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Optimization of conditions for deep desulfurization of heavy crude oil and hydrodesulfurized diesel by *Gordonia* sp. IITR100

Jyoti Adlakha [a], Pooja Singh [a], Saurabh Kumar Ram [a], Manoj Kumar [b], M.P. Singh [b], Dheer Singh [b], Vikram Sahai [a], Preeti Srivastava [a,*]

[a] Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, Delhi, Hauz Khas, New Delhi 110016, India
[b] Biotechnology Department, Indian Oil Corporation Limited Research and Development Center, Faridabad 121007, Haryana, India

HIGHLIGHTS

- A maximum of 76% sulfur reduction in heavy crude oil has been achieved by *Gordonia* sp. IITR 100.
- Viscosity of the heavy crude oil was reduced by 31%.
- The sulfur content decreased by 98% and 70% in diesel from two different sources.
- The hydrocarbon content was not affected by the bacterium.

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ABSTRACT

The reserves of conventional crude oil are depleting and therefore there is a growing interest in utilizing the large amounts of unconventional heavy crude oils. However, these are rich in organosulfur compounds, which contribute to viscosity and other problems such as pipeline corrosion and environmental pollution. Most of the studies on heavy crude oil biodesulfurization (BDS) have been limited to microorganisms, which can either desulfurize aromatic or aliphatic organosulfur compounds. In the present study we have determined the efficacy of *Gordonia* sp. IITR100, which can desulfurize both aliphatic and aromatic organosulfurs, towards BDS of heavy crude oil and diesel. Here, we report 76.1% reduction in total sulfur of heavy crude oil by growing cells of *Gordonia* sp. IITR100. The viscosity of the heavy crude oil was reduced by 31%. The results of GC-FID of heavy crude oil before and after treatment suggest that the bacterium does not affect the hydrocarbon content of crude oil. BDS of hydrodesulfurized diesel from two different sources showed 98% and 70% reduction in total sulfur using this microorganism. To our knowledge this is the first report of a bacterium showing such high levels of desulfurization of heavy crude oil. Moreover, the present results show that *Gordonia* sp. IITR100 can potentially be used for upgrading of heavy crude oil and diesel.

1. Introduction

There are large reserves of heavy crude oil also known as unconventional crude oil, which are underutilized because of the high sulfur content, heteroatoms and high viscosity. Aromatic organosulfurs constitute up to 62% of the total sulfur content [1]. The one, two or three sulfur atom containing compounds in heavy crude oils are present at concentrations of 74%, 11% and 1% respectively [2]. Conventionally, after fractionation of petroleum, hydrodesulfurization (HDS) is used to remove sulfur, but it is an energy-intensive as well as cost-intensive process [3]. Moreover, it is not capable of removing sulfur from recalcitrant compounds such as 4,6 dimethyl dibenzothiophene, other alkylated dibenzothiophenes and benzothiophenes where the sulfur atom is sterically protected. BDS is an attractive alterative to HDS [4]. Gallagher and coworkers reported the selective removal of sulfur by 4S pathway [5]. Since then, considerable research on the BDS of model organosulfur compounds, diesel, gasoline and crude oil by different microorganisms have been done. BDS of diesel has been demonstrated using both growing and resting cells. The diesel with initial sulfur content in the range of 250–3000 ppm was used in the earlier studies and the reduction up to 47–94.5% was observed after BDS [4]. Studies were carried out at both shake flask level as well as in bioreactors. McFarald et al. used resting cells of *Rhodococcus*

* Corresponding author.
E-mail addresses: preetisrivastava@hotmail.com, preeti@dbeb.iitd.ac.in (P. Srivastava).

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sp. for BDS of diesel in a continuous stirred tank reactor and observed 50–70% reduction in sulfur [6]. Similarly, using *Pseudomonas delafieldii* R-8 in a 5 L reactor, 47% sulfur removal from diesel was observed [7]. About 50% removal of sulfur from diesel was observed when *Gordonia alkanivorans* RIPI 90A was used in an airlift bioreactor [8].

Studies on BDS of heavy crude oil are rather limited. The maximum reduction in sulfur content achieved with different microorganisms using heavy crude oil as the sulfur source are in the range of 47–68% [9–15]. A biphasic media utilizing hydrophobic biocatalysts was used in all the studies [16].

In the present study, *Gordonia* sp. IITR100 [17] was selected for BDS of heavy crude oil and hydrodesulfurized diesel. It was isolated previously by selective enrichment of hydrocarbon contaminated soil collected from Koyali refinery, Gujarat, using organosulfur compounds as the only sulfur source. It was selected for the following reasons (a) *Gordonia* has high mycolic acid content contributing to the hydrophobicity at the outer surface [18], (b) the bacterium could desulfurize aliphatic sulfides such as dibenzyl sulfide [19] and aromatic Dibenzothiophene (DBT), Thianthrene [20], and (c) the bacterium did not show any growth in media containing organosulfurs as the only carbon source. A lot of studies on fossil fuel desulfurization have been done with different microorganisms, but only limited studies have been done with *Gordonia* spp. [21]. Thus, the objective of the study is to determine the BDS ability of *Gordonia* sp. IITR100 towards heavy crude oil and hydrodesulfurized diesel and to optimize the conditions for improved biodesulfurization.

