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Isolation and characterization of native microorganism from Turkish lignite and usability at fungal desulphurization

Pınar Aytar [a,*], Derya Oz Aksoy [b], Yağmur Toptas [a], Ahmet Çabuk [c], Sabiha Koca [b], Hüseyin Koca [d]

[a] Department of Biotechnology and Biosafety, Graduate School of Natural and Applied Sciences, Eskisehir Osmangazi University, 26480 Eskisehir, Turkey
[b] Department of Mining Engineering, The Faculty of Engineering and Architecture, Eskisehir Osmangazi University, 26480 Eskisehir, Turkey
[c] Department of Biology, Faculty of Arts and Science, Eskisehir Osmangazi University, 26480 Eskisehir, Turkey
[d] Anadolu University, Porsuk Technical College, 26470 Eskisehir, Turkey

HIGHLIGHTS

- Lignite studied is inconvenient and inadequate to clean with physicochemical methods.
- Microbial desulphurization as a complementary approach was applied.
- With a strain of *Alternaria* sp., 52% of sulphur removal was performed.
- Several analyses before and after process were carried out.

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ABSTRACT

In this study, microorganisms were isolated from Mihaliccik region (Eskisehir, Turkey) lignites with low rank and high ash and sulphur content.

A series of heavy liquids (from 1.3 g/ml to 1.9 g/ml) were used to determine the washability characteristics of lignite sample. Washability data indicated that the physical cleaning of the sample would be difficult. Therefore, microbial desulphurization through native microorganism was performed owing to inadequate physical cleaning.

After isolation studies, six different bacteria, five different molds and seven different yeasts were obtained. Desulphurization studies showed that the most effective isolate was a fungus and its molecular identification of the 18S rRNA gene showed that this fungus was *Alternaria* sp. Cf1 isolate with accession number KF564051. After screening of desulphurization studies, optimisation experiments with this fungal isolate were performed with Taguchi's methodology. The parameters such as particle size, pulp density, initial pH, inoculum amount, incubation time were investigated during optimisation studies and optimum values were found as −0.106 + 0.038 mm; 1% of pulp density; pH 4; 2%; and 12 days, respectively. The results demonstrated that the treatment with lignite-derived inocula removed 52% of total sulphur. After fungal desulphurization, organic sulphur and sulphate removal were obtained as 38% and 51%, respectively.

1. Introduction

Inorganic materials and sulphur that are responsible for the generation of ash and SO_2, respectively are the main unwanted materials in the coal structure. Sulphur dioxide, one of the causes of air pollution, is particularly formed as a byproduct of coal combustion. For reducing such environmental problems, coal should be cleaned with alternative methods. Thanks to clean coal technologies, it is predicted that coal will have a larger share in the near future global market.

Coal cleaning prior to combustion could be obtained by physical and chemical methods. Coal cleaning by physical methods has been widely used in industrial scale. The success of physical separation depends on washability characteristics of coal that could be determined by float and sink analysis [1]. Besides only physical and chemical methods for reducing sulphur content of coal are not very effective at eliminating organic sulphur, biological methods may also be used due to the fact that they are cheaper and eco-friendly [2]. The studies on biodesulphurization by bacteria such as *Acidithiobacillus* sp. [3,4] and *Sulfolobus acidocaldarius*[5] and fungi [6] or their metabolites [7] have been reported.

Although coal is also the most significant indigenous and cheaper fossil fuel for Turkey, this fossil fuel is generally excavated as

* Corresponding author. Tel.: +90 222 239 37 50x2757; fax: +90 222 239 35 78.
E-mail address: pinaraytar@gmail.com (P. Aytar).

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low ranked lignites owing to being low calorific value and high sulphur. Turkey's energy production meets nearly 28% of its total primary energy consumption. Air pollution has become a great environmental concern in Turkey because of SO_2 emission [8]. Therefore, clean coal technologies should be utilised to decrease of high sulphur content.

Native microorganisms from coal can be used as inocula at biodesulphurization experiments due to the interactions between the microbial population and coal. Some researchers have published that the bacteria capable of desulphurizing coal inhabited on different coal types [9,10]. If coal naturally carries microorganisms capable of desulphurization, the use of them to obtain clean coal seems to be a good way of avoiding problems such as the adaptation of them [11].

This work covers first part of a research project that was carried out to obtain a clean lignite product from Koyunagili region lignites by utilising physical, physico chemical and biological method. Koyunagili lignite deposite, which is located in the mid-west of Turkey, has over 60 million tons of reserves and a small amount is produced annually for local domestic heating. An electricity generation plant is being erected to utilize these lignites. It is expected to start operation in early 2014.

The aim of this study is first to determine the washability characteristics of studied region lignites and at the same time to isolate native microorganisms living on lignites. Among all the isolated microorganisms, the most effective isolate for desulphurization was used in optimisation experiments using Taguchi's approach.

