JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2012. Vol. **6**(2), p. 717-729

Bacterial Desulfurization of Organic Sulfur Compounds Exist in Fossil Fuels

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(Received: 15 July 2011; accepted: 11 September 2011)

Air pollution by sulfur oxides as a result of burning and combustion of fuels that contain sulfur compounds play a significant role in increasing the rate of some diseases and problems in each ecosystem. Hydrodesulfurization (HDS) have been the most popular method of desulfurization. Incapability of this technology to remove polyaromatic sulfur heterocyclies (PASHs) has led to establish another alternative technology that is called Biodesulfurization (BDS). The most important advantage of this process is that BDS does not have any effect on the caloric value of fossil fuels, as this process is able to remove sulfur atom from sulfur compounds selectively. Dibenzothiophene (DBT) as the most abundant sulfur compound in the fossil fuels have been more taken into account in BDS researches and two main pathways have been defined for DBT biodesulfurization which are Kodama and 4S. The purpose of this study was to understand the desulfurization methods and review the desulfurizing bacterial characteristics in terms of biodesulfurization activities and growth conditions of them. As the 4S pathway is the main pathway of removing sulfur selectively from DBT by some bacteria, biodesulfurizing bacteria that are able to follow this pathway were considered more in this study.

Key Words: Biodesulfurization (BDS), Hydrodesulfurization (HDS), DBT, 4S pathway, Organic sulfur compounds, Desulfurizing bacteria.

Nowadays fossil fuels are the most significant energy resources in the world. Indeed, around 85% of our energy comes from fossil fuels, 8% from nuclear power, and 7% from other sources of energy (Gupta *et al.*, 2005). Sulfur, as a notable element in petroleum after hydrogen and carbon, plays a significant role in producing pollutions in the environment.

Burning and combustion of sulfuric compounds in crude oil lead to release sulfur oxides (SO_x) in the atmosphere. Releasing SO_2 can be one of the main causes of lung cancer, cardiopulmonary and respiratory problems such as bronchial irritation and asthma attacks in prone human (Mohebali and Ball, 2008). In addition, corrosion and erosion of refinery pipes and metal fittings, creating acid rains, demolishing of forest and agricultural ecosystems through pH reduction, washing metal ions from soil, damaging the alimentary chains ecosystems, destroying of buildings and constructions and, reducing the value of crude oil are other problems caused by releasing sulfur oxides in atmosphere (Gou *et al.*, 2005; Monticello, 1998).

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It is certainly true that having clean atmosphere or decreasing air pollution cannot be achieved without removing sulfur from fossil fuels used in transportation systems, factories and even in domestic usage. Indeed, there is not any determined strategy of reducing air pollution that can be successful without sulfur removing to around zero level.

For the above mentioned reasons and because of notable role of reducing sulfur compounds in fossil fuels, the strategy of reducing sulfur content of diesel from 500 ppm (2006 regulation) to 15 ppm for the year 2012 has been taken into account by the United States Environmental Protection Agency (EPA) in the regulatory announcement (Borgne and Quintero, 2003; EPA, 2005).

Sulfur Compounds in Fossil Fuels

Sulfur is the most abundant element after carbon and hydrogen in petroleum. This element can be found in three phases of material, which are gas, solid and liquid. The amount of sulfur concentration in crude oil varies and it is different from 0.03% to 7.89% wt (Mohebali, 2008). However, the value of 13.95% sulfur concentration has been reported in some cases (Grossman *et al.*, 1999). In addition, the amount of sulfur in natural gas exist as much as 5% wt, in forms of H_2S, thiols and volatile sulfides. The percentage of existing sulfur in light oil, heavy oil, and solid petroleum (asphalt) is about 0.5%, up to 6% and 6%, respectively (Borgne and Ayala, 2010).

Organic sulfur compounds that are hydrocarbon molecules contain atoms of sulfur are considered more in biodesulfurization. Disulfides, polysulfides, sulfones, mercaptans, thiols, thioethers, thiophenes, and other more complex compounds are the examples of organic sulfur compounds in petroleum (Kayser *et al.*, 1993). Thiophenes (C_4H_4S) and its alkyl-substituted isomers that include different aromatic rings, with heterocyclic structure, (i. e. PASH such as thiophene, benzothiophene, dibenzothiophene, and Benzonaphthothiophene), are carcinogenic compounds (Khadem Haghighat *et al.*, 2003; Ansari, 2008), and among other types of organic sulfur compounds have been more considered for desulfurization from fossil fuels. Inorganic sulfur compounds such as pyrites (FeS_2) besides elemental sulfur and hydrogen sulfide can also be present in fossil fuels (Marcelis, 2002).

Due to the fact that nearly up to 60% of existing sulfur compounds in petroleum products is DBT and its alkyled derivatives (Monticello, 1998) and also persisting of this compound in the environment for more than three years (Xu *et al.*, 2006), the most considerable sulfur compound in petroleum in terms of desulfurization, clean fuel and environmental researches is DBT. Moreover, the availability and abundance of pure DBT in sulfur rich fractions has led to use this compound in biodesulfurization researches widely.

