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REVIEW

Microbial Petroleum Desulfurization

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The increasing use of fossil fuels is polluting our environment at a constantly increasing rate. Besides carbon dioxide, a green house effect gas, the use of petroleum and coal generate huge quantities of sulfur oxides, which play a major role in the formation of acid depositions. Environmental regulations in every country around the world are becoming more stringent in order to reduce the sulfur oxides emissions. To meet there more stringent regulations high quality crude oil with low sulfur content can be used, but the availability of low sulfur crude oil is low. Another alternative approach is to remove sulfur from the fuel.

The sulfur content of crude oil ranges up to 5% (Table 1) and about 200 organic sulfur compounds have been identified in crude oil. They include sulfides, thiols and thiophenes (Fig. 1). Light fractions boiling below 200°C contain mainly sulfides and thiols. These can easily be removed by chemical methods. Thiophenes remaining in the heavier fractions are resistant to chemical processes. Derivatives of benzothiophene (BT) and dibenzothiophene (DBT) are the major sulfur compounds in certain types of crude oil. Thiophenes comprise up to 70% of the sulfur compounds present in Texas gas oil and up to 60% of the sulfur compounds in Middle East gas oil, respectively. These sulfur compounds end up in heavy petroleum products such as diesel and bunker-C oil. Thiophenes are also the major sulfur compounds found in gasoline produced by cracking heavy oil.

Petroleum desulfurization processes currently in use are inadequate at removing thiophene based sulfurs, which require special catalysts, and extremely high temperature and pressure, making the processes very expensive. In addition, the quality of the petroleum products produced is reduced by the process known as 'deep desulfurization'. Microbial processes are therfore being studied actively as potential alternatives to the 'deep desulfurization process'.

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MICROBIAL METABOLISMS OF ORGANIC SULFUR COMPOUNDS

Organic sulfur compounds are synthesized biologically and abiologically in nature. Various microorganisms metabolize these sulfur compounds as their sulfur, carbon, energy sources or electron acceptor (Table 2).

Microorganisms Utilizing Thiols and Sulfides

A variety of alkyl sulfides are present in the environment (79). Methanethiol (MSH), dimethylsulfide (DMS), dimethyl disulfide (DMDS) are produced by marine plants. Higher alkyl sulfides are present in animal manure and are found in sewage sludge treatment plants. They are degraded in aerobic conditions as well as in anaerobic environments.

Various aerobic organotrophs and some lithotrophs use methylated sulfur compounds such as MSH, DMS, DMDS, dimethyl sulfoxide (DMSO) and dimethyl sulfone ($DMSO_2$) as their energy and sulfur sources. They include *Pseudomonas acidovorans* (84), a methylotrophic bacterium (29), *Hyphomicrobium* sp. (8) and *Thiobacillus* sp. (69). The methylated sulfur compounds are also metabolized by denitrifiers (79), sulfate-reducers (73), methanogens (17), fermentative halophiles (30) and anaerobic phototrophic bacteria (77).

DMS is oxidized by DMS monooxygenase to MSH and formaldehyde, and MSH oxidase oxidizes MSH in *Hyphomicrobium* sp. (8) and *Thiobacillus* sp. (13) (Fig. 2a). A facultative anaerobe, *Thiobacillus* sp. ASN-1 was thought to use C1 carrier to metabolize DMS in a similar pathway in the absence of molecular oxygen (78). This facultative anaerobe can use oxygen and nitrate as the electron acceptors to oxidize DMS. DMS is used as a sulfur source by *Rhodococcus* sp. SY-1 (58) and *Thiocystis* (83). These bacteria convert DMS to methane, methanol and sulfate through a pathwsy involving DMSO, $DMSO_2$ and methanesulfonate (Fig. 2b).

A methanogenic mixed culture reduces dibenzyldisulfide to toluene (51). The mechanism of the reductive reaction in the culture is not known. But the reduction was not inhibited by sodium sulfide or by 2-bromoethane.

These aerobic and anaerobic bacteria have not been tested either for their abilities to metabolize thiols and sulfides of higher molecular weight, which are found in petroleum or for their ability to remove other sulfur compounds in petroleum. Most of these bacteria are found in biofilters used to remove gaseous sulfur compounds (3).

