Research on Coal Desulfurization of *Pseudomonas Stutzeri*

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Abstract: The burning of high-sulfur coal releases sulfur dioxide and causes environmental pollution. An efficient desulfurization strain is the key to the development of coal biological desulfurization. In this study, *Pseudomonas stutzeri* LH-42 was employed as the experimental bacterial, and the mutant strain *Pseudomonas stutzeri* ZW-15 with the highest desulfurization efficiency was selected by UV mutagenesis. The mutant strain ZW-15 was applied to the bioleaching experiment for coal from Liupanshui mine, Guizhou Province, China, and the result showed that 41% of the total sulfur and 93.25% of the organic sulfur in coal was removed in a 15-days experiment, which indicated that the mutant strain ZW-15 do have an application potential for the biodesulfurization of high-sulfur coal.

1 INTRODUCTION

China is one of the countries with the largest production and consumption of coal in the world. Chinese coal consumption accounted for half of the world's total in 2015 (Yang et al., 2012, Liu et al., 2016). However, the combustion of high sulfur coal will cause serious environmental problems, such as acid rain. (Burns et al., 2016). With the depletion of high-quality coal resources, the proportion of high-sulfur coal consumption is getting higher. Therefore, how to reduce sulfur content of high-sulfur coal has become a hotspot in environmental science research(Zhang et al., 2013).

The best method to limit the amount of sulfur oxides emitted into the atmosphere is to reduce the content of sulfur in coal before combustion(He et al., 2012). Coal sulfur has inorganic and organic sulfur in two forms. Inorganic sulfur mainly exists in the form of pyrite in coal, organic sulfur is mainly in the form of Dibenzothiophene (DBT) (Mishra et al., 2017). In the past years, we ofen took chemical and physical methods to remove the sulfur from the coal, however, these ways are high-cost, energy-intensive and inefficient for removing organic sulfur(Gonsalvesh et al., 2012). Thus, more and more attention has been focused on the biodesulfurization of high sulfur coal since it offers a clean alternative method to remove sulfur from coal (He et al., 2012, Khanna et al., 2011, Kodama et al., 2000).

At present the biodesulfurization technology is still in the laboratory research stage, because the stable and efficient desulfurization strain is not easy to obtain, there are some challenges in industrial applications of coal biodesulfurization. However, coal biodesulfurization technology still has great potential for development and application prospects with the exploitation of desulfurization strains and improvement of biotechnology desulfurization process.

In this article, *Pseudomonas stutzeri* LH-42 was employed as the experimental bacterial. And we got mutant strain ZW-15 with the highest desulfurization efficiency after UV mutagenesis, and described its characteristics of coal leaching desulfurization experiment, and demonstrated the possibility of its application for the biodesulfurization of high sulfur coal.

2 EXPERIMENTAL SECTION

2.1 Coal Sample

The coal sample used in the experiment was collected from Liupanshui, Guizhou Province, China. X-ray diffraction (XRD) was used to analyze the component of coal sample. These results showed

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in Table 1. The total sulfur of coal samples is 4.973 %. The sample was pulverized in a ball mill and size to less than 0.5 mm for desulfurization test (Cara et al., 2003).

Table 1: Phase analysis results of sulfur in raw coal.

Sulfur forms	Content (%)	Proportion (%)
Total sulfur	4.973	
Organic sulfur	1.979	39.795
Sulfate	0.144	2.896

2.2 Media

The BSM medium was used for cultivation of *Pseudomonas stutzeri* LH-42 contained: 2.44 g of KH_2PO_4, 12.03 g of $Na_2HPO_4•12H_2O$, 0.36 g of $MgCl_2•6H_2O$, 2.00 g of NH_4Cl, 0.004 g of $MnCl_2•4H_2O$, 0.001 g of $FeCl_3•6H_2O$, 0.001 g of $CaCl_2$, 1.62 g of glycerin, and one liter of deionized water (Zhang et al., 2017). Final pH value was 7.2. Sulfur sources, DBT, were added to the medium at the concentration of 0.3 mM. The BSM medium with 0.3 mM of DBT was labeled as BSM(D) in the following experiments.

2.3 Microorganisms and Cultivation

The strain Pseudomonas stutzeri LH-42 originally isolated from the petroleum-contaminated soil was routinely maintained in our laboratory(Yang et al., 2013). The strain was inoculated into 100 ml medium and cultured at 30℃ with constant agitation (170 rpm) for 48h.