Desulfurization Methods

Being aware of SO_x harmful side effects on human health and ecosystems have led to establish some regulations and laws in the world to reduce the level of existing sulfur compounds in fossil fuels, since early 1990s (Borgne and Ayala, 2010). The main goal of these regulations is controlling the amount of SO_x releasing in the atmosphere. Hydrodesulfurization and biodesulfurization are the main methods of removing sulfur compounds in the fossil fuels (Soleimani *et al.*, 2007). Hydrodesulfurization could be effective in pre-combustion desulfurization in refineries, but disability of this method to remove some sulfur compounds such as DBT and its alkylated derivatives, which is 70% of existing sulfur compounds in fuels (Borgne and Ayala, 2010; Kertesz and Wietek, 2001), forced researchers to use other complimentary methods.

Hydrodesulfurization

A traditional method for desulfurization of oil compounds such as kerosene and gasoline is hydrodesulfurization, that is catalytic reaction between hydrogen and organic sulfur to remove it at the pressure between 5 up to 10 MPa and in the range of 300 to 350°C temperature, depending on the required level of desulfurizing (Shafi and Hutchings, 2000). Indeed, this is a physico-chemical method of removing sulfur bonds in sulfuric compounds (Marcelis, 2002).

The efficiency of hydrodesulfurization is related to the chemical variety of hydrocarbons. Mercaptans, thioethers, and disulfides among other sulfur compounds are easily removed by hydrodesulfurization due to their instability. But hydrodesulfurization is not able to remove and get deep desulfurization of the oils that contain some other molecules specially the polyaromatic sulfur

heterocyclies (PASHs) that present in the heavier fractions (Guerinik and Al-Mutawah, 2003). In addition, high costs of operation and building, unwanted secondary effects, inherent chemical limitations associated with hydrodesulfurization , discharging the high amount of CO_2 in the environment as a result of doing hydrodesulfurization under high temperature and pressure conditions and significant octane number reduction of fuels led to investigate and using complementary biological methods for sulfur removing from oils and petroleum (Ishii *et al.*, 2005; Khadem Haghighat *et al.*, 2003; Li *et al.*, 2005; Ansari, 2008).

Moreover, deep hydrodesulfurization may cause other several problems that make this process inconvenience; 1) reducing the caloric value of treated oil in the high temperature during hydrodesulfurization, 2) producing carbonaceous coke on the catalysts by extreme conditions that are applied in hydrodesulfurization, 3) generated H_2S during hydrodesulfurization is a poison for catalysts and truncate their functional life (Mohebali and Ball, 2008).

Biodesulfurization

ZoBell in 1953 used anaerobic sulfate reducing bacteria for the first time to remove organic sulfur compounds in petroleum by producing H_2S as a final metabolite (cited in Borgne and Ayala, 2010). The first bacterial strain with the ability of removing sulfur from DBT without effecting on hydrocarbon structure was identified in the late 1980s (Kilbane, 1990). After that, the crude Kuwait oil was desulfurized by *D. desulfuricans M6* by production of H_2S as a final metabolite (Kim *et al.*, 1990). Armstrong *et al.* (1995) and McFarland (1999) reported that *Desulfovibrio*, *Desulfotomaculum* and *Desulfomicrobium* species are able to use DBT as a sole source of sulfur and converting it to H_2S and biophenyl.

The main advantage of biodesulfurization compared to hydrodesulfurization is that this process does not need high reaction conditions such as high temperature and pressure and it can happen under normal pressure and temperature condition. In addition, biodesulfurization compare to hydrodesulfurization has some other merits such as capital and operation cost saving, capability and flexibility to treat a wide variety of petroleum streams, more rapid engineering and construction time, more energy efficient process, safer and milder condition and sulfur selectively removal (Kargi and Robinson,1982; Xiaojuan *et al.*,2008).

Aerobic Biodesulfurization

Biodesulfurizing microorganisms are able to use and remove sulfuric compounds into three ways, which are oxidative C-S cleavage, oxidative C-C cleavage, and reductive C-S cleavage (Gupta *et al.*, 2005). Biodesulfurization can occur in aerobic and anaerobic conditions. The aerobic and anaerobic mechanisms can breakdown the carbon-sulfur bonds in sulfur heterocyclic compounds such as thiophene, benzothiophene and dibenzothiophene (Bressler *et al.*, 1998). The rate of biodesulfurization under anaerobic condition is too slower than that in aerobics biodesulfurization and the low rate reaction of anaerobic biodesulfurization has been already proven (Ohshiro *et al.*, 1999).

Desulfurization of DBT can be performed in two pathways by aerobic bacteria, which are called Kodama and 4S pathways. The mechanism of DBT degradation and desulfurization through Kodama and 4S pathway are shown in the figure 1 and 2.

