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Biodesulfurization of Sour Crude Oil

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Abstract

Crude oil is one of the most important types of fossil fuel in the world. It is an economically important commodity that is massively used in many industrial activities. The poor quality of crude oil is related to high sulfur content, which translates to lower profit margins and negatively impacts air quality standards. Polyaromatic sulfur heterocycles (PASHs) that exist in crude oil requires an efficient reduction method to achieve significant desulfurization levels. Recently, biodesulfurization (BDS) is gaining greater attention attributed to its environmentally benign bioprocess; possible benefits of BDS include lower capital and processing costs. Studies have reported that BDS is urgently needed for desulfurization of recalcitrant organic sulfur relative to traditional approach, hydrodesulfurization (HDS). The establishment of commercial scale biorefining technology relies on major advancement with respect to less expensive and sufficient production of highly active and stable biocatalysts that can be adapted to intense conditions encountered in petroleum refineries. In this paper, a review on BDS processes for removing recalcitrant thoiphenic components from sour crude oil is conducted, covering the aim of most studies concerning desulfurizing bacteria, which enables a deep desulfurization of organosulfur compounds by 4S pathway, maintaining the caloric value of fuel.

Keywords: hydrodesulfurization, biodesulfurization, biocatalysts, biorefining

1. Introduction

The existence of sulfur compounds in final crude oil products has serious impacts on a balanced ecosystem. For example, pre or post combustion of fuels with sulfur produces sulfur oxides and the emission of SO2 and SO3 produces low pH fogs with acid rain (Shahaby & El-din, 2017; Prayuenyong, 2001). The occurrence of acid rain leads to the corrosion of historical buildings, and affects marine life through the lowering of the pH of water bodies such as rivers and lakes, which in turn is harmful to plants (EPA, 2006; Soleimani, Bassi, & Margaritis, 2007). In addition, high corrosiveness of H_2S implies a high maintenance cost for oil refineries due to a regular wear and tear of steel pipes, storage tanks, stove containers, compressors, and pipelines for transportation and other metallic equipment (Duissenov, 2012; 2013). There are different techniques of desulfurizing sour crude oil in the literature covered by Javadli and Klerk (2012). However, each technique has its associated advantages and disadvantages (Javadli & Klerk, 2012; Javadli, 2011). Researchers focused on desulfurization of sour heavy oil have reviewed and evaluated the feasibility of different desulfurization techniques. For example, the study by Mužic and Sertić-Bionda (2013), highlighted hydrodesulfurization (HDS), as well as, biodesulfurization (BDS) techniques on sour heavy oil. Other researchers combined HDS, BDS, and desulfurization ionic liquids (ILs) techniques as an optimal solution for future refining process (Soleimani et al., 2007); due to the fact that the properties of sour heavy oil, such as high sulfur content, high viscosity, low API, and high boiling point, require unconventional approaches for high performance (Javadli & Klerk, 2012).

2. Sour Crude Oil

Sulfur content in crude oil is characterized as either sweet or sour. Crude oil is considered sweet if its sulfur content is at most 0.5 wt. %, while sour crude has sulfur content of more than 0.5 wt. % (ICCT, 2011). On the other hand, crude oil ranges from sweet crude oil $\leqslant$ 0.05wt. % to sour crude oil $\geqslant$ 1 wt. % (ICCT, 2011).

Indeed, sweet light crude oil is more commercially lucrative compared to sour heavier brands. This is mainly due to low-sulfur content, thus, requiring less refining and handling processes (Duissenov, 2012; 2013). Attributed to this, sour crude oil is associated with lower commercial demand and sold at discounted prices compared to its light sweet grade with a typical yield product (Tossot & Welte, 1994; 2013).