Microorganisms Utilizing Thiophenes

DBT has been used as a model compound for recalcitrant aromatic sulfur compounds in fossil fuel. Pseudomonads utilizing aromatic compounds have been known to oxidize DBT for some time (52). Since a DBT oxidizing bacterium, *Rhodococcus rhodochrous* IGTS8 was isolated (31) using a medium with DBT as a sole sulfur source, many DBT utilizing aerobes were enriched and isolated. They include *Brevibacterium* sp. DO (74), *Rhodococcus erythropolis* (24, 59, 59a, 61, 80), and Corynebacterium sp. SY-1 (57).

Mixed cultures of anaerobic bacteria consisting of *Desulfovibrio* sp., *Bacillus* sp., and *Clostridium* sp. (15,

Table 1. Organic sulfur contents of some crude oils.

Source	% sulfur
Kuwait	2.6
Venezuela	1.7
Mississippi	1.6
Middle East	1.5 – 3
California	1
Canada	0.44
East Texas	0.26
North Africa	0.18
Far East	0.1
West Texas	0.05 – 5

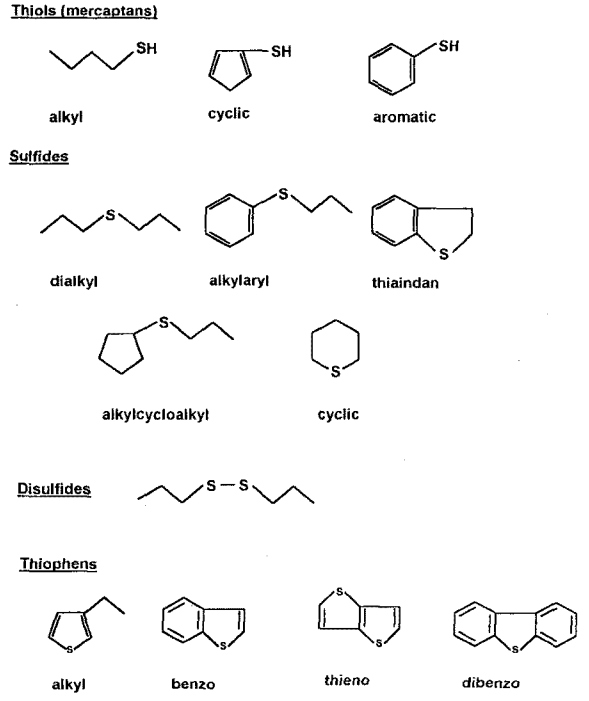

Fig. 1. Major organic sulfur compounds found in the petroleum.

Table 2. Organic sulfur compounds metabolizable by microorganisms.

Strains	Sulfur compounds metabolized	References
Pseudomonas sp.	DBT	11, 23, 43, 44, 52, 54, 64
Beijerinckia sp.	DBT	47
Rhizobium	DBT	50
Acinetobacter	DBT	50
Hyphomicrobium sp.	MSH, DMS, DMDS, DMSO, $DMSO_2$	8
Sulfololobus acidocaldarius	DBT, thianthrene thioxanthene	26, 27
Thiocystis sp.	DMSO, $DMSO_2$	13, 83
Thiobacillus sp.	DMS	69, 78
Brevibacterium sp. DO	DBT	73
Cunninghamella elegans	DBT	6
Pseudomonas acidovorans	MSH, DMS, DMDS, DMSO, $DMSO_2$	84
Rhodococcus rhodochrous IGTS8	DBT, sulfides, sulfones, mercaptans, sulfoxides	28, 32
Rhodococcus erythropolis	DBT	24, 59, 60, 61, 80
Corynebacterium sp. SY-1*	DBT, DMS, DMSO, DBSO, sulfonates, CS_2	57, 58
Desulfovibrio desulfuricans M6	DBT, BDS, BS, BT, thiols, crude oils	33, 39
Desulfotomaculum orientis	DBT	49
D. desulfuricans	DBT	49
Thermodesulfobacterium commune	DBT	49

* reidentified as *Rhodococcus*.

BDS, benzyldisulfide; BS, benzylsulfide; DBSO, dibenzylsulfoxide; DBT, dibenzothiophene; DMDS, dimethyl disulfide; DMS, dimethyl sulfide; DMSO, dimethyl sulfoxide; $DMSO_2$, dimethyl sulfone; MSH, methanethiol.