Kodama Pathway (Oxidative C-C cleavage)

The first reported aerobic pathway of DBT biodesulfurization is Kodama pathway (McFarland *et al.*, 1999; Gupta *et al.*, 2005). This pathway, which is oxidative C-C cleavage, involves the ring cleavage of one aromatic ring in sulfur compounds because of initial oxidative reaction. As it is shown clearly in figure 1, Kodama pathway is done into three main steps; i) hydroxylation, ii) ring cleavage and iii) hydrolysis. In fact, Kodama pathway is an incomplete ring destructive of DBT without sulfur removing. *Pseudomonas jijani* and *P. abikonesis* were reported by Kodama in 1970 (cited in Gupta *et al.*, 2005) that are able to follow Kodama pathway to degrade DBT.

Hydrocarbon ring cleavage and destruction are the result of aerobic biodesulfurization via Kodama pathway. Moreover, formyl benzothiophene remains after finishing biodesulfurization through this pathway, and sulfur specific releasing is not carried out at the end of this pathway.

In fact, Kodama pathway is not considerable and suitable mechanism of biodesulfurization because of producing water-

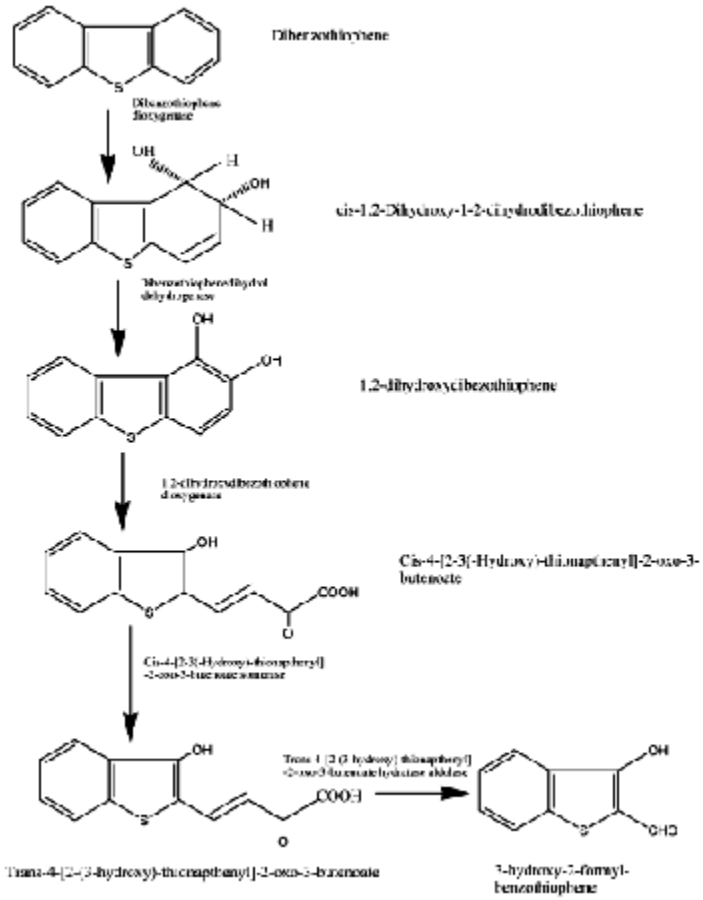

Fig. 1. Kodama Pathway of DBT Desulfurization (Gupta *et al.,* 2005)

soluble sulfur compounds, which are not burnable, at the end of this process (Mohebali *et al.,* 2007). Therefore, because of producing these water-soluble sulfur compounds in fuels after biodesulfurization via Kodama pathway, caloric value and thermal unit of petroleum are reduced and the result is not acceptable and economical.

4S Pathway (Oxidative C-S Cleavage)

In 1990, the 4S pathway was suggested firstly by Kilbane, in which microorganisms are able to oxidize sulfur atoms in DBT without any breakage of C-C bonds (Bressler *et al.,* 1998). In this method, sulfur atoms are released from DBT and hydrocarbon parts of DBT are protected.

The 4S pathway is a sulfur specific DBT desulfurization. This pathway is called 4S because it involves four intermediate sulfur compounds as it is shown in figure 2. This pathway of desulfurization is more considerable in biodesulfurization compare to Kodama pathway because of its neutral influence of this type of removing sulfur on hydrocarbon structure and caloric value of fuels.

4S pathway is a DBT sulfur specific desulfurization pathway that contain consecutive sulfur oxidation of DBT by breaking down the S-C bonds that leads to 2-hydroxybiphenyl (2-HBP) and sulphate formation and leaving hydrocarbon skeleton unchanged (Labana *et al.,* 2005). This pathway involved in four steps of enzymatic reactions. These enzymes attack the sulfur part of DBT selectively and release the sulfur from hydrocarbon parts.

Complete reaction of DBT desulfurization

through 4S pathway, can be divided into three stages from the chemical reaction point of view, which are: (i) activation of thiophene ring for cleavage caused by oxidation of the sulfur moiety; (ii) an aromatic sulfinate formation because of thiophene ring cleavage; (iii) sulfinate group removal. HBP and sulfate are the final products of 4S pathway of DBT desulfurizing (McFarland, 1999; Gray *et al.*, 2003).

According to figure 2, to do biodesulfurization via 4S pathway, two cytoplasmic monooxygenases supported by flavin reductase and a desulfinase are involved. First two sequential monooxygenation reactions that lead to conversion of DBT to DBT sulfoxide (DBTO; dibenzothiophene 5-oxide) and then to DBT sulfone ($DBTO_2$; dibenzothiophene 5,5-dioxide) are catalysed by the mono-oxygenase, DszC enzyme.