2.1 Sulfur Compounds in Crude Oil

Sulfur is the third most abundant element in petroleum after carbon and hydrogen (Soleimani et al., 2007) and can exist in petroleum in two main forms: organic and inorganic sulfur compounds (Javadli & Klerk, 2012). Crude oil contains various types of inorganic sulfur, appearing either as a suspension or dissolved substances in the oil (Soleimani et al., 2007). Nevertheless, elemental sulfur is rarely found in oil because it usually reacts with hydrocarbons when crude oil is heated (Wauquier, 1995). One of the sulfur compounds, hydrogen sulfide (H_2S), is usually formed during crude oil processing operations such as catalytic cracking, hydrodesulfurization, thermal cracking, and thermal decomposition during distillation (Wauquier, 1995). Pyrite with the exception of low molecular weight compounds (Tossot & Welte, 1994; 2013). In general, organic sulfur compounds exist in crude oil in two forms namely, heterocyclics and non-heterocyclics. However, there seems to be more research attention on heterocyclic sulfur compounds compared to non-heterocyclic sulfur compounds (Mohebali & Ball, 2008). Figure 1 shows organic sulfur compounds can be classified as acidic sulfur compounds (which include mercaptanorthiols) and non-acidic as sulfides (such as disulfides and thiophene) (Duissenov, 2012; 2013). Mercaptans usually contain thiol group (SH) from 0.1 to 15 % mass of the total content of sulfur compounds (Ryabov, 2009). The sulfides are chemically neutral compounds which are represented in high amounts of 50-80% of the total sulfur compounds. Another form of chemical compounds found in small quantities in crude oil is disulfides, in ranges of 7-15% of overall sulfur content of oil (Ryabov, 2009). Other compounds found in crude oil such as thiophene and its derivatives are neutral cyclic and temperature resistant compounds with five-membered rings. They do not dissolve in water and exhibit chemical properties similar to aromatic hydrocarbons (Duissenov, 2013). These aromatic sulfur among all organic sulfur compounds are considered recalcitrant, which require deep desulfurization processes in order to remove their sulfur atom.

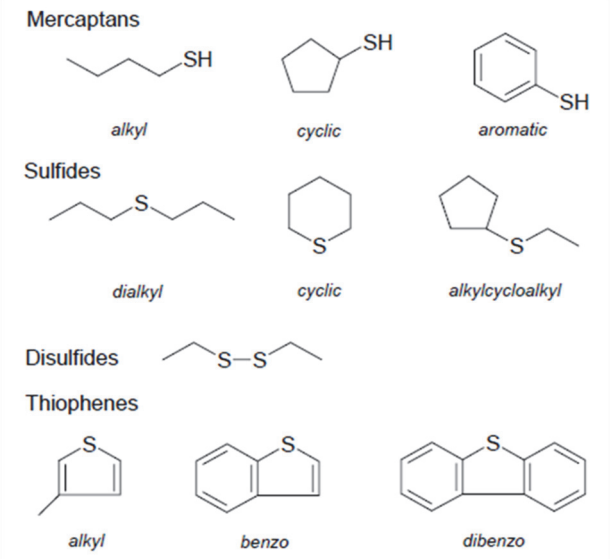

Figure 1. Acidic and Non-Acidic Organic Sulfur Compounds in Crude Oil

3. Hydrodesulfurization (HDS) Processes

Hydrodesulfurization (HDS) is the most commonly used method in refineries for transforming sulfur into H_2S. Conventional HDS process requires intensive energy usage, costly, and ineffective as the ratio and forms of organosulfur compounds increase (Mohebali & Ball, 2008). This technology is widely used in the oil industry and requires extreme industrial conditions such as 200-340ºC temperature and 5 MPa pressure (Egorova, 2003) Here, because the desulfurization level is dependent on the type of petroleum, the sour heavy oil requires deep hydrogenation at very high pressure and temperature of 200-425ºC and 1 to 18 MPa (Javadli, 2011). Consequently, this process under extreme conditions with longer residence time could lead to lower fuel value of the oil product (Egorova, 2003). The conversion of organosulfur compounds into H_2S can further increase the

cost of oil production (Javadli, 2011). Nevertheless, while the HDS procedure can be used to remove sulfur compounds such as thiols, sulfides, and disulfides, it is so difficult in removing when the number of the rings and methyl substituents is increased (Zhao, 2009), as shown in Figure 2.