2.4 UV Mutagenesis

A mutant was obtained by UV mutagenesis to increase the efficiency of the organic sulfur degradation of *Pseudomonas stutzeri* LH-42. A cell suspension (1 loopful/ml) was spread on the surface of the agar plate. Then, the cells were irradiated by UV light (30 W) for 0s, 10s, 15s, 20s, 25s, 30s, 45s, 60s (All experiments were run in triplicate) at a distance of 30 cm, and cultivated at 30°C.

2.5 Coal Desulfurization Comparison Test

The row coal used in the experiment were sterilized and the biodesulfurization process of different mutants was carried out in flasks with a volume capacity of 100 mL in 250 mL of BSM medium, 15% w/v pulp density, the initial cell concentration of 1.0×10^6 cells/mL and processing time of 20 d. The wild strain was used as control. The sulfur content of coal in this biodesulfurization system were detected in the first five days, and after that, the sulfur content was detected in every five days. The best mutant strain was chosen to the following experiment by comparing the desulfurization efficiency.

2.6 Coal Bioleaching Experiment

To remove the sulfur of coal, the best mutant strain Mutant ZW-15 cells were inoculated into column containing 2L sterilized culture medium supplemented with 1000g coal sample (the initial cell concentration was 1.0×10^6 cells.mL−1), and were incubated at room temperature, processing time of 15 days. At the bottom of the column, layered 1000g of coal which was 10 Tyler mesh, and 1000g of coal which was 20 Tyler mesh. The leaching system sprayed for 1 minute per hour, and 2.5 L liquid per minute. Triplicate leaching experiments were performed under identical conditions. Parallel experiments (without cells; but the same culture medium) were prepared as sterile control. The pH value and the redox potential of bioleaching system were determined every day. After the bioleaching, the sulfur content was detected again to calculate the efficiency of biodesulfurization.

2.7 X-ray Diffraction Analysis of Coal

In order to further study the influence of sulfur removal in coal of the induced strain ZW-15. Coal sample of the initial coal and biological desulfurization sample were tested by X-Ray diffraction analysis (XRD), respectively.

3 RESULTS AND DISCUSSION

3.1 Purification and culture bacteria.

In order to obtain individual bacterial colony, Pseudomonas stutzeri LH-42 was cultured in BSM(D) solid medium. And several single colonies were identified by analyzing their 16S RNA sequences. The result of BLAST indicated that this strain is *Pseudomonas stutzeri* (Yang et al., 2013). The growth curves of the strain LH-42 which cultured in BSM liquid medium containing 0.3 mmol/L of DBT was shown in Figure 1.

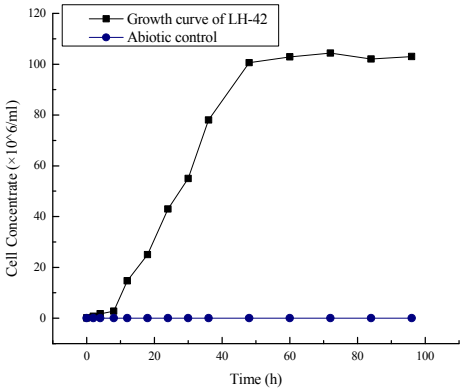

Figure 1: Growth curves of the strain LH-42.

3.2 The results of UV mutagenesis

Pseudomonas stutzeri LH-42 was cultured in BSM(D) solid medium and treated by Ultraviolet ray. Lethality rate of spores of *Pseudomonas stutzeri* LH-42 by Ultraviolet ray was shown in Figure 2. The lethality rate of LH-42 could achieve 89.3% when the strain radiated for 15 seconds, and it could be further improved to 100% by increased the induced time to 30s.

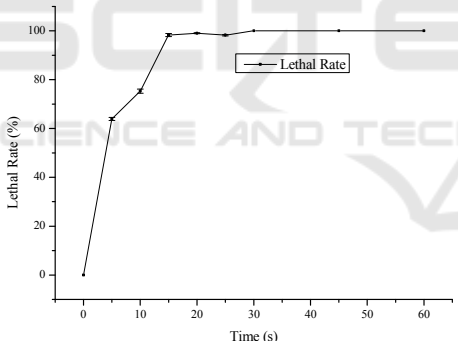

Figure 2: Lethality rate of spores of *Pseudomonas stutzeri* LH-42 by Ultraviolet ray.

3.3 The results of coal desulfurization comparison test

The UV-mutated colony whose diameter was greater than 2mm was selected to be inoculated into BSM(D) medium supplemented with sterile coal sample and cultured for 20 days. Desulfurization rates of wild and mutant stains were shown in figure 3. By comparing the efficiency of desulfurization, we found that a mutant strain named Mutant ZW-15 was the most efficient one. A comparison test between wild strain and Mutant ZW-15 was proceed to verify whether the mutant strain actually more efficient. The desulfurization rate of Mutant ZW-15 was 49.00% compared with wild strain's rate 21.36% (shown in Figure 3). Especially in the first five days, the desulfurization rate of Mutant ZW-15 increased massively and there was not significant change in the total sulfur desulfurization after 10 days' leaching, which suggested that the period of biodesulfurization could be shortened greatly.

