positive effect on the desulphu-

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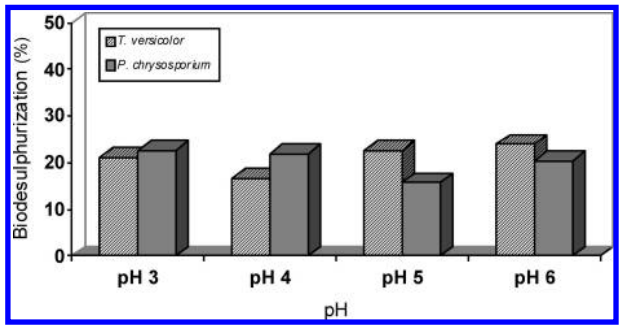

Figure 1. Influence of pH on biodesulphurization.

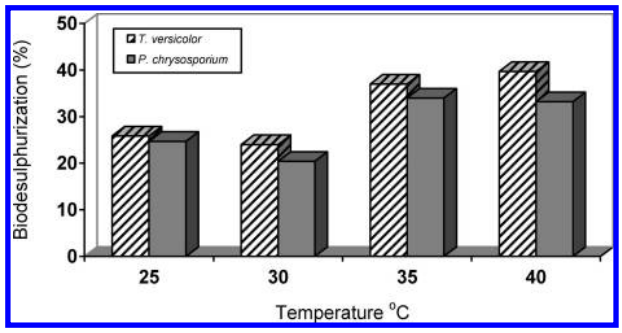

Figure 2. Effect of temperature on the desulphurization of coal.

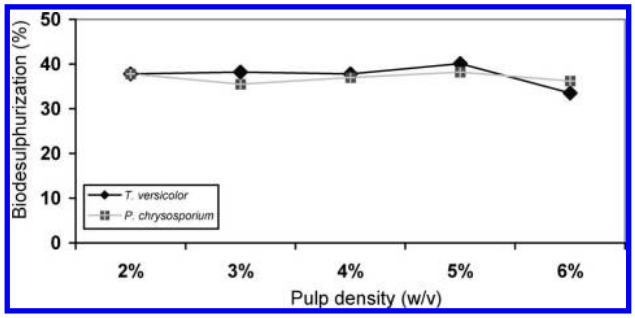

Figure 3. Influence of pulp density on the microbial desulphurization of coal.

rization process in acidic medium has been defined also in the literature.[23] Therefore, a pH of 6 was preferred in all of the desulphurization experiments to observe only the effects of fungal desulphurization.

3.2. Optimization of Temperature. Incubated cultures at 25, 30, 35, and 40 °C were investigated for the extent of sulfur removal. As can be seen in Figure 2, the biodesulphurization of lignite with studied fungi was high at 35 and 40 °C. But, 35 °C is a more convenient temperature than 40 with regard to the industrial scale. Therefore, we preferred to study at 35 °C. Acharya et al. also carried out studies at 33.5 °C for the fungal desulphurization process with *Aspergillus*.[24]

3.3. Influence of Pulp Density. It is important to take into consideration the coal amount during the desulphurization operation for evaluating the economy of the process. Towards this aim, different pulp densities including 2, 3, 4, 5, and 6% were studied. As may be deduced from Figure 3, a 5% (w/v) pulp density was determined as greatest extent of desulphurization for the examined fungi. When the amount of pulp density was greater than 5%, the desulphurization became reduced. Several researchers have also investigated the effect of pulp density on the desulphurization processes by using different microbial biomass, and similar results have been reported. For

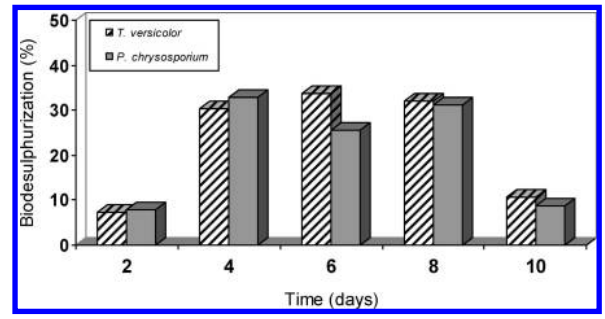

Figure 4. Incubation time optimization on the microbial desulphurization of coal.