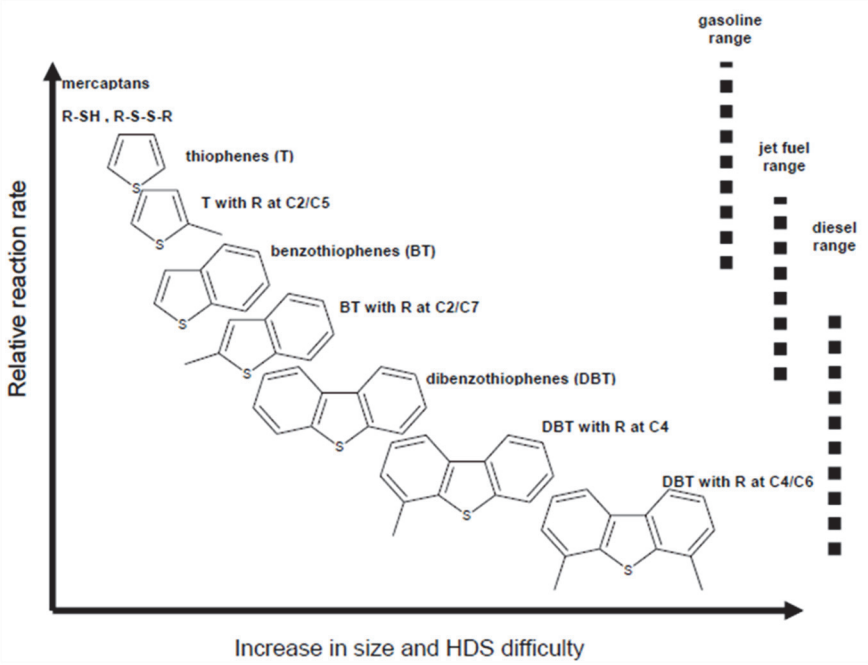

Figure 2. The Reverse Linkage Between Hydrodesulfurization and Structure of Organosulfur Compound

4. Biodesulfurization (BDS) Processes

In fact, the BDS unit is considered as a complementary technology to deep desulfurize all forms of sulfur compounds present in fossil fuels with numerous advantages (Monticello, 1998). A biocatalytic desulfurization is a new approach of sulfur elimination in crude oil introduced to replace HDS in order to enhance energy savings. Thus, BDS is carried out at mild temperature, low pressure, and low emission with safe reaction products (Mohebali & Ball, 2008). Furthermore, the desulfurization process results to a negligible level of undesirable products without lowering the calorific value of the fuel (Soleimani et al., 2007; Etemadifar, Cappello, & Zarkesh-Esfahami, 2014). BDS is also a human friendly method of sulfur elimination in that it utilizes natural bacteria to metabolize organic sulfur. As such, the genetic engineering technology can be used to increase its efficiency (Ma, 2010). Due to these many prospects of the BDS technique, numerous research works have been carried out towards upgrading the HDS process using alternative technologies based on the ability of microorganisms to attack organic sulfur in petroleum that cannot be removed easily using the HDS procedure (Gray, 2010). As well as this process in the presence of species microbial has the specific mechanisms serving as catalyst for desulfurize crude oil and fossil fuels (Soleimani et al., 2007; Gray, 2010)