After that, the third monooxygenation reaction that is transformation of DBT sulfone to HBP sulfinate (HBPS; 2-[2'-hydroxyphenyl benzene sulfinate]) is catalysed by monooxygenase DszA enzyme. In the last stage, another desulfinaze enzyme, which is DszB, catalyses releasing of sulfur atom from HBP sulfinate to produce 2-HBP and free sulfate. In other words, DszB that is an aromatic sulfinic acid hydrolase, form 2-HBP by affecting the nucleophilic attack of base-activated water molecule on the sulfinate sulfur.

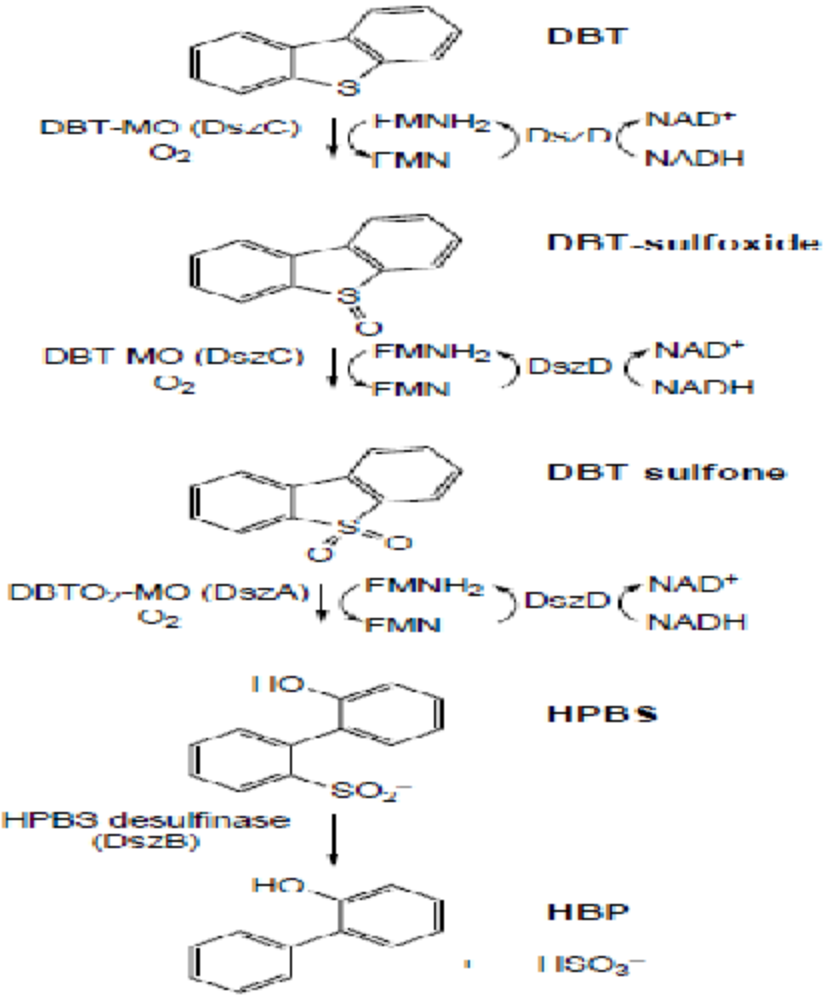

Fig. 2. Sulfur-Specific Desulfurization of DBT through the 4S Pathway by *R. erythropolis* IGTS8

Anaerobic Biodesulfurization

Anaerobic desulfurization can be performed in the presence of hydrogen or nitrogen gases by anaerobic bacteria. H_2S gas and organic sulfur compounds production are accompanied by anaerobic biodesulfurization (Kim *et al.*, 1995). Zobell reported the first anaerobic biodesulfurization of oil in 1953 by recruitment of *D.desulfuricans* (Sohrabi, 2008). One of the advantages of anaerobic biodesulfurization is that it is more compatible with traditional desulfurizing methods such as hydrodesulfurization (Sohrabi, 2008). Reducing equivalent requires for occurring reductive desulfurization, reductive C-S cleavage, to reduce DBT to biphenyl and H_2S to release sulfur from DBT (Kim *et al.*, 1995). Nowadays, there is no significant anaerobic biodesulfurization process in large and industrial scale because of its drawback and difficulties such as cost of required hydrogen and difficulties in providing and maintaining anaerobic conditions (Gupta *et al*,. 2005).

Desulfurizing Genes and Enzymes involve in 4S Pathway

The first name designed for desulfurizing gene was *sox* (sulfur oxidation). However, as other unrelated genes was labelled by *sox*, *dsz* (desulfurization) designation has been accepted generally to avoid any confusing (Gupta *et al.*, 2005). Four *dszA,B,C,D* genes in *R. erythropolis* IGTS8 are encoding four enzymes that catalyse 2-HBP formation from DBT in four stages. *R. erythropolis* IGTS8 is able to separate sulfur from DBT and converts it into 2-HBP (Denome *et al.*, 1993 and 1994; Piddington *et al.*, 1995). *dszABCD* genes code the DszA (monooxygenases), DszB (desulfinase), DszC (monooxygenase), DszD (flavin reductase) enzymes for catalysing DBT desulfurization during 4S pathway. Enzymatic reaction of DBT biodesulfurization by *R. erythropolis* IGTS8 is portrayed in figure 2.