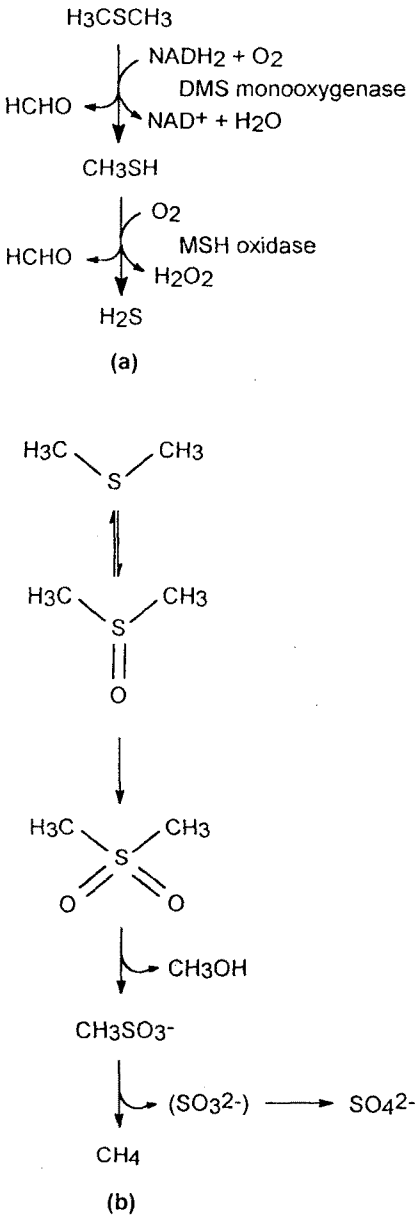

Fig. 2. Dimethylsulfide (DMS) degradation pathway in thiobacilli (13) and hyphomicrobia (8) (a) and in *Rhodococcus* sp. SY1 (58) (b).
DMS is metabolized through similar pathway to (a) in an anaerobic thiobacilli, where methyl group is transferred to a C1 carrier for the oxidation in the absence of molecular oxygen.

45, 46), *Desulfovibrio desulfuricans* M6 (33, 37-40), and *Desulfotomaculum orientis, Desulfovibrio desulfuricans* and *Thermodesulfobacterium commune* (49) have been reported to degrade DBT. Lizama *et al.* (49) defined DBT as an electron acceptor.

DBT is metabolized through different pathways depending on the organisms (Fig. 3). *R. rhodochrous* employs

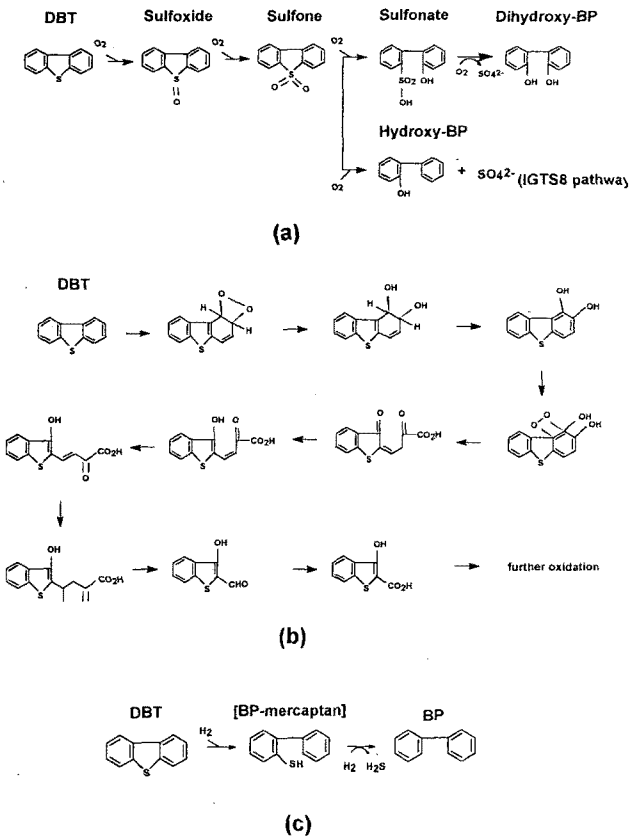

Fig. 3. Propsed pathway of DBT degradation by oxidation (a, b) and reduction (c).
(a) oxidative C-C bond cleavage (Kodama) pathway in *Pseudomonas* sp (43), (b) oxidative 4S pathway in *Rhodococcus rhodochrous* (19), and (c) reductive pathway in *Desulfovibrio desulfuricans* (37).

