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RESEARCH TITLE

Biodesulfurization of Al-Masila (Hadramout – Yemen) Crude Oil using *Pseudomonas aeruginosa*

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Abstract

Sulfur compounds represent one of the most common impurities present in crude oil. The combustion of these compounds in fossil fuels tends to release sulfur dioxide into the atmosphere, which leads to acid rain and reduces the life of the engine due to corrosion. The process of biodesulfurization rationally exploits the ability of certain microorganisms in the removal of sulfur prior to fuel burning, without loss of calorific value. The recent study focused on reducing the total sulfur of Al-Masila (Hadramout–Yemen) crude oil, by the biodesulfurization process using two sources of *Pseudomonas aeruginosa* bacterial strains (one isolated clinically from wounds and the other procured from American type culture collection, ATCC 27853). All the results showed that *P. aeruginosa* bacteria can be used to remove the sulfur from Yemeni crude oil.

Keywords: Biodesulfurization, Crude oil, P. aeruginosa

عنوان البحث

النزع الحيوي للكبريت في نفط خام المسيلة (حضرموت-اليمن) باستخدام بكتيريا الزائفة الزنجارية

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المستخلص

تمثل مركبات الكبريت واحدة من مواد عدم نقاوة النفط الخام. يؤدي احتراق هذه المركبات إلى إطلاق ثاني أكسيد الكبريت إلى الغلاف الجوي، مما يؤدي إلى تكون المطر الحامضي وتقصير العمر الافتراضي للمحركات بسبب تآكلها. تستخدم عملية النزع الحيوي للكبريت باستخدام الكائنات المجهرية في إزالة الكبريت مع الحفاظ على القيمة السعرية قبل احتراق الوقود عملية بالغة الأهمية. ركزت الدراسة الحالية على اتباع الطريقة الحيوية لنزع الكبريت للتقليل من الكبريت الكلي في خام نفط المسيلة (حضرموت–اليمن) وذلك عبر استخدام مصدرين (معزولة سريريا من الجروح أو جاهزة) من بكتيريا الزائفة الزنجارية. اظهرت النتائج إمكانية استخدام هذه النوع من البكتيريا في نزع الكبريت من النفط الخام اليمني.

الكلمات المفتاحية: النزع الحيوي للكبريت، النفط الخام، الزائفة الزنجارية

1. INTRODUCTION

Petroleum can be defined as a complex mixture of hydrocarbons, non-hydrocarbons, and heteroatom-containing compounds (Overton et al., 2016). Petroleum consists of carbon, hydrogen, and heteroatoms like sulfur, nitrogen, oxygen, metals, etc. Among heteroatoms, sulfur is the most abundant with around 0.03–6 wt% in natural gas and crude oils (Bajia et al., 2017; Shahaby & Essam-El-din, 2017). When the total amount of sulfur is <0.42 %, it is called sweet crude oil, while when the amount of sulfur is more than around 0.42 %, it is called sour crude oil. Sulfur-containing compounds are classified into different types, as shown in Fig.1(Saleh, 2020). It is recommended that sulfur compounds are removed in the refining process as they cause the deactivation of the catalysts used in crude oil processing and corrosion problems in pipelines, along with the pumping, and refining equipment. From an environmental point of view, the sulfur left in fuels may cause the emission of toxic gases that react with water and cause acid rain. Thus, these gases or acid products can damage buildings and other materials (Das et al., 2020; Elwan et al., 2020; Jumina et al., 2021).