Requiring NADH as a cofactor and its effects on the rate of desulfurization activities of *R. erythropolis* strain D-1 have been reported and investigated by researchers (Izumi *et al.*, 1994; Ohshiro *et al.*, 1994; Ohshiro and Izumi, 1999). DszD enzyme, which is coded by *dszD* gene (Xi *et al.*, 1997; Gray *et al.*, 1996), is an oxidoreductase enzyme and has NADH:FMN flavin oxidoreductase activity. Indeed, the contribution of $FMNH_2$ involved in monooxygenase reaction is guaranty by DszD enzyme (Caro, 2008).

DszD enzyme provides necessary NADH, reduced FMN ($FMNH_2$) for DszC and DszA enzymes, and raises the rate and efficiency of desulfurization activity by this method (Gray *et al.*, 1996; Matsubara *et al.*, 2001; Ohshiro *et al.*, 1994). DszA and DszC do not use NADH directly but use $FMNH_2$ from a NADH:FMN oxidoreductase (DszD). Therefore, DszD is coupled by oxidation of NADH and substrate oxygenation by DszA and DszC.

Desulfurizing Bacteria

Microorganisms are able to degrade and remove sulfur compounds in oil and fossil fuels that result in improving the quality of fuels without altering the caloric values of them. Bacteria are more noticeable among all microorganisms because of their unique characteristics such as their size and their abilities in growing under specific conditions that exist in the presence of fossil fuels. For example, many petroleum tanks and pools contain the high level of NaCl that can be tolerated by some bacteria (Van Hamme *et al.*, 2003).

Wide variety of bacterial strains that are able to remove existing organic sulfur compounds in petroleum have been isolated (Mohebali and Ball, 2008). However, the rate of their desulfurizing activity is not sufficient to use them commercially and for different sulfur compounds (Yang and Marison, 2005). In order to improve desulfurizing activities and using bacterial abilities in a large scale, the rate of biodesulfurization should be increased around 500-fold of currently used bacteria (Kilbane, 2006).

First pathway of desulfurization that is Kodama pathway was shown by Monticello (1985) for *Psedomonas alcaligenes* and *P.putida*. Kodama pathway has not been considered as a sulfur removal pathway for using it in biodesulfurization process because of its effects on caloric value of fuels. Therefore, researchers attempted to isolate and identify the bacteria, which are able to remove sulfur compounds non-destructively and selectively.

Rhodococcus erythropolis IGTS8 strain was isolated by Kilbane in gas institute technology (Kilbane and Jackowski 1992; Olson *et al.*, 1993; Omori *et al.*, 1992). This aerobic strain was able to do DBT desulfurization through the 4S pathway and it was the first isolated and identified sulfur

specific desulfurizing bacteria that were able to cleavage C-S bond in DBT (Marcelis, 2002).

Several bacteria have been suggested to be used in biodesulfurization process. However, most of researches and studies have been done on the issue of selective desulfurization of organic sulfur compounds by *Rhodococcus* strains especially *R. erythropolis* IGTS8 and other bacteria such as *Mycobacterium* (Ishii *et al.,* 2005). The percentage of removing sulfur compounds that was done by these strains was reported around 30-70%, 49-86% and 24-33% for middle distillates, HDS treated diesel oils and crude oil, respectively (Ishii *et al.,* 2005).

The ability of *R. erythropolis* strain IGTS8 in DBT degradation without effecting and decreasing the caloric value of fuels has led to use it in biotechnology industry (Gray *et al.,* 1996). *Rhodococcus* strain IGTS8 among other strains of this genus is able to use a wide variety of sulfur compounds, such as thiophene, sulfides, disulfides, mercaptans, sulfoxides and sulfones (Kayser *et al.,* 1993), as a sole source of sulfur.

One of the newest desulfurizing bacterium that was isolated by Li *et al.* (2005) is *Mycobacterium goodii* X7B. The most important ability of this thermophilic bacterium is extending the 4S pathway to produce 2-methoxybiphenyl (2-MBP) from 2-hydroxybiphenyl as it is shown in figure 3. Other two bacteria that are able to extend 4S pathway to produce small amount of 2-MBP are *Microbacterium* sp. ZD-M2 (Li *et al.,* 2005) and *Microbacterium* sp. ZD-19 (Chen *et al.,* 2009).