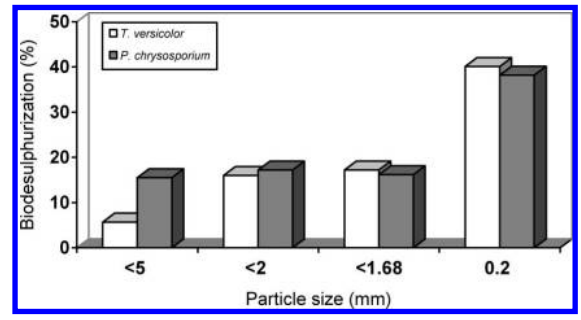

Figure 5. Influence of particle size on fungal desulphurization.

instance, Sukla et al. reported that 20% pulp density inhibited the growth of *Thiobacillus ferrooxidans*, a microorganism used in a biodesulphurization process.[25] According to Chaudhury, this decrease may be due to stress on the microorganisms on account of deterioration and buildup of compounds leached from coal.[26]

3.4. Optimization of Incubation Time. Coal samples of 200 μm size were subjected to fungal desulphurization using 5% (w/v) pulp density for incubation durations. The effect of incubation time was investigated in the range of 1−10 days (Figure 4). The extents of biodesulphurization of the two biomasses increased up to the fourth day of the incubation period. The rate was not significantly observed to change up to 8 days. Interestingly, the extent of desulphurization was sharply decreased at 10 days of incubation. *T. versicolor* laccase activity was the highest at the 10th day. However, the sulfur present in the lignite did not reduce at this day. Factors which play a role in biodesulphurization process may be in cooperation with a number of enzymes in the lignin degrading system.[15] The total sulfur reduced to 33.7% for *T. versicolor* after 6 days and to 32.9% for *P. chrysosporium* after 4 days.

3.5. Influence of Particle Size on Biodesulphurization Processes. The particle size of the lignite is a significant factor in the abilities of microorganisms to remove sulfur from coal. If the particle size of coal is small, the accessibility of the microorganism to pyrite is high.[25] Among studied particle sizes such as <5.0, <2.0, <1.68, and 0.2 mm, the best removal of sulfur was achieved by the fraction of 200 μm in coal samples (Figure 5). The results show that the higher the degree of sulfur elimination from coal, the smaller the coal grain size.

3.6. Role of Sterilization on Biodesulphurization. At this stage of experiments, the medium was sterilized in an autoclave at 120 °C for 15 min. According to Figure 6, for unsterilized coal, a higher extent of biodesulphurization was observed when treated by both *T. versicolor* (TV) and *P. chrysosporium* (PC). The positive effect on the desulphurization process with

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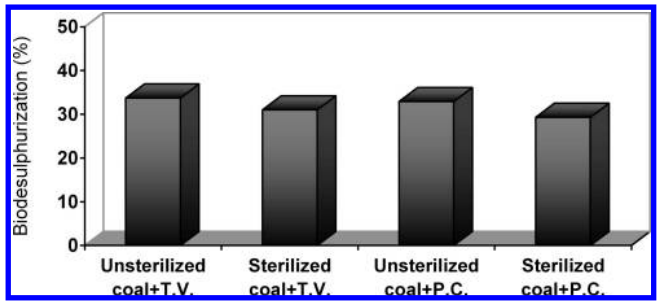

Figure 6. Comparison between unsterilized and sterilized coal for percent biodesulphurization.