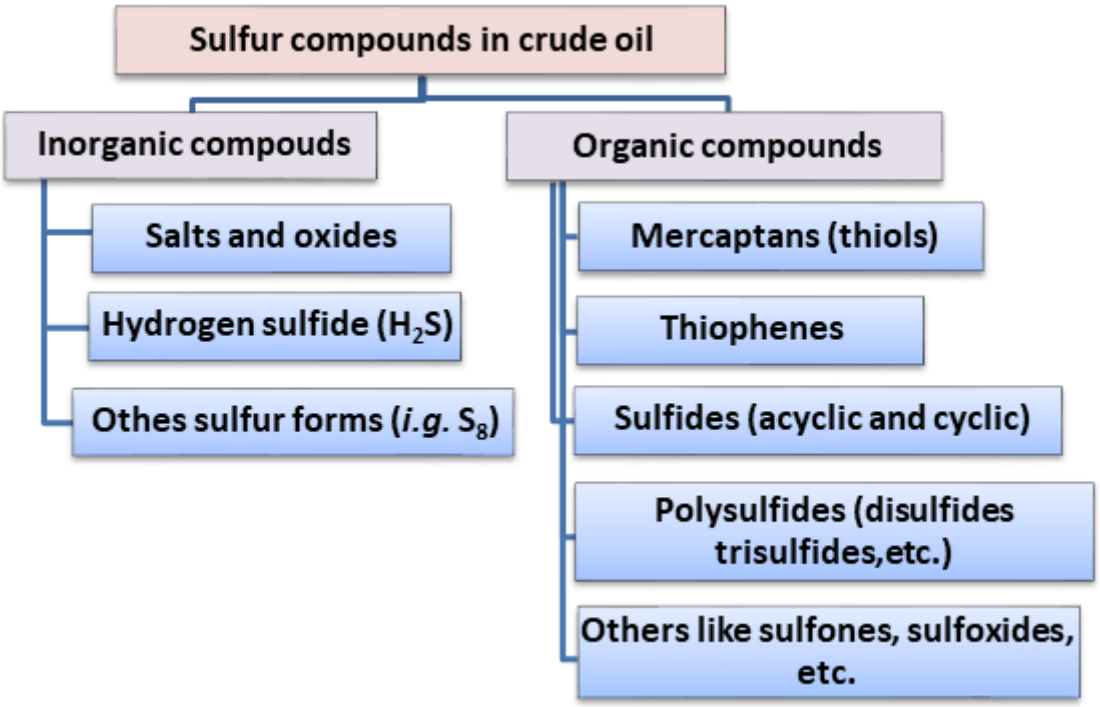

Fig. 1. Inorganic/organic sulfur compounds in crude oil (adopted from Saleh, 2020)

Currently, hydrodesulfurization (HDS) is employed to remove sulfur from fossil fuels. HDS operates at high temperature and pressure, and besides, it cannot remove completely sulfur (Nezammahalleh, 2015). Biodesulfurization (BDS) through microbial activities can solve this problem. Compared with the HDS process, the biodesulfurization (BDS) process using microorganisms and or enzymes could be carried out more safely, under mild conditions. This process of microbial desulphurization or biodesulfurization is expected to overcome the technical and economic problems associated with HDS as it has the potential benefits of lower capital and operating costs and will produce lesser greenhouse gases (Adlakha et al.,

2016; Al-Bidry & Azeez, 2020).

Many microorganisms have been reported to use various petroleum hydrocarbons and sulfur compounds, as their sole carbon and energy substrate, despite their extreme insolubility in the aqueous phase. It is possible to desulfurize crude oil directly by selecting appropriate microbial species (Javadli & de Klerk, 2012). Numerous genera of bacteria are known as good hydrocarbon degraders such as *Pseudomonas*, *Rhodococcus*, *Bacillus*, *Mycobacterium*, *Klebsiella*, *Enterobacter*, *Actinomycetes*, *Acinetobacter*, etc. (Al-Zahrani & Idris, 2010; Ban et al., 2013; Bhatia & Sharma, 2012; Izumi et al., 1994; Kirimura et al., 2001; Raheb et al., 2010). However, biodesulfurization has become an alternative way to remedy crude oil and refined products, where the addition of specific microorganisms or enhancement of microorganisms already present, can improve desulfurizing efficiency (Austin & Callaghan, 2013). In order to develop environmental technologies for crude oil desulfurization, it is necessary to isolate and characterize specific microbial species for evaluation of their efficacy in the utilization of sulfur compounds before application to crude oil (Shahaby & Essam-El-din, 2017).