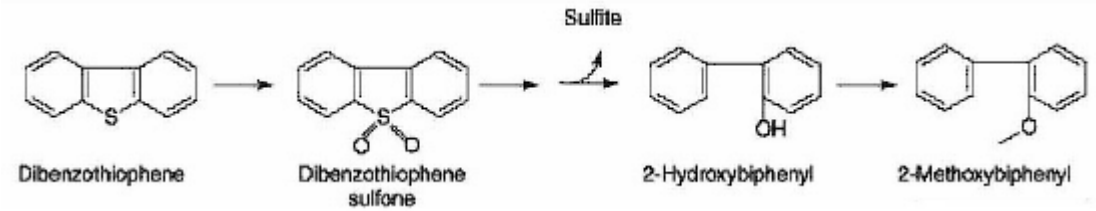

Fig. 3. Proposed mechanism of 2-MBP formation during 4S pathway extending by *Mycobacterium goodii* X7B

Characteristics of some sulfur specific DBT desulfurizing bacteria

Researchers have investigated the growth of isolated bacteria on the different concentration of DBT and other sulfur compounds. For example Gou *et al.* (2002) reported that strain *Rhodococcus erythropolis* LSSE8-1 is not able to growth on the culture media that contain more than 10 mmol/l DBT. In addition, this strain cannot grow well in the medium that contain less than 0.01mmol/l DBT concentration. In this experiment the best growth was related to the concentration of 1.00 mmol/l DBT followed by 0.5 mmol/l, 5 mmol/l, 0.1 mmol/l. Therefore, the high concentration of DBT (more than 10 mmol/l) effects on this strain and results in prevention of biodesulfurization and the concentration less than 0.01 mmol/l can provide the minimum sulfur requirement for this strain to do desulfurization.

Gordona strain CYKS1was characterized by Rhee *et al.* (1998) in terms of DBT and other organic sulfur compounds desulfurization. It was reported that under 0.3 mM of DBT this strain had the maximum growth rate at 100h of cultivation. In addition, decreasing in the desulfurizing activity and growth by 23% was seen because of reduction in pH from 7.4 to 5.9. Moreover, 95% of DBT converted to 2-HBP by this strain and desulfurization of DBT continued just under 70h and after that although the growth of bacterium continued, the concentration of DBT remained constant. Rhee *et al.* (1998) have proven the suppressive action of sulfate on DBT desulfurization. In case of using 2-HBP as a source of carbon, it was found that this metabolite was not used up to the concentration of 0.25 mM and at this concentration the generation time of this bacterium increased by 50%. However, this bacterium could grow at the presence of 0.3 mM 2-HBP by longer generation time.

Another DBT sulfur specific desulfurization was done by *Paenibacillus* sp.

strain A11-2 at 50°C (Konishi *et al.*, 1997). The effect of temperature on DBT desulfurization and growth rate by *Paenibacillus* was tested in the range of 30 to 60°C and the results showed that the growth of this bacterium was depended on temperature and it reached at the peaked at 58°C. In addition, they found that the lowest growth rate of *Paenibacillus* was in 54°C and they did not observe any growth of this bacterium at 63°C. Moreover, they observed that increasing the temperature of culturing increased the rate of 2-HBP producing by *Paenibacillus* and the most specific desulfurizing activity was seen at 54°C.

In another experiment, Furuya *et al.* (2001) investigated the effect of temperature in the range of 20 to 50°C on *Mycobacterium phlei* WU-F1 to desulfurize DBT and found that the resting cell of this strain had the most DBT degradation activity and 2-HBP production at 50°C and 45°C respectively. In addition, this bacterium compared to mesophilic *Rhodococcus* sp. IGTS8, thermophilic *Paenibacillus* sp. A11-2 and *Bacillus subtilis* WU-S2B showed the highest desulfurizing activity at the temperature of 40 to 50°C.

Ohshiro *et al.* (2005) compared desulfurizing enzymes properties that were purified from moderate thermopilic and mesophilic *B. subtilis* WU-S2B, *R. erythropolis* D-1 and KA2-5-1 and *Paenibacillus* sp. A11-2. They reported that *Paenibacillus* has the highest thermal stability and optimum temperature. In addition, moderate thermophiles have higher thermal stability and optimum temperature compare to those in *Rhodococcus erythropolis*.

Pantoea agglomerans D23W3 showed the ability of DBT desulfurizing via 4S pathway in Bhatia and Sharma (2010) experiment. This strain could remove nearly 93% of DBT at the initial concentration of 0.54 mM after 24h and the complete degradation of DBT was observed after 96h. An interesting characteristic of this strain was that the accumulation of 2-HBP was observed after 120h of cultivation and it can be the advantage of this bacterium that make it valuable compare to other desulfurizing bacteria that produce 2-HBP after 48h of cultivation. Therefore, this characteristic leads to delay the 2-HBP inhibitory roles on DBT degradation. This strain also was able to remove some other organic sulfur such as 4,6-DM-DBT and BT completely.

Another bacterium that has been isolated by Davoodi Dehghani *et al.* (2010) is *R. Erythropolis* SHT87 and it was compared to *R. Erythropolis* IGTS8. This bacterium continued its growth until 120h in the presence of 0.25 mM DBT and showed the ability of desulfurizing DBT via 4S pathway. In addition, the production of 2-HBP, as a final metabolite of 4S pathway, was less than the consumption of DBT. The variety of sulfur compounds that can be utilized by this strain as sulfur sources is similar to that reported for IGTS8 and other strains of *R. Erythropolis*.