the oxidative 4S pathway where C-S bonds of DBT are cleaved by an oxygenase, finally yielding hydroxybiphenyl and sulfate (19, 31, 32, 57, 74). 4S refers to the reaction intermediates of this pathway; sulfoxide, sulfone, sulfonate and sulfate. Pseudomonads use oxygenases to oxidize the aromatic rings of DBT to water soluble 3-hydroxy-2-formyl-benzothiophene through the oxidative C-C bond cleavage pathway (Kodama pathway). In this pathway sulfur is not mineralized to inorganic forms (6, 23, 27, 50, 52, 54, 64). Some bacteria degrade DBT through the mixed pathway in which C-C and C-S bonds are cleaved by oxygenase (43, 44, 47, 74). *D. desulfuricans* has the reductive pathway in which the C-S bond of DBT is cleaved by hydrogen and finally reduced to produce biphenyl and hydrogen sulfide (38).

DBT metabolized as a sulfur source. Bacteria isolated based on their abilities to use DBT as their sulfur source oxidize DBT through the 4S pathway (Fig. 3). They belong to the genus *Rhodococcus* (Izumi, personal communication) although initially they were identified differently. These bacteria oxidize DBT and other organic

sulfur compounds to sulfite which is further oxidized to sulfate chemically. Some of them cannot oxidize the carbon part of DBT, and convert it to 2-hydroxybiphenyl (24, 31, 32, 80), or 2,2'-dihydroxybiphenyl (19, 56). On the other hand, *Brevibacterium* sp. DO (74) oxidize the carbon part of DBT complete to CO_2 via benzoate. The latter organism possesses the ability to oxidize the aromatic ring in addition to 4S pathway. Most of the DBT oxidizing aerobes are able to metabolize thiols and sulfides (28, 58) as their sulfur sources.

Reduced pyridine nucleotide (NADH) or flavins are required for the oxidation of DBT (59, 61). These suggest that the oxidation is catalyzed by a monooxygenase. Recently, 3 genes possibly related to the DBT desulfurization were identified and sequenced from *R. rhodochrous* IGTS8 (10, 11, 62). The genes showed no significant level of homology with known genes including oxygenases. These genes are plasmid-borne and expressed as an operon with a control by sulfur repression.

Oxidation to water soluble organic sulfur compounds. Many bacteria are able to oxidize complex aromatic sulfur compounds with the formation of water-soluble sulfur containing products via the Kodama pathway. Some bacteria such as *Pseudomonas* sp. (23, 52, 54, 64), *Rhizobium* sp., *Acinetobacter* sp. (50), and *Sulfololobus acidocaldarius* (27), and a fungus *Cunninghamella elegans* (6) are able to oxidize only the peripheral aromatic ring of DBT since formyl-BT or hydro-BT remain as dead-end metabolites in the culture fluid. DBT can also be used as a sulfur source via pathways similar to the 4S pathway by other bacteria such as *P. jianii, P. abikonensis* (43, 44), *Beijerinckia* sp. (47), and *Brevibacterium* sp. DO (74). These processes might be of practical value given that soluble organic sulfur-containing compounds can be separated from oil. Oxidation of DBT to these compounds is analogous to the initial steps of naphthalene degradation.

Denome *et al.* (11) has identified and sequenced oxidation (*dox*) genes from *Pseudomonas* sp. C18. The genes were found to encode the complete DNA sequence of the upper naphthalene catabolic pathway that is responsible for the conversion of naphthalene to salicylate.

Anaerobic metabolism of DBT. After Zobell (85) showed that an anaerobic sludge containing sulfate-reducing bacteria with strong hydrogenase activities could reduce organic sulfur to hydrogen sulfide, reductive desulfurization of fossil fuel was also studied. Kurita *et al.* (46) enriched cultures containing Gram-negative anaerobes which produce hydrogen sulfide from organic sulfur compounds in a medium containing lactate in an N_2 or H_2 atmosphere, and reported that methyl viologen as an electron mediator was essential for the desulfurization. Desulfurization was monitored by measuring H_2S but there was no documentation on the desulfurized end product.