Sulfur removal through a biodesulfurization process could take place through C─C cleavage (Kodama pathway) or C─S cleavage (4S pathway) (Chen et al., 2019; Lateef et al., 2019). In the Kodama pathway, the biochemical reactions happen in the aromatic rings of dibenzothiophene as presented in Fig. 2 (a). Initially, the dibenzothiophene is oxidized to form cis-1- 2-dihydroxy-1,2-dihydro dibenzothiophene with the help of oxygen gas and NADH. The cis-1,2-dihydroxy-1,2-dihydro dibenzothiophene is then oxidized to form 1,2-dihydroxy dibenzothiophene. The 1,2-dihydroxy dibenzothiophene is oxidized again to form cis-4-[2-(3-hydroxy)- thionaphthenyl]-2-oxo-3-butenoic acid and afterward oxidized to pyruvic acid and 3-hydroxy-2-formylbenzothiophene. These two compounds are water-soluble and thus are spontaneously ejected from the crude oil phase (Das et al., 2020). In contrast, the sulfur element is removed as sulfite anion (SO_3^{-2}) from the aromatic hydrocarbon framework of dibenzothiophene in the 4S pathway Fig. 2 (b). At the start, dibenzothiophene-sulfoxide forms by oxidation dibenzothiophene compound and finally leads to produce dibenzothiophene sulfone in the presence of NADH, $FMNH_2$, DszC, and DszD enzymes. Dibenzothiophene-sulfone compound is further oxidized to form 2-hydroxybiphenyl-2-sulfinate in the presence of oxygen gas, $FMNH_2$, NADH, DszA, and DszD enzymes. Subsequently, the 2-hydroxybiphenyl-2-sulfinate is hydrolyzed to 2-hydroxybiphenyl and sulfite anion with the help of DszB enzymes. The 2-hydroxybiphenyl is a valuable by-product that can be used as a disinfectant and fungicide (Jumina et al., 2021).