Yu *et al.* (2006) compared biodesulfurization rate of *Rhodococcus erythropolis* XP, *Rhodococcus* sp. strain SDUZAWQ and *Mycobacterium goodii* X7B and found desulfurization rate (for analysed control crude oil sample) of 100%, 64% and 87% for these strains respectively.

Another experiment was done by Li *et al.* (2007) that led to know that *M. goodii* X7B could grow better in the medium contain DBT at concentrations less than 0.5 mM. However, the least cell yield of this bacterium was achieved at the DBT concentration of 0.05 mM due to inadequate amount of sulfur source. Li *et al.* (2007) could observe the highest yield of the cell with culturing this bacterium at the DBT concentration of 0.2 mM and they selected this concentration for their future experiment. In addition, it was reported that this bacterium was able to degrade 90% and 75% of 0.5 and 1 mM initial concentration of DBT within 48h respectively. *M. goodii* X7B could not grow at 5 mM concentration of DBT due to the toxicity effect of DBT on the growth of this bacterium. The study that was done for investigation of the effects of 2-HBP concentration on the DBT desulfurization and growth of the cell showed that the desulfurization activity and growth rate of this bacterium was declined by increasing the concentration of 2-HBP. Moreover, this bacterium was able to reduce the sulfur content of crude oil by 59% from 3600 ppm to 1478 ppm after three days (Li *et al.* 2007).

Rhodococcus erythropolis XP was isolated by Yu *et al.* (2006) and was grown on DBT, 4-Methyl-DBT (4-M-DBT), 4,6-dimethyl-DBT (4,6-DM-DBT), benzonaphthothiophene (BNT), and 3-M-BT separately as a sole source of sulfur (the concentration of each sulfur model compound was

0.5 mM) and the following results was achieved.

The presence of aromatic hydroxyl groups was proven by Gibbs assay and therefore this bacterium is able to follow 4S pathway to desulfurize DBT. It was reported that this bacterium is able to desulfurize DBT and its alkyl substituted DBTs in hydrodesulfurized diesel oil by 94.5% reduction of total sulfur. In addition, α-hydroxy-β-phenyl-naphthalene and 2-hydroxybiphenyl (2-HBP) were detected as final products of BNT and DBT metabolisms, respectively. 2-hydroxy-3¹-methyl-biphenyl and 2-hydroxy-3-methyl-biphenyl were detected as metabolites of 4-M-DBT. The end products of 3-M-BT metabolism were detected as 3-M-BT sulfone and 2-isopropenylphenol and in case of 4,6-DMDBT metabolisms 2-hydroxy-3, 3-dimethyl-biphenyl was found as a final metabolite.

Characteristics of some organic sulfur compounds desulfurizing bacteria

Gou *et al.* (2002) examined *Rhodococcus erythropolis* LSSE8-1biodesulfurization of 0.2 mmol/l thianaphthene and phenyl sulphide. The results show that the strain was able to remove 100% and 80% of sulfur from phenyl sulphide and thianaphthene, respectively. In addition, 5 mmol/l 4,6-DMDBT was used as a alkyl dibenzothiophenes to study desulfurization by LSSE8-1 strain. It was shown that this strain was able to remove sulfur from this compound and decrease the concentration of 4,6-DMDBT by 0.3 mmol/l after 4 days.

Ishii *et al*. (2005) showed that *Mycobacterium phlei* WU-0103 was able to degrade a wide variety of heterocyclic sulfur compounds such as naphto[2,1-*b*]thiophene (NTH), benzo[*b*]thiophene (BTH) and dibenzothiophene (DBT) derivatives that exist in diesel oil. At this experiment, this bacterium grew at 50°C and at the presence of 0.54 mM concentration of NTH, DBT and BTH as a source of sulfur separately. This bacterium could remove NTH which is an asymmetric structure of DBT after 5 days completely at 50°C. In that research Ishii *et al.* (2005) could detect 2¹-hydroxynaphthylethene (HNE) and naphto(2,1-*b*)furan (NFU), sulfur free compounds, as final products of NTH and NTH sulfone sulfur degradation. In addition, 2-HBP (2-hydroxybiphenyl) was detected as a metabolite of DBT. Furthermore, BTH sulfone and benzofuran was detected as BTH metabolites. Thus, this bacterium could desulfurize NTH to NFU via NTH sulfone or it can do it through sulfur specific pathway by the final producing of HNE.

It was also reported that *Mycobacterium phlei* WU-0103 is able to degrade NTH in the range of different temperature between 30 and 50°C by the optimal temperature of 45°C. Another good aspect of that research was the investigation of degradation of DBT, BTH and NTH derivatives by the growing cells of *M.phlei* WU-0103. This bacterium was cultured in the mediums that were contained 0.54 mM of each heterocyclic sulfur compounds as source of sulfur and it allowed growing for 4 days in 45°C. In case of DBT derivatives degradation, the most compound that could be desulfurized was DBT sulfone by 90% degradation (the most degradation rate) followed by DBT sulfoxide by 88% degradation.