4.1 Aerobic Desulfurizing Bacteria

By the late l980s, *R. erythropolis* IGTS8, which is a unique strain, showed the highest sulfur removal capability (Kilbane, 1990; Kilbane & Jackowski, 1992). Here, the bacteria of interest is the genus *Rhodococcus* within the Actinobacteria class, Actinomycetales order, ocardiaceae family, and *Rhodococcus* species (Kayser, 2002). The *Rhodococcus* species are obligate aerobic, esophilic, chemoorganotropic, rod gram positive, branched mycelia, non-motile, non-endospore, and have oxidative type of metabolism. Furthermore, they possess a wide variety of catabolic enzymes, have small circular to large plasmids, which give them greater biotransformation across a wide range of compounds (Desomer, Dhaese, & Van Montagu, 1988; Kayser, 2002). In additional, the cell wall of Rhodococci with the peptidoglycan layer of long aliphatic mycolic acid are chained, therefore, it becomes hydrophobic and attaches the oil/water interface in aqueous–hydrocarbon system (Borole et al,, 2002: Gallagher, Olson, & Stanley,1993). The strain *Rhodococcus erythropolis* IGTS8 formerly called *R. rhodochrous* IGTS8, was successful in the first attempt to isolate from soil by Kilbane (1990). While the first reported *Rhodococcus erythropolis* IGTS8 involved in DBT-desulfurising via 4S pathway by Kilbane and Bielaga (1990). According to Kilbane (1990), the strain Rhodococcus erythropolis IGTS8 can utilize DBT not as a source of carbon but as a

source of sulfur. Also Gallagher et al. (1993) reported, *Rhodococcuse rythropolis* IGTS8 strains are able to utilize DBT and remove organic sulfur compounds selectively without cleavage of C-C bonds. The authors further reported that from 1992 until 2017, many strains of *Rhodococcus* sp followed the 4S pathway. These include, for example, *R. erythropolis* D-1 (Ohshiro, Hine, & Izumi,1994), *R. erythropolis* H-2 (Ohshiro et al.,1994), and *Rhodococcus* sp. ECRD-1 (Grossman et al., 2001), *R. globerulus* DAQ3 (Yang et al., 2007), *R. erythropolis* DRA (Q.Li et al., 2008), *R. erythropolis* LSSE8-1 (F.Li et al., 2007), *R. erythropolisstrain* SHT87 (Davoodi-Dehaghani, Vosoughi, & Ziaee 2010), *Rhodococcus* sp. strain SA11, and *Rhodococcus* sp. strain SA31 (Mohamed, Al-Yacoub, & Vedakumar, 2015). A recent study identified *Rodococcus erythropolis* and *Rodococcus ruber* (Shahaby et al., 2017). Briefly, there is much evidence that all of these strains are very closely related to *R. erythropolis* IGTS8. The last strain among of the species of *Rhodococcus* has received an increased attention in studies because it has higher efficiencies (Prayuenyong, 2001).

4.2 Desulfurizing Bacteria from Many Genera

However, different isolates of these bacterial elements such as *Rhodococcus* could attack the heterocyclic sulfur compounds and desulfurizing derivatives of thiophene from the petroleum (Mohebali & Ball, 2007; Kilbane, 2006). For instance, *Agrobacterium* strain MC501 was first reported to be a gram-negative bacterium capable of desulfurizing DBT to produce 2-HBP (Constanti, Giralt, & Bordons, 1996). Similarly, *Mycobacterium* strain G3, which also follows the 4S pathway (Nekodzuka, Nakajima-Kambe, Nomura, Lu, & Nakahara, 1997), and gram positive thermophile bacillus strain Al 1-2 have been reported to exhibit desulfurizing capabilities at 50°C (Konishi, Ishii, Onaka, Okumura, & Suzuki 1997). Similar studies were conducted by other researchers such as *Gordonia* sp. (Rhee, J.H.Chang, & Y.K.Chang, 1998), *Nocardia* sp. (J.H.Chang, Rhee, Y.K.Chang, & H.N. Chang, 1998), and *G. alkanivorans* RIPI90A (Mohebali & Ball, 2008), *Sphingomonassu* barcticaT7b (Gunam et al., 2013), and *Stenotrophomonas* sp.strain SA21 (Mohamed et al., 2015). However, authors who reported the isolation of biodesulfurization bacteria from many genera and compared the efficiency in the removal of organosulfur compound, such as Ismail et al. (2016) reported biocatalytic desulfurization organosulfur compounds including *Sphingobacterium*, *Klebsiella*, *Pseudomonas, Stenotrophomonas*, and *Arthrobacter*, *Mycobacterium*. Meanwhile, Shahaby et al. (2017) reported five genera comprising *Bacillus*, *Pseudomonas*, *Rodococcus, Mycobacterium*, and *Klebsiella*, which showed desulfurization of crude oil and oil products.