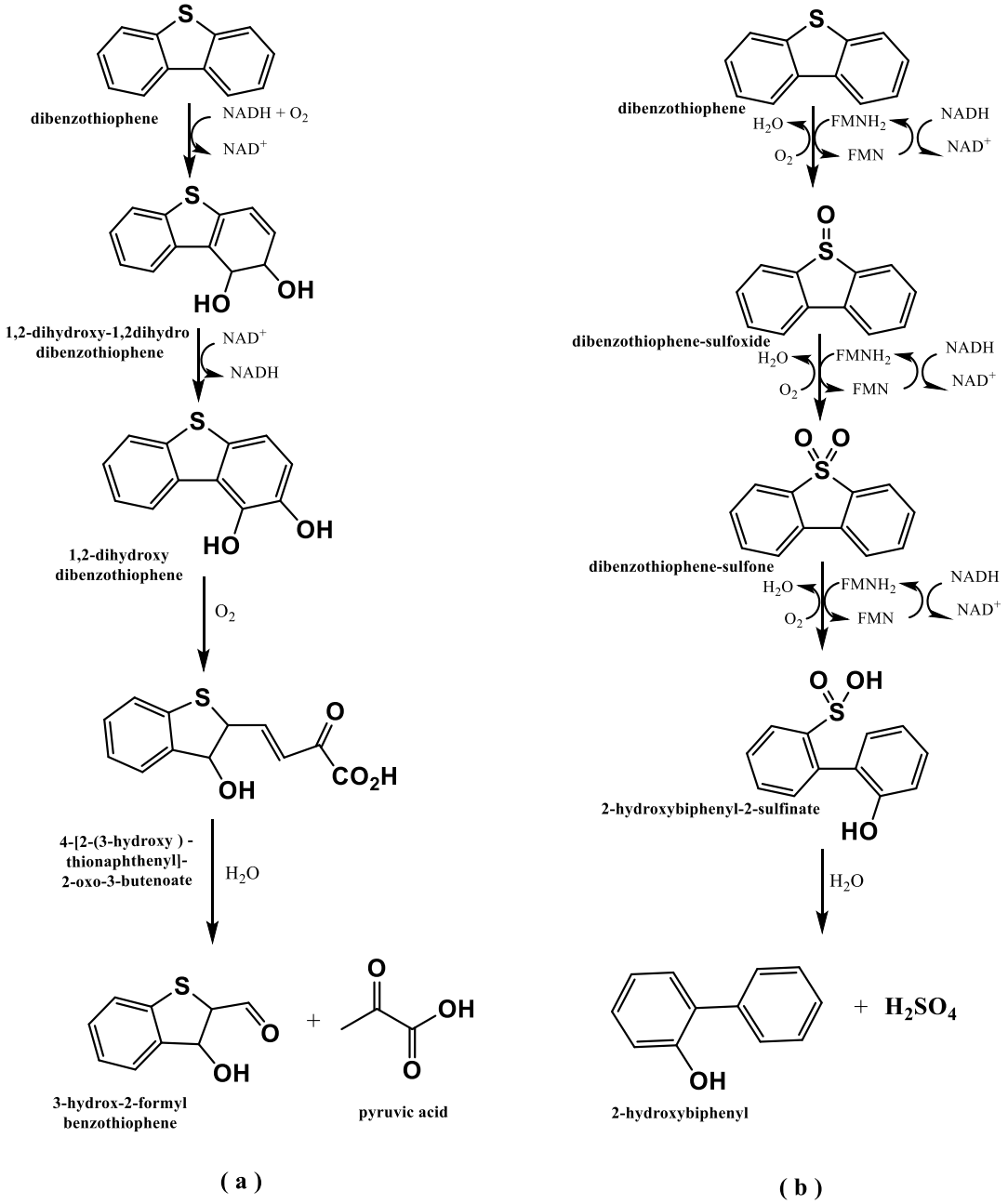

Fig. 2. (a) Kodama and (b) 4S pathways on the biodesulfurization of dibenzothiophene (Jumina et al., 2021)

This work represents a continuation of our research in petroleum biodegradation technology. The study aims to use/isolate *Pseudomonas aeruginosa* bacteria. In addition, describing the ability of the selected bacterial strains to desulfurize crude oil and its refined products, and comparing the isolated local strains with reference commercial strains.

2. MATERIAL AND METHODS

2.1 Material

2.1.1 Microorganisms

The microorganisms used in this study were clinically isolated as characterized in Table (1).

Table (1). The categorizations of microorganisms

Microorganisms	Isolated and diagnosed by
***P. aeruginosa* (from wounds)**	Laboratories of The Supreme Board of Drug & Medical Appliances- Aden.
***P. aeruginosa* ATCC 27853**	Doctors Without International Borders - Yemen.

2.1.2 Crude oil

Al-Masila crude oil, Hadramout –Yemen. Some chemical and physical properties are given in Table (2).

Table (2). Some properties of Al-Masila crude oil

Test description	Test method	Result
Gravity	(D-4052 ASTM, 2019)[1]	33.1
Specific Gravity at 15.5 °C	(D-4052 ASTM, 2019)	0.8599
Vapor Pressure (kPa)	(D-5191 ASTM, 2019)	5.1
Total Sulfur (wt %)	(D-4294 ASTM, 2019)	0.4015
Carbon Residue (wt %)	(D-189 ASTM, 2019)	0.25
Pour Point ^{0}C	(D-97 ASTM, 2019)	-11
Kinematic Viscosity at 40^{0}C mm^2/s	(D-445 ASTM, 2019)	4.897
Water Content (Vol %)	(D-95 ASTM, 2019)	0.05
Element Concentration (ppm)[2]		
Vanadium (V)		25
Nickel (Ni)		13
Lead (Pb)	IP 501(IP, 2019)[3]	NIL
Sodium (Na)		3
Calcium (Ca)		1
Zinc (Zn)		NIL
Iron (Fe)		2
Magnesium (Mg)		1
Copper (Cu)		NIL

[1]ASTM: American Society for Testing and Materials; [2]ppm: part per million; [3]IP: Institute of Petroleum.

2.2 Method of Analysis

The total sulfur content of the untreated and treated crude oil samples was determined by SELFA-2800 sulfur-in-oil analyzer (Horiba, USA). The test method is based on ASTM D-4294 (ASTM, 2019). All the experiments of total sulfur measurements were performed at Aden Refinery Company's laboratory. The concentrations of some elements (Table 2) in the studied crude oil were determined using inductively coupled plasma hyphenated to optical emission spectrometry (ICP-OES) model Thermo Scientific iCAP 6000 Series, USA at Central Processing Facility Laboratory of Al-Masila Petroleum Exploration and Production Company, Hadramout –Yemen.