2. Material and methods

2.1. Chemicals

Tween80 and Dimethyl sulfoxide (DMSO) were obtained from Merck Limited, Mumbai, India; DBT was obtained from Fluka Chemicals, ⩾98% (GC), Sigma-Aldrich, St. Louis USA. All the other chemicals were of analytical grade, available commercially and used without further purification. Two different hydrodesulfurized diesel samples were used in the present study, one was obtained from Indian Oil Research and Development Center, Faridabad (hydrodesulfurized diesel source (1) with a sulfur content of 70 ppm or 70×10^{-3} mass% and one was obtained from a petrol pump (hydrodesulfurized diesel source (2) with a sulfur content of 50 ppm or 50×10^{-3} mass%. Heavy crude oil used in all shake flask experiments was procured from Indian Oil Research and Development Center, Faridabad. The sulfur content of heavy crude oil was found to be 4.53 mass%. The heavy crude oil used in all experiments was diluted 5-fold in *n*-hexadecane (Spectrochem Pvt. Ltd., Mumbai, India); final sulfur content of heavy crude oil diluted in *n*-hexadecane was (1.083 ± 0.5%) mass%.

2.2. Bacterial strain and growth conditions

BDS experiments were performed with *Gordonia* sp. IITR100, which was isolated from petroleum contaminated soil by enrichment culture using 4,6 dimethyl dibenzothiophene as the sulfur source (Gen bank accession number GU084407) [17].

BDS studies, on heavy crude oil and hydrodesulfurized diesel, were performed in shake flask and in bench top bioreactor (5 L Infors AG) equipped with monitoring and control of standard environmental variables. An optimized minimal salt media supplemented with sucrose as a carbon source and heavy crude oil as the only sulfur source was used. The basal salts media (BSM) comprised of the following components (in 1000 mL): 2 g Na_2HPO_4, 1 g KH_2PO_4, 4.25 g Ammonium oxalate, 0.4 g $MgCl_2$ and 17.1 g sucrose, trace elements solution (0.1% v/v) and vitamins solution (0.1% v/v) Kao and Michayluk solution, Sigma chemical co, St Louis, USA were supplemented. Trace elements solution consisted of the following components per litre: 0.05 g KI, 0.05 g LiCl, 0.8 g $MnCl_2{\cdot}4H_2O$, 0.5 g H_3BO_3, 0.1 g $ZnCl_2$, 0.1 g $CoCl_2{\cdot}6H_2O$, 0.1 g $NiCl_2{\cdot}6H_2O$, 0.05 g $BaCl_2$, 0.05 g $(NH_4)_6\ Mo_7O_{24}{\cdot}2H_2O$, 0.5 g $SnCl_2{\cdot}2H_2O$, 0.1 g $Al(OH)_3$.

Resting cells were prepared in potassium phosphate buffer (0.1 M, pH 7.0) with the following composition: 6.15 mL of K_2HPO_4 (1 M) and 3.85 mL of KH_2PO_4 (1 M) and the final solution was made up to 100 mL.

2.3. BDS of heavy crude oil with growing cells in shake flasks

Pre-inoculum was prepared from single colonies grown in 20 mL BSM medium containing DBT as the sulfur source at 30 °C, 180 rpm for 7 days. For optimizing the oil water ratio, 20 mL heavy crude oil was used as sulfur source and the quantity of media (BSM) added varied depending upon the different oil/water ratios. Cells were inoculated at an initial OD_{600nm} of 0.1. The bioreaction flasks were set in triplicate and incubated at 30 °C, 180 rpm for 7 days. Heavy crude oil was separated by centrifugation at 4000 rpm (2057 g), 10 min and sulfur content was estimated.

For sequential desulfurization of heavy crude oil, triplicates of each samples treated in the oil/water (o/w) optimization (samples 1:3 and 1:9) were pooled and used with fresh culture and BSM media as described above.

For determining the effect of surfactant, 0.5 g/L Tween 80 was used.

For optimization of rotation speed, the experiment was set in triplicate at the optimal oil/water ratio 1:3. Heavy crude oil 20 mL and 60 mL of media was used in 500 mL flask and incubated at 30 °C for 7 days at different rotational speeds (120, 150, 180, 210, 250, and 300). Heavy crude oil was separated and sulfur content was estimated.

2.4. BDS of heavy crude oil with resting cells in shake flasks

The cells were grown to an $OD_{600nm} \sim 1.5$ in BSM supplemented with DBT. Cells were harvested by centrifugation at 5000 rpm for 10 min, washed twice and suspended in 45 mL potassium phosphate buffer (0.1 M, pH 7.0) at a final $OD_{600nm} \sim 20$. Five millilitre heavy crude oil was added and incubated at 30 °C, 180 rpm. Heavy crude oil was extracted after 24 h, 48 h, and 72 h and sulfur content was estimated.

Different concentrations of DMSO (0.2% (v/v), 1.0% (v/v) and 8.0% (v/v)) were added along with 5 mL of heavy crude oil. The volume was made up to 50 mL by phosphate buffer containing cells at an $OD_{600nm} \sim 20$. Effect of DMSO on the cell permeabilization was tested through the decrease in sulfur content over a period of 72 h.

2.5. Viscosity reduction of heavy crude oil in shake flasks

For viscosity reduction, only the heavy crude oil sample which gave maximum reduction in sulfur content using growing cells in shake flasks was analyzed as described in analytical methods.

2.6. BDS of hydrodesulfurized diesel in shake flasks

Preinoculum was prepared as described above and an o/w ratio 1:3 was used. Thus, 20 mL of HDS diesel source 1 along with 60 mL of culture media was used. Incubation was done at 30 °C, 250 rpm for 7 days. Extraction of diesel oil was done by centrifugation at 4000 rpm for 15 min. to separate the organic and aqueous layers and sulfur content estimated with XRF. GC-SCD and GC-FID of the samples was also done.