Köhler *et al.* (45) reported that mixed cultures containing sulfate-reducing bacteria removed sulfur from a variety of model compounds, including DBT, BT, DBS, DBDS and petroleum preparations. Eckart *et al.* (15) used a mixed culture consisting of *Desulfovibrio* sp., *Bacillus* sp., and *Clostridium* sp. in a study of microbial petroleum desulfurization.

Microbial desulfurization utilizing anaerobic bacteria is a reductive process. Kim *et al.* (37) reported that a sulfate-reducing bacterium *D. desulfuricans* M6 could reduce DBT to biphenyl and hydrogen sulfide in a hydrogen atmosphere as shown in Fig 3. They also investigated the possibility of providing reducing equivalents electrochemically rather than through hydrogen gas and microbial desulfurization by the sulfate-reducing bacterium, and suggested that the electrochemically generated reducing equivalents can be incorporated into the normal electron metabolism of the anaerobic bacterium through the mediation of methyl viologen (40).

The enzymes responsible for the reductive degradation of DBT are not yet known. As stated in the earlier studies, hydrogenase activity is closely related to desulfurization activity. Hydrogenase oxidizes hydrogen to reduce cytochrome c_3, a low redox potential ferrocytochrome which plays a key role in the energy transduction process of *Desulfovibrio* (20, 22). BT, but not DBT was degraded by reduced cytochrome c_3 of *D. desulfuricans* M6 (Park and Kim, unpublished data). The calculation of the free energy change showed that the reduction of DBT by cytochrome c_3 is an exergonic reaction (Park and Kim, unpublished data). Lizama *et al.* (49) showed that DBT is used as an electron acceptor by various sulfate-reducing bacteria such as *Desulfotomaculum orientis, Desulfovibrio desulfuricans* and *Thermodesulfobacterium commune.*

Other hemoproteins are reported to degrade thiophenes. DBT was oxidized by hemoglobin, cytochrome *c*, and myoglobin in the presence of hydrogen peroxide as an oxidizing agent (42, 81). The hemoproteins have been reported to be able to readily oxidize DBT to its S-oxide and S-dioxide. Duhalt *et al.* (14) showed that cytochrome c from horse heart is also able to catalyze the oxidation of some heterocyclic sulfur compounds and sulfide. It would be of interest to investigate if oxidation and reduction of sulfur compounds are general properties of hemoproteins.

Comparisons of DBT degradation activity. Due to the diversity of sulfur compounds in petroleum it is neccessary to use a simple model compound in desulfurization experiments. DBT is the most widely used model compound. DBT degradation activities of various microorganisms are compared in Table 3. Since the desulfurization activities of these organisms were measured under different conditions, the activities cannot be

Table 3. Microbial degradation of DBT.

Organism used	Reaction conditions			Overall degradation (%)	Reference
	DBT Conc. (mM)	Temp. (°C)	Time (Days)		
S. acidocadarius	0.50	70	15	85	26, 27
	1.10	70	15	78	
	1.63	70	15	70	
	2.70	70	15	30	
Rhodococcus rhodochrous IGTS8	0.11	30	8.3	100	28
Rhodococcus erythropolis D-1	0.13	30	2	100	24
	2.20	30	0.1	100	59, 61
Desulfovibrio desulfuricans M6	5.40	35	6	42	39
Desulfovibrio thermophilus KCTC2482	5.40	60	6	68	39

compared directly. From the table, however, it can be said that *R. rhodochrous* D-1, *D. desulfuricans* M6 and *D. thermophilus* KCTC 2482 are better candidates for process application than others.

PROCESS DEVELOPMENT

The development of economically feasible biotechnological processes depends on biological factors as well as physico-chemical issues such as mass transfer, temperature, pH, *etc.* The cost of a biocatalyst be the most important factor in an economic evaluation of the biological desulfurization of petroleum using a genetically modified *Pseudomonas alcaligenes* (21). It should also be noted that many envisioned bioprocesses may be limited in their commercialization by traditional engineering processing constraints rather than by biological constraints.