2.2.1 Preparation of nutrient broth media (NB)

13.0g of nutrient broth powder was added into one liter of distilled water in a flat-bottomed conical flask. The mixture was heated with frequent agitation and boiled for one minute to completely dissolve the media. The flask was then tightly closed using cotton wool and further covered with aluminum foil. The mixture was autoclaved for 15 minutes at 120±1˚C after which it was left to cool down to room temperature.

2.2.2 Experimental procedure

By using a sterile cotton swab, a few bacterial groups were taken and put into the test tube containing sterile saline water, and the turbidity was adjusted to meet 0.5 McFarland standard which contains approximately 1.5×10^8 colony formation unit/ mL(CFU) of bacteria (Tan, 2015). and was taken using a 1ml pipette to a 250 mL Erlenmeyer flask containing 50 mL of sterilized nutrient broth media. The contents were shaken to hydrolyze and then allowed to stay for 6 hours. For the growth, about 1 mL of broth was sampled after 6 h to measure the optical density of cell growth with a spectrophotometer at 600 nm (Hidayat et al., 2017). After that, 10 ml of crude oil was added and incubated at 30±1 °C. The sulfur content was measured at different times.

3. RESULTS AND DISCUSSION

3.1 Effects of Cell Concentration

To study the effect of *Pseudomonas aeruginosa* concentration on the desulfurization rate of Al-Masila crude oil with a starting concentration of 0.4015wt%, the reaction solution containing 50 mL of sterile nutrient broth medium, 10 mL of crude oil, and a known amount of two types of *P. aeruginosa* bacteria were tested for 24h. First type isolated from wounds in the laboratories of the Supreme Board of Drug & Medical Appliances- Aden. The second type obtained from Doctors Without International Borders -Yemen is (*P. aeruginosa* ATCC 27853). The reaction solution was centrifuged to separate the oil from the aqueous phase after the end of the reaction.

Figure 3 indicates the removal of sulfur from Al-Masila crude oil with different concentrations of bacteria. There is no significant difference between the two types of bacteria. The highest percentage of sulfur removal, about 48.5% at a concentration of 1.3×10^8 CFU, was coined by isolated bacteria of the wounds, while the highest percentage of removal sulfur by *P. aeruginosa* ATCC 27853 was 39.8 % at a concentration of 1.3×10^8 CFU.

Sulfur crude oil is often found buried in supra structures formed by several aromatic rings, which are linked by aliphatic bridges. Possibly, all the easily accessible sulfur was utilized in the round of desulfurization and the available sulfur in crude oil was either buried or difficult for uptake and desulfurization by the bacterium (Adlakha et al., 2016).