In addition, the least degradation percentage was for removing just 8.9% of 4,6-Dipentyl DBT sulfone. Ishii *et al.* (2005) also reported that the strain *M.phlei* WU-0103 compared to two other strains *Rhodococcus* sp.WU-K2R and *M.phlei* WU-F1 has higher activity in terms of NTH biodesulfurization.

Mycobacterium phlei GTIS10 was isolated and well characterized by Kayser *et al.* (2002). This bacterium was able to grow in the presence of DBT as a sole source of sulfur in the different temperature at 45 – 65°C. In addition, the most desulfurizing activity of this bacterium was observed at 45-52°C to convert DBT to 2-HBP by considering the fact that the most desulfurizing activity of *R. erythropolis* IGTS8 was at 30°C. It was also observed that this bacterium was able to utilize a wide variety of organic sulfur compound as a sole source of sulfur at high temperature that makes this bacterium valuable in biorefineries process.

Rhodococcus sp. Strain WU-K2R, which was isolated by Kirimura *et al.* (2002), were able to degrade 0.27 mM NTH during a week. In addition, this bacterium did not use NTH and BFU as the sources of carbon. The experience of growing this strain on several sulfur compounds as a sole source of sulfur indicated that *Rhodococcus* sp. strain WU-K2R were not able to use DBT, DBTO2, or 4,6-dimethyl-DBT as a sole source of sulfur. The desulfurizing activity of *Rhodococcus* sp. strain WU-K2R was much higher than the desulfurizing

activity of *M. phlei* WU-F1 since the degradation rate of desulfurizing 0.27 mM NTH were 80% and 39% after 5 days by *Rhodococcus* sp. strain WU-K2R and *M. phlei* WU-F1, respectively.

Li *et al*. (2005) tested the growth of *Mycobacterium goodii* X7B on different organic sulfur compound. The degradation of 0.5 mM of different organic sulfur such as dimethyl sulfoxide, 4,6-dimethyl-DBT, DBT sulfone, DBT, BTH, 5-methyl-BTH, 2-thiopheneacetic acid, 2-thiophene carboxylic acid, 4-Methyl-DBT propylmercaptan and 3,3¹-thiodipropionic acid by this strain at 45°C showed the following results.

For the first eighth sulfur compounds, this strain could use them as a sulfur sources and grew after 24h and it grew well on the ninth sulfur compound after 48h. For the last sulfur compounds, X7B could use it as a sole source of sulfur and grew well after 72h incubation. In addition, Li *et al*. (2005) approved the degradation of these compounds by this strain.

Kitauchi *et al.* (2005) proved that strains *Pseudomonas stutzeri*, *Rhizobium* sp., *Shingomo-nas* sp., and *Xanthobacter polyaromaticivorans* strain 127W were able to degrade 50 mg/l DBT completely by via Kodama pathway in three days under aerobic conditions after 3 days.

In another experiment that was done by DS *et al.* (2009) the degradation of DBT and BT by *Desulfobacterium anilin* was measured. It was shown that this bacterium was able to remove 82% and 81% of DBT and BT of this diesel compounds after three days (the examined diesel was contain 9.006 mg/L of benzothiophene and 157.031 mg/L of dibenzothiophene).

CONCLUSION

Many studies (Mohebali and Ball, 2008; Ohshiro *et al.*, 2005; Borgne and Quintero, 2003; Matsui *et al.*, 2001; Monticello, 1998; Bell *et al.*, 1998) have been done to improve the efficiency of biodesulfurization. The mixture of microorganisms may be required for running of biodesulfurization in the large scales and commercially purposes (Marcelis, 2002). Regarding this issue, researchers are attempting to identify and isolate the genes, which are involved in desulfurization pathways, followed by cloning them to raise the biological potential of them to remove sulfur compounds.

The metabolic and genetic engineering strategies aim to take advantages of the desirable properties of strains without any desulfurizing activities. In fact, they are recruited to produce desulfurizing biocatalysts that are made in isolated bacteria with low efficiency desulfurizing activities due to their properties. In this manner, the desulfurizing efficiency of isolated enzymes can be improved by cloning their genes in other suitable host strains.

On the other hands, the effects of host strains properties such as physical properties, growth properties, or higher basic metabolic rate on the expression of desulfurizing genes that are introduced from isolated bacteria are not completely known and more researches should be done to investigate them. Therefore, understanding the factors and properties that can be effective on the rate of biodesulfurizing pathways and increasing the level of desulfurizing enzymes gene expression can be achieved by isolation and identification of new strains of bacteria and identification of their biocatalysts (Kilbane, 2006).

Increasing the number of desulfurizing genes copies, manipulation of desulfurizing genes to raise the products with more activity and efficiency and increasing the rate and amount of gene expression can help researchers to improve biodesulfurization efficiency (Hirasawa *et al.*, 2001; Matsui *et al.*, 2001a; Kertesz and Wietek 2001; Li *et al.*, 1996). Therefore, the key factors to enhance biodesulfurization efficiency are increasing the specific desulfurization activities, desulfurization at extremely hard condition such as high pressure and temperature and isolation of new bacterial strain with noticeable desulfurizing activities to remove a wide variety of sulfur compounds that are present in fossil fuels.

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