2. Materials and methods

2.1. Lignite samples

Lignite samples from Mihaliccik open mine and washing plant were used for washability, biodelphurization and microorganisms isolation studies [4]. For washability and biodelphurization studies, samples were collected from open and underground pits, and mixed homogenously in accordance with their production amount to obtain a composite sample. These samples were crushed to −0.500 mm and sieved into −0.500 + 0.212, −0.212 + 0.106 and 0.106 mm fractions. Preliminary studies revealed that −0.038 mm fraction was not effectively used in the float and sink analysis. Therefore these fines were removed by wet sieving.

For isolation of microorganisms, samples were carefully collected from open pit, underground pit, washing plant feed and products by hand. The lignite types in this study were run-of-mine, washed, air dried and oven dried.

2.2. Float and sink analysis

Heavy liquids (from 1.3 g/ml to 1.9 g/ml) were prepared by ZnCl solution. The sample was introduced into the liquid of lowest density. The floats product is collected as a separate fraction at each heavy liquid and remaining sinks product was removed and transferred to the liquid of next higher density and so on. All the floats fractions and final sink product were drained, washed, dried and weighed, separately. All fractions were analysed for ash and sulphur content [1]. This gives the density distribution of the sample by weight.

2.3. Isolation procedure

In our previous study, two procedures were followed for bacteria and fungi isolation [14]. Several media were investigated to isolate more microorganisms. According to first procedure, the standard isolation procedure, the used solid media were ferrous sulphate agar (Fe_{sol}), ferrous iron-galactose agar (FeG_{sol}), 9 K agar ($9K_{sol}$) and Malt extract agar ($Malt_{sol}$). Apart from Malt extract agar, pHs of all media were adjusted to 2.5, agar amount were 20 g/l. For second isolation procedure, five enrichment media were prepared and 5% of lignite samples were added. These media were ferrous sulphate (Fe_{liq}), sulphur (S_{liq}) and ferrous iron/galactose (FeG_{liq}) [15,16], 9 K medium ($9K_{liq}$) and malt broth ($Malt_{liq}$). For all of media, the pH was adjusted to 2 with 98% of sulphuric acid (0.1 M); except for malt broth. After 15 days of incubation period, contents of liquid cultures were utilised as inocula for solid media. These solid media used for transferring were Fe_{sol}, FeG_{sol}, FeS_{sol}, $FeTh_{sol}$ and $Malt_{sol}$ streak plates [16]. While pH values of some media such as Fe_{sol}, FeG_{sol} and FeS_{sol} were adjusted to 2.5, the medium pH of ferrous sulphate/sodium thiosulphate was altered to 4.5 and pH of $Malt_{sol}$ was 4.8. Agar and mineral salts were separately autoclaved and agar was added to acidic media at nearly 45 °C immediately. Therefore agar hydrolysis was prevented. Bacterial and fungal plates were incubated at 30 °C and examined regularly for colony growth and mycelia development was followed for fungal plates. The mold and yeast isolates were further purified using malt extract agar.

2.4. Biodesulphurization screening studies

The obtained isolates were used for biodesulphurization screening studies. Each isolate was screened at own media which on isolated medium before in view of biodesulphurization ability. Koyunagili lignite with high sulphur content was utilised in all biodesulphurization experiments. For bacterial and fungal isolates, the conditions at screening studies were 100 ml of working volume, 32 °C, pH 2 (pH 4.5 for fungal isolates), 15 days of incubation time, 5% of pulp density, 1% of inoculum amount and −0.212 + 0.106 mm of particle size [13]. An inoculum concentration of 2.5×10^5 spores/ml was used. Before screening and optimisation of biodesulphurization experiments, all coal samples were sterilized. For screening and optimisation stages, at the end of incubation time, the coal samples were separated by filtration and then washed with hot water to separate from the cells. The coal samples were dried at 45 °C and analysed for total sulphur content.

2.5. Classical and molecular identification of efficient fungal isolate

As the result of the screening study, the effective isolate in terms of sulphur removal from lignite was identified as a fungus. "Illustrated Genera of Imperfect Fungi" [17], "Introduction to Food and Airborne Fungi" [18] "Fungi and Food Spoilage" [19] were used for classical identification at the genus level taking into account its macroscopic and microscopic features.

Genomic fungal DNA was extracted using CTAB protocol. The fungal internal transcribed spacer (ITS1-5,8S rDNA region) was amplified with PCR. This reaction was performed in 50 μl reaction mix including 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.4 mM dNTP, 1.25 unit Taq DNA polymerase, 0.8 mM of primer (final concentration of each component), and 1 μl DNA template. The PCR program consisted of initial denaturation step at 94 °C for 3 min, followed by 40 cycles of 94 °C for 35 s, 52 °C for 35 s, 72 °C for 45 s followed by a final extension step at 72 °C for 7 min. The PCR products were visualised by gel electrophoresis on an agarose gel and then ITS region was visualised [20]. PCR products were then purified and done sequence analysis. Sequence similarity was investigated using BLAST tool from National Center for Biotechnology Information [21].

2.6. Determination of optimum biodesulphurization conditions for effective isolate

A fungal isolate grown on malt broth was investigated for desulphurization. Optimisation of sulphur removal activity by