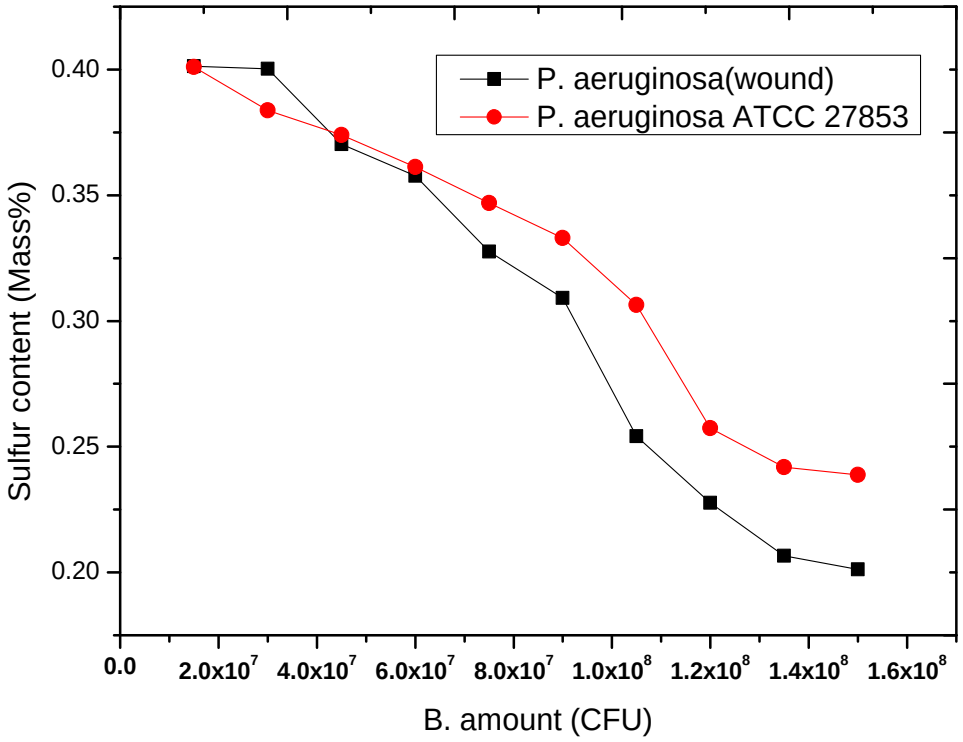

Fig. 3. Effects of *P. aeruginosa* cell concentrations on the desulfurization

3.2 Effect of Time

Figure 4 represents the effect of different periods on the desulfurization of Al-Masila crude oil using *Pseudomonas aeruginosa*. When 10 ml of the crude oil sample and 50 mL of sterile nutrient broth medium were treated with 1.5 x10^8 CFU *P. aeruginosa* (wounds) and *P. aeruginosa* ATCC 27853, the sulfur removal rate from the crude oil was 48.2 % and 41.2 %, respectively, and after 48h the removal rate was 53. 9% and 44.3%, correspondingly. Nevertheless, after 72h, there was no significant change in the results where the percentage of sulfur removal was 54% and 47%, respectively. It is noted that the percentage of the removed sulfur from crude oil was high by bacteria isolated from wounds, and the highest results were obtained after 24h. After that, the percentage of removal sulfur was low.

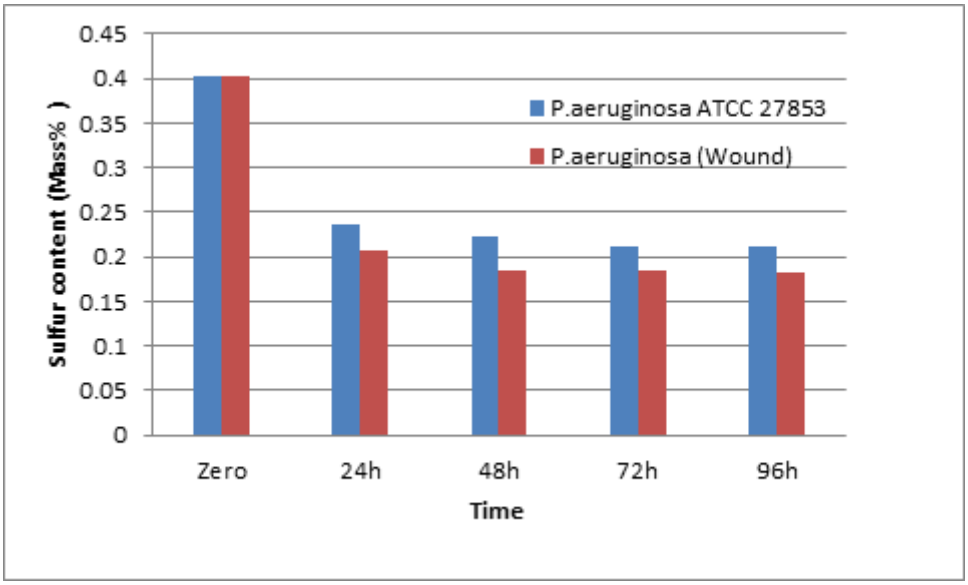

Fig .4. Final sulfur content measured at zero, 24, 48, 72, and 96 h desulfurization by *P. aeruginosa*

4. CONCLUSION

In contrary to the previous study (Saeed & Ahmed, 2021), this research applied *P. aeruginosa* bacteria to eradicate sulfur from Yemeni crude oil. The bacterial species decreases the total sulfur content of the crude oil to about 54% during 24h. Biodesulfurization offers an attractive alternative to conventional hydrodesulfurization due to the mild operating conditions and reaction specificity afforded by the biocatalyst. Nonetheless, further research in characterizing the kinetics of desulfurization and development of strain has to be carried out before realistic assessments in pilot plant studies.

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