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The Isolation Desulfurizing Native Thermophilic Bacteria Bacillus thermoamylovorance and Optimization Culture Medium

Narges Etemadi[1], Abbas Akhavan Sepahi[1]*, Ghasemali Mohebali[2] and Fatemeh Yazdian[3]

1. Microbiology Group, Faculty of Life Science, North Tehran Branch, Islamic Azad University, Iran

2. Microbiology and Biotechnology Research Group, Inviroment and Biotechnology Research Division, Research Institute of Petroleum Industry (IRPI), Iran

3. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Iran

A_akhavan@iau-tnb.ac.ir

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INTRODUCTION

On combustion of the fossil fuels, sulfur dioxide is released into the environment and causes air pollution and acid rain. Therefore, for limiting the amount of sulfur dioxide emitted into the atmosphere, it is necessary that the sulfur in the fossil fuels be decreased [1]. There are different manners to eliminate sulfur from fossil fuels; one of the manners is hydro-desulfurization (HDS) which is the most commonly used [1]. In addition, bio-desulfurization is deliberated frequently as a possible alternative to the HDS method applied commonly in refineries. In this manner, microorganisms eliminate organic sulfur from oil fractions without breaking down the carbon skeleton of the organic sulfur derivatives via 4S pathway [2]. Moreover, thermophilic bacteria are important for commercial bio-desulfurization. The use of thermophilic bacteria has some advantages since it is not necessary to cool-down the oil fractions following the HDS, which it makes this process less expensive [3]. In this study, we describe the isolation and identification of a novel thermophilic desulfurizing *Bacillus thermoamylovorans* capable of utilizing DBT at up to 55 °C as the sole sulfur source for growth

with the aim of more efficiency in commercial bio-desulfurization. Finally, it is helpful to achieve microorganisms which illustrate much higher desulfurization capability at high temperatures.

MATERIALS AND METHODS

About 40 samples including oil, oil-polluted wastewater, and oily soil samples were harvested from Iranian Oilfields. In addition, samples were inoculated in enrichment BSM, including DBT (50 mgl-1) as the sole sulfur source at 55 °C. To identify the cell growth and bio-desulfurization activity, basal salt medium containing DBT was used. Primary enrichment cultures were provided by adding 10 g samples to BSM (100 ml) supplemented with DBT (50 mgl-1) at 150 rpm at different temperature 30, 42, 50, 55 °C for 20 days. For microbial strain selection, each enrichment culture was spread on to ENA agar and incubated at 55 °C. Moreover, the cultures were prepared by adding colonies to BSM (100 ml) supplemented with of DBT (50 mgl-1) at 150 rpm and 55 °C separately. For primary selection of desulfurizing microbial strain(s), the Gibb's assay was done. The isolated strain that showed a higher ability to grow in DBT was selected. For classification of this strain, a range of morphological, biochemical, and molecular methods were done according to standards for microbial identification in Bergy's manual of systematic bacteriology. Isolates were identified with gram reaction, spore formation, cellular and colonial morphology, metabolic products, catalase reaction, oxygen requirement and motility test. Identification of this strain was performed by PCR amplification and sequencing of 16S rRNA gene. For optimization of the culture medium, different carbon and nitrogen source and also the different concentrations of sulfur source were used. Furthermore, optimization of BDS capability and cell growth was carried out in different concentrations of carbon and nitrogen source.

RESULTS AND DISCUSSION

From 40 sampling, enrichment, and screening assessment, several thermophile bacteria strains were selected as DBT desulfurizing strain. Strain EAMYO was isolated from an oily soil collected from oilfields. This strain was found to be appropriate for further investigation based on its more effective bio-desulfurization capability. Until now, different microorganisms have been isolated and recognized as desulfurizing DBT via 4S pathway [2]. One of this important microorganisms is thermophilic microorganisms. In addition, these microorganisms are important for commercial bio-desulfurization. In this effort, we describe the capability of a novel isolated thermophilic bacterium, *Bacillus thermoamylovorans* to desulfurize and utilize DBT as the sole sulfur source for growth via 4S pathway at up to 55 °C. Morphological and biochemical characterization of the isolates indicated that it is Bacillus sp. In addition, species level confirmation of the isolate was done by 16S rDNA sequencing. Based on BLAST search analysis of the 16S rRNA gene indicated that this strain was related most closely to *Bacillus thermoamylovorans* (97 %) having accession number NR117028.1. The growth rate and desulfurizing capability of strain EAMYO on DBT (100 mgl^{-1}) as the alone sulfur were studied as follows: the maximum cell concentration on DBT was achieved after incubation for 96 h. The maximum desulfurizing capability and production HBP were observed

after 72 h in the exponential phase. Moreover, B. thermoamylovorans grew in BSM medium with DBT at 55 °C and showed maximum growth (OD_{660} = 0.850) after 72 h of cultivation. Also, the maximum producing 2-HBP after 72 h was 26.13 ± 0.12 mgl^{-1} according to the curve calibration Gibbs assay.

Finally, the findings have illustrated that the maximal cell growth has achieved after 96 h incubation. The Gibbs results have shown that this strain has eliminated the sulfur in DBT by 4S pathway and 2-hydroxybiphenyl, as the end product of the desulfurization process was produced at the maximal concentration (26.1 mgl^{-1}) at 72 h. In addition, the results of research have illustrated that the isolated thermophilic strain is capable of eliminating sulfur in DBT, and this process may be improved by optimization of the culture media.

CONCLUSIONS

The Bio-desulfurization is based on 4S pathway. In the pathway, the carbon skeleton of the organic sulfur derivatives such as DBT is not break down, but converted to 2-HBP thus caloric value of the fossil fuels is not decreased. Thermophiles microorganisms have been isolated from geothermally heated soils, hot springs, and oil reservoirs. Bio-desulfurization is currently most attractive as a step following the HDS, which in turn requires elevated temperatures. If bio-desulfurization has been performed around 45 °C and higher, it would be unessential to chill the HDS-treated diesel oil to medium temperatures. In addition, it has advantages of increased enzymatic rates and diminished contamination by undesirable bacteria [4]. Also, the bio-desulfurization process would be increased due to the higher mass transfer rate at higher temperatures [5]. In the commercial bio-desulfurization process, it is helpful to achieve microorganisms which illustrate much higher desulfurization capability at high temperatures (around 55 °C). Although bio-desulfurization at thermophilic temperatures have shown high removal efficiency, the high cost of associated energy use makes the process uneconomical. Finally, in this study, the new thermophilic strain capable to desulfurize DBT at 55°C has been described by us.

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