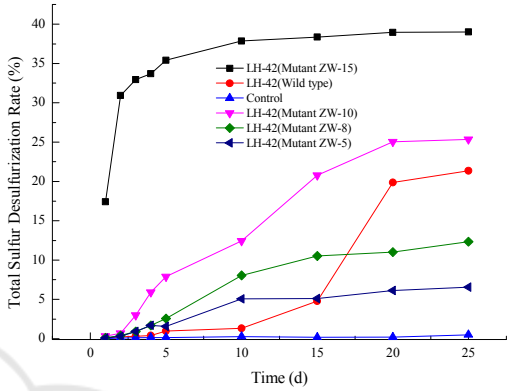

Figure 3: Comparison of desulfurization rates between wild and mutant.

3.4 The change of pH and redox potential of coal bioleaching system

The change of pH value and the redox potential of leaching desulfurization system were shown in Figure 4. Compared with the control group, the pH value of the leaching system showed significant downward trend. The coal sample itself contains a certain amount of humic acid which caused the sharp decrease of pH in the first one hour after the sample's dissolved in water. The pH value of test group dropped to 4.3 finally, which might because the bacterial released H^+ while decomposed the organic sulfur of coal. The slow oxidation of the pyrite in coal also contributed to the reduction of pH.

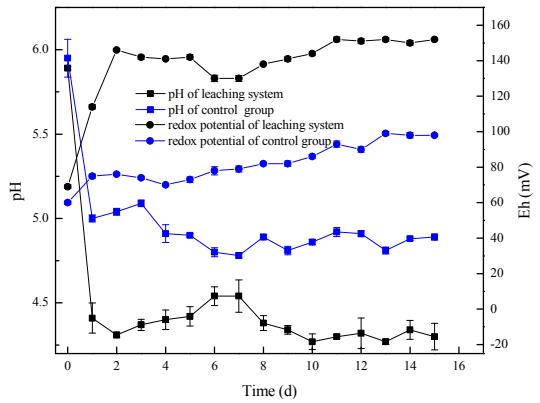

Figure 4: The pH and redox potential of leaching desulfurization test.

Compared with the control group, the redox potential of the leaching system changed significantly. The initial redox potential of test group was 69 mv, but after 2 days the potential jumped to 152 mV. Due to the initial stage of leaching system was unstable, there had been a small range of fluctuation of redox potential. Along with the process of bioeaching, the bacteria released metal ion like ferric ion and ferrous ion while decomposed the organic sulfur of coal, and it would increased the redox potential(Hu et al., 2006).

3.5 Desulfurization rate of coal bioleaching system

The mutant strain Mutant ZW-15 showed the better performance compared with the wild strain in desulfurization within 15 days, it degraded 41% total sulfur and 93.25% organic sulfur from the coal sample.

3.6 The results of mineralogical X-ray diffraction analysis of coal

The XRD graphs collected from the bioleached particles are shown in Figure 5. The Figure shows that the pyrite content was high before leaching but reduced after 15 days leaching process. The results further validated that biodesulfurization by induced strain ZW-1 can effectively remove inorganic sulfur from coal.

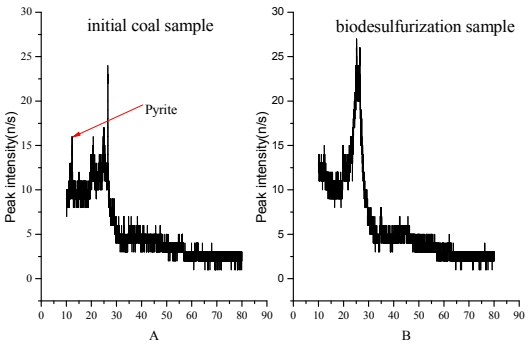

Figure 5: The XRD results of initial coal sample (A) and biodesulfurization sample (B).

4 CONCLUSIONS

The application of biodesulfurization is still premature so far, however, it has shown some enormous potential in the development of energy industry and environment protection. More active microbial cultures with higher desulfurization efficiency which can degrade a wide variety of sulfur compounds are needed for process development.

The mutant strain *Pseudomonas stutzeri* LH-42(Mutant ZW-15) 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 the efficient strain in the coal biodesulfurization.

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