5. Metabolic Pathways of Desulfurziing Aerobic Bacteria

The future directions of BDS procedure include the use of aerobic bacteria to remove organosulfur from petroleum without degrading the carbon skeleton (Kareem, Aribike, Nwachukwu, & Latinwo, 2012; Oldfield, Pogrebinsky, Simmonds, Olson, & Kulpa, 1997). On the same basis, several aerobic microorganisms present the abilities to metabolize sulfur in petroleum, but the advances in biodesulfurization is to find appropriate microorganisms to remove sulfur atoms from polyaromatic heterocyclic compounds, which do not serve as sole carbon and energy source (Fedorak, 1990; Kareem et al., 2012: Sohrabi et al., 2012).

5.1 4S Pathway (Oxidative C–S Cleavage)

4S pathways have recently attracted significant research attention for applications involving biodesulfurization. This procedure involves the release of sulfur atoms from aromatic rings in such a way that the hydrocarbon parts of the DBTs are protected, and as a result, protects the caloric value of the fuel (Aggarwal, Karimi, & Ivan, 2013; Sohrabi et al., 2012). However, the 4S pathway is considerably a non-destructive desulfurization because it attaches C–S bonds in a sequence of sulfur oxidation steps without breaking the hydrocarbon skeleton C– C (Mohebali & Ball, 2008; Etemadifar et al., 2014). *R. erythropolis* IGTS8 (dszABC genes) is another common desulfurization process, involving three gene structures namely, dszA, dszB, and dszC, which encode the enzyme through four steps to convert DBT to 2-hydroxyphenyl (2-HBP) and sulfite (Kayser, 2002; Etemadifar et al., 2014). The first and second steps involved in the process are converting DBT to DBT-sulfone in two consecutive monooxygenation reactions of DszC through the monooxygenase catalyzing activation of thiophene ring. This is followed by converting DBT-sulfone to HBP sulfinate through a second monooxygenase (DszA enyme) through oxidative cleavage of thiophenering (carbon-sulfur bonds). Finally, DszB is desulfinased through the desulfination of HPBS and release of sulfur atoms for producing 2-HBP and sulfite (Etemadifar et al., 2014; Matsubara et al., 2001). Figure 3 shows the steps of the 4S pathway.

6. Model Compounds for Biodesulfurization Systems

Thiophenic sulfur is the most common organosulfur compounds found in petroleum and oil fractions, and usually constitutes 50–95% of the total sulfur contents (Zhao, 2009; Kilbane & Le Borgne, 2004; Monticello, 1998). For this reason, dibenzothiophene model and dibenzothiophene with substitutions (DBTs) have been frequently used as a polyaromatic sulfur model for the investigation of microbial desulfurization of petroleum

and coal (Kilbane 2006; Pryanyong 2010). Dibenzothiophene has a general molecular formula of $C_{12}H_8S$ and average mass 184.257mol/g. Figure 4 shows DBT consisting of two benzene rings and sulfur atom in the central thiophene ring, which exhibits a similar structure when present in coal, oil, or contaminated environments (Olson, 1993).