Biocatalysts

Important characteristics of the biocatalyst include specific activity, productivity or yield during fermentation, and stability. Specific activity of the biocatalyst is a critical factor for the development of a practical biological desulfurization process (52). As shown in the previous sections (Table 3), many bacteria, either aerobic or anaerobic, have been shown to remove sulfur atoms of the heterocyclic compounds in the form of sulfate or hydrogen sulfide, respectively. Among these, bacteria such as *D. desulfuricans* M6, *D. thermophilus, R. erythropolis* D-1, *R. rhodochrous* IGTS8 showed very high activities (Table 3). The specific activities of these bacteria can be further improved through either genetic or culture condition modifications.

Although pure cultures of these bacteria showed efficient degradation of DBT, some of them were not successful in desulfurization of petroleum (48). This seems due to the fact that these bacteria were enriched and isolated for their abilities to degrade DBT and that petroleum contains a wide spectrum of organic sulfur compounds. As sulfur compounds in petroleum are present in many different forms, the enzyme specificity for a substrate of the bacteria limits their application in petroleum desulfurization. For this reason a biological desulfurization process is being developed as an alternative to deep desulfurization processes. Mixed bacterial cultures can be considered as candidates to improve the reactivity of the biocatalyst (15, 45, 48).

Productivity of the biocatalyst determines the manufacturing cost of the biocatalyst in terms of capital investment as well as running cost. The stability or the life span of the biocatalyst contributes to the processing cost. For economic desulfurization, repeated uses of a biocatalyst are desirable. According to Hartdegan *et al.* (21), the improvement in these two factors along with shorter residence time of petroleum in the reactor could greatly reduce the cost of biocatalyst. But available information regarding the productivity and stability of the biocatalyst is very scarce (63).

Factors Affecting the Process

In aerobic desulfurization processes using the *Rhodococcus* species, organic sulfur compounds are oxidized by a monooxygenase, which requires reduced pyridine nucleotides or flavins and molecular oxygen as substrates (61). Consequently, these electron carriers have to be continuously reduced by supplying electron donors such as the growth substrate. A recent bacterial isolate, *Rhodococcus erythropolis* H-2 is known to use aliphatic hydrocarbon as its carbon and energy source, and to use DBT as its sole sulfur source (60). An adequate supply of oxygen is also required in this process. Reductive desulfurization is possible by either growing or resting cells (37, 46). In the presence of hydrogen, resting cells reduced sulfur atom in the organic compounds to hydrogen sulfide.

Engineering Considerations

Biological desulfurization of petroleum is a multiphase reaction. It comprises an aqueous phase with a biocatalyst, an organic oil phase with sulfur compounds, and a gaseous oxygen or hydrogen phase. For desulfurization, sulfur compounds in the organic phase should be available to the biocatalyst in the aqueous phase. Hydrocarbons and other hydrophobic compounds are known to affect the mass transfer of the sulfur compounds between the different phases. Hexadecanoic acid increased the degradation of DBT at high DBT concentrations, and inhibited it at low concentrations. The alkanoic acid affects DBT degradation as a carrier or barrier by forming micelles with the sulfur compound (66). This is an example which shows the importance of physico-chemical factors such as mass transfer between each phase governing the desulfurization efficiency along with the activity of the biocatalyst. Addition of *n*-alkanes enhanced the biodegradation of DBT or sulfur compounds in heavy oil by *Pseudomonas* sp. through the action of solubilization and emulsification (67). *n*-alkane which could be used as a carbon source surrounds the bacteria with a higher concentration of organic sulfur compounds than that of the medium and increases the availability of sulfur compounds to the bacteria. But the presence of *n*-alkane in the growth medium also inhibits BT degradation by growing anaerobic bacteria such as *D. desulfuricans* since it prevented the uptake of carbon source by the bacterium (68).

The availabilitiy of hydrogen or oxygen to the biocatalyst in the processes are other examples of mass transfer limitations. Under standard conditions, the solubility of hydrogen in distilled water is 0.8 mM. To avoid hydrogen limitations, Kim *et al.* (40) suggested an electrochemical desulfurization process where *D. desulfuricans* M6 reduced DBT or organic sulfur compounds in oil to hydrogen sulfide using electrons directly supplied from the electrode through an electron mediator, methyl viologen.

Biodesulfurization in non-aqueous media was also studied to overcome the mass transfer limitation (16). Desulfurization of DBT by bacteria adapted to an organic solvent showed a sigmoidal mode to cell concentration which implies that diffusion of DBT is not a rate-limiting step in the desulfurization process.