Table 2. Initial and Ultimate Chemical Analyses of Lignite (200 μm)

analyses	untreated coal	treated with *T. versicolor*	treated with *P. chrysosporium*
ash (%)	20.47	15.09	15.65
GCV (cal/g)	5182	5330	5270
iron (mg/L)	5.4 ± 0.4	3.62 ± 0.1	5 ± 0.2
zinc (mg/L)	740 ± 2.4	639 ± 6.3	466 ± 11.7
manganese (mg/L)	30.5 ± 5.1	22.8 ± 0.8	17.8 ± 0.3
copper (mg/L)	1.8 ± 0.2	0.9 ± 0.0	0.6 ± 0.1
nickel (mg/L)	27 ± 0.4	19 ± 0.3	18.8 ± 0.6
chromium (μg/L)	286 ± 53.4	165 ± 0.3	183 ± 2.3
cobalt (μg/L)	661 ± 37.9	442 ± 6.0	560 ± 4.5
cadmium (mg/L)	38.1 ± 0.1	3.9 ± 0.2	10.1 ± 0.4
lead (μg/L)	822 ± 1.3	241 ± 2.6	208 ± 4.7
magnesium (mg/L)	1520 ± 3.4	1240 ± 10.9	875 ± 13.9
aluminum (mg/L)	3.8 ± 0.4	1.75 ± 0.1	3 ± 0.1

microorganisms living at native medium of coal is defined also in the literature.[27] Therefore, working with unsterilized coal, seems more suitable due the need to facilitate the study of the industrial scale which is possible in future.

3.7. Quality of the Coal Treated. Chemical analyses of lignite were evaluated before and after fungal treatment (Table 2). The immediate analyses which are calorific value (gross calorific value also known as the higher calorific value) and ash content for treated lignite with white rot fungi were carried out together with the determination of metal content before and after fungal treatment. As can be observed in Table 2, calorific value did not change, although a little increase was observed after fungal desulphurization. Given that the biodesulphurization process dissolves mineral matter, the coal becomes more concentrated, which results in a higher calorific value.[28,29] Furthermore, there was decrease in the ashes, suggesting the presence of a relationship between the extent of desulphurization of lignite and the ash eliminated. But, it should be stressed that the reduction of ash content is usually because of a chemical process rather than fungal treatment.[30] The reduction in ash content may be due to dissolution of mineral matters. This is considered to be advantageous because of the problems related to heavy metals present in the bottom ash and fly ash after combustion of untreated coals.[24] So, sulfur content was not only reduced but also the metal content existing in coal was changed positively with fungal treatment.

4. Conclusions

The abilities of white rot fungi strains, *T. versicolor* and *P. chrysosporium*, on the biodesulphurization of coal in the shake flasks have been investigated. The results showed that the most suitable pH for the growth of fungi used in this work was in the range of 6. The optimum temperature for the specific fungal growth rate was found to be 35 °C. The preferred incubation time for *T. versicolor* was 6 days, and for *P. chrysosporium*, the most convenient incubation time was observed to be 4 days. The conditions optimized for the maximum removal of sulfur were a particle size of 200 nm and a pulp density of 5% (w/v). Chemical studies showed a reduction in ash and metal content. The calorific value has not changed after microbial treatment significantly.

This is a rationalist notion because the representative structures of lignin and coal are closely related. *T. versicolor* and *P. chrysosporium* are considered to have good potential for application in the biodesulphurization of coal.[16]

The occurred metabolic actions seemed to be a chemical stress response against exogenously added lignite involving sulfur. These metabolic reactions were optimized under ligninolytic conditions, also suggesting the occurrence of a fungal xenobiotic response.[18] The fungi used in this study may prefer sulfur as a nutrient sulfur source for growth.

Biodesulphurization of coal is contemplated gradually for commercial evaluation. Further progress is being made in the direction to establish mechanisms, improve the extent of biodesulphurization, and evaluate, in terms of the pilot scale, fungal desulphurization of coal.

Acknowledgment. This study is based partly on the MSc thesis of P. Aytar. One of the authors (P.A.) would like to thank TÜBİTAK (The Scientific and Technical Research Council of Turkey) BIDEB for a grant. Also, we thank Coal Quality and Testing Laboratory from Garp Lignite Administrations for coal analyses.

EF700664T

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