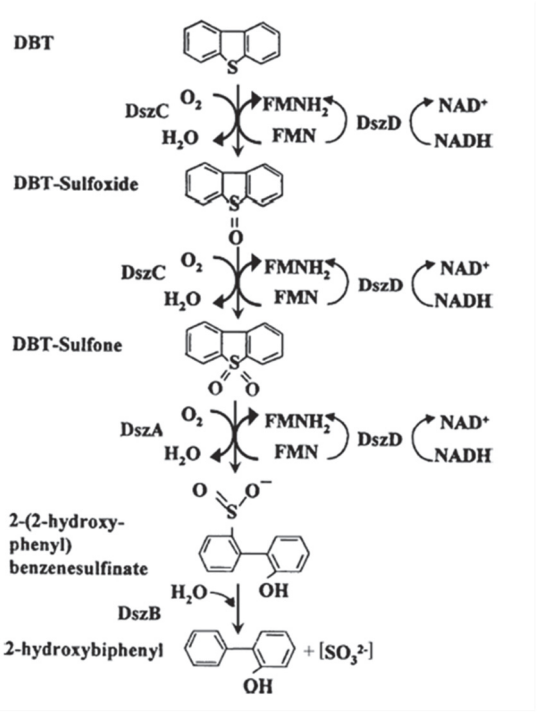

Figure 3. 4S Pathway of Microbial Desulfurization Process of DBT to 2HBP and Sulfite

However, many studies focused on the identification of 4S metabolites of microorganism by using thiophenic compounds, particularly DBT (Abbad-Andaloussi, Warzywoda, Monot, 2003; Gray, Pogrebinsky, Mrachko, Monticello, & Squires, 1996). Several investigation has used dibenzothiophene (DBT) concentration ranging from 0.2 to 0.5 mM as model or analogy to elucidate the metabolic pathway (4Spathway) of microbial strain (Abbad-Andaloussi et al., 2003; Mohamed et al., 2015). Adopting such methods, DBT is evaluated by the Gibbs test in the presence of aromatic hydroxyl group (Sohrabi et al., 2012). Kayser reported, desulfurization activity is indicated by blue color when reacted, producing 2-hydroxy biphenyl (2HBP) as the final product with Gibbs reagent (Kayser, Bielaga-Jones, Jackowski, Odusan, & Kilbane, 1993). Additionally, HPLC is confirmed of its DBT desulfurization ability of microorganism (Fedorak, 1990: Prayuenyong, 2001). Microorganisms can utilize condensed thiophenes as pure organosulfur compounds, but cannot serve as sole carbon and energy sources (F.Li, Zhang, Feng, Cai, & Xu, 2007). Studies investigated the capability of bacteria to selectively desulfurize alkylated dibenzothiophenes (DBTs) using the 4S pathway. For example, *Rhodococcus* sp IGTS8 was utilized on dibenzothiophene as a sole sulfur source in coal and crude oil (Kilbane & Bielaga, 1990; Nekodzuka et al., 1997). Here, the isolated *Rhodococcuc erythropolis* D- 1 has been shown to selectively remove sulfur by oxidizing dibenzothiophene (Ohshiro et al., 1994). A similar study reported that the *R. erythropolis* H-2 was capable of attacking the sulfur atoms in 3,4-benzo DBT, 2,8-dimethy DBT, and 4,6-dimethyl DBT (Ohshiro et al., 1996), while the *Rhodococcus erythropolis* SHT87 completely desulfurized DBT (Davoodi-Dehaghani et al., 2010). Also, other species have been reported, such as the isolated Arthrobacter species, which can desulfurize model compounds like 4,6-diethyldibenzothiophene, yielding 2-hydroxy-3,3'-diethylbiphenyl, and liberated sulfur as the salfite (Lee, Senius, & Grossman, 1995). Likewise, Mycobacterium sp.strain G3 was also utilized to desulfurize isolates to 4,6-dimethyl DBT (Nekodzuka et al., 1997), selective cleavage C-S bonds from DBT, and its derivatives. In addition, a moderate thermophile, *Mycobacterium phlei* GTIS10, also converts DBT to 2-hydroxybiphenyl (Kayser, 2002).

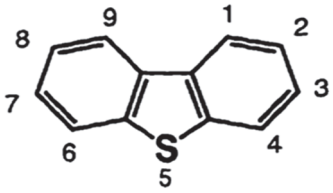

Figure 4. Dibenzothiophene (DBT) structure

7. Biocatalytic Desulfurization of Petroleum

In fact, there exist not many sensible sources of energy that can replace crude oil for sustainable economic growth (Kiboub, 2011). A new challenge in increasing the quality standard of crude oil is to improve its qualities through advanced BDS (Kareem et al., 2012). For decades to come, it is expected that the global demand for petroleum will grow exponentially. Oil companies report that most reserved oil has a high content of sulfur which lead to investigations to overcome this problem. Treatment procedures must be devised or improved to reach sulfur levels below 10 ppm (König, Herding, Hupfeld, Richter, & Weidmann, 2001; Song, Hsu, & Mochida, 2000). The World Health Organization has recognized negative impacts of indoor and outdoor burning of fuels on the atmosphere, which have degraded human health in various regions of the world (WHO, 2006). For this reason, the Environmental Protection Agency of the United States suggested that the sulfur level of diesel oil should be decreased from 500 to 15 ppm, with a target of 10 ppm (Kilbane, 2006). Consequently, researchers consider BDS as an important choice for treatment of sour heavy crude oil from new reservoirs, through potential desulfurizing bacterial activity. Table 1. Lists desulfurizing bacteria and their desulfurization yields.

Table 1. Desulfurization of Petroleum by Aerobic Bacteria

Desulfurizing bacteria	Desulfurization yield	References
R. erythropolis I-19	67% desulfurization of petroleum	Folsom, Schieche, DiGrazia, Werner, & Palmer, 1999
R. sp. strain ECRD-1	669 p.p.m desulfurization middle distillate oil	Grossman et al., 2001
R. sp. and *Athrobacter sulfurcus*	50% desulfurization of diesel oil	Labana, Pandey, & Jain, 2006
R.erythropolis XP	94.5% desulfurization of diesel oil	Yu, Xu, Shi, & Ma, 2006
R. globerulus DAQ3	1500 ppm desulfurization of diésel oil	Yang et al., 2007
Gordon sp. strain CYKS1	Reduction of sulfur from 0.15% (wt/wt) to 0.06% (wt/wt) of middle distillate unit feed MDUF	Rhee et al., 1998
P. delafieldii R-8	313 desulfurization from 591 mg/L of diesel oil	Guobin et al., 2006
*Nocardi*a sp. CYKS2	0.3 - 0.24 wt.% desulfurization of diesel oil	Chang et al., 1998
Mycobacterium sp. X7B	86% desulfurization diesel oil	Li et al., 2003
Mycobacterium phlei WU-0103	52% desulfurization gas oil fraction from 1000 to 475 ppm	Ishii et al., 2005
Mycobacterium goodii X7B	59% desulfurization of Liaoning crude oil from 3600 to 1478 ppm.	F.Li et al., 2007

8. Conclusions

The advances in biodesulfurization are to find appropriate microorganisms to remove sulfur atoms from polyaromatic heterocyclic compounds, which do not serve as sole carbon and energy sources. Thus, the higher is the authors' knowledge about selective pathway to metabolize recalcitrant sulfur in a particular sour crude oil. Petroleum and other fossil fuels exhibit varying sulfur content as well as nature of mixture that makes sulfur removal more challenging. Biorefining is a new concept, a sustainable alternative to the petroleum refineries, which is critical to the future of the oil industry. This technique is designed against the backdrop that biorefinery can be integrated into microorganisms in the current oil refining process to produce high-quality oil products and

fuel. The suitability of BDS as the best option for current oil refineries has been reported in the literatures. In the next few years, biocatalysts with high potential will make it possible to treat crude oil in industry production level.

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