Biological Desulfurization Processes

Various processes for biological petroleum desulfurization have been suggested, but all of these were conceptual. Zobell (85) described a batch process using a reactor resembling a storage tank supplying hydrogen from the bottom of the tank. Anaerobic bacteria in the lower aqueous phase reduce the sulfur complex dissolved from the upper oil phase to hydrogen sulfide. For a continuous process, a contact tower-type reactor with plates and bubble caps was suggested. Processing oil enters the reactor from the bottom and exits from the top, while bacteria are supplied from the top. Hydrogen bubbles up from the bottom.

Hartdegen *et al.* (21) suggested a reactor in which the oil phase was kept separate from the aqueous phase. In this configuration, immobilized whole cells were sandwiched between two streams and separated from each by a permeable membrane. With this reactor, two effluent streams are obtained without separation.

Monticello and Kilbane (53) described a conceptual design for batch and continuous desulfurization processes, which has been further developed to a pilot scale test. In this design, the bacteria are grown separately and processed prior to interacting with the oil. In the batch process, the biocatalyst is combined with the oil in the bioreactor. The processed oil is separated from the aqueous phase containing the biocatalyst, which is reused. Yasui (82) also proposed a similar batch reactor system for anaerobic desulfurization. For a continuous process, the immobilized biocatalyst is used in a fixed bed or fluidized bed mode.

A commercial BDS unit is known to be operating as of 1996 (Hydrocarbon Processing, May 1995, 19-20), but much informatio remains as trade secrets.

By-products/waste Treatment

Sulfur atoms of organic sulfur compounds in petroleum are converted to either sulfate or sulfide during the biological desulfurization process. Sulfide can be processed by existing processes such as those employed in the HDS processes. Sulfate can be neutralized to ammonium or calcium salts. Alternatively dissolved sulfate can be reduced to sulfide by sulfate reducing bacteria (7, 12, 65, 75) and elemental sulfur can be recovered from sulfide either biologically, or physico-chemically. Biological recovery of sulfur from sulfide can be conducted by the phototrophic (5, 34-36) or chemoautotrophic bacteria (1, 2, 18, 70-72).

PROSPECTS

Increasingly stringent sulfur emission standards around the world compel refiners to make lower-sulfur products. Sulfur management will be a critical concern of the petroleum processing industry for the next 20 years. And it may determine the fate of many small refiners in the next ten years. Industry experts estimate that refiners will spend over $25 billions for new desulfurization capacity and an additional $6 billions in annual operating expenses between now and the year 2000 to produce the required low-sulfur products. Much of this capacity will be required, regardless of the implementation of new regulations, as a consequence of lower availability of low-sulfur crude oil. This trend is expected to continue for the next several years. Demand for new, low-cost desulfurization technologies has led to

renewed interest in biological desulfurization since bioprocessing offers the potential for a low-temperature and low-pressure desulfurization process which reduces both capital and operating costs (ASM News, 1993, vol. 59, 387-388).

Technical Feasibility

In BDS, the specificity and activity of the biocatalyst and the oil/water volume ratio seem to be the technical bottlenecks. Aerobic microorganisms degrade a wide variety of sulfur compounds in petroleum. There is evidence that anaerobic bacteria can remove sulfur attached directly to aromatic rings, but aliphatic organic sulfurs are resistant to anaerobic bacterial reduction (33). Aliphatic sulfur compounds are removed easily through existing HDS processes. This means that BDS is an alternative for deep desulfurization processes (53). Another limit for the industrial application of BDS is the critical ratio of the organic and aqueous phases which affects the sizes of the reactors and the storage tanks. At present, this ratio is not over 1/1(V/V).

One way to overcome some of these difficulties would be the breeding of improved biocatalysts through genetic manipulation, which identifies the specific genes for the desulfurization steps and clones them into a suitable host. Recently, Denome *et al.* (9) and Piddington *et al.* (62) have cloned the genes for DBT desulfurization in *R. rhodochrous* IGTS8 and shown that increased specific activity for desulfurization of DBT can be obtained by increasing the copy number of those genes and that sulfur repression can be alleviated by promoter replacement. Similar work is in progress using *D. desulfuricans* M6 in the authors' lab.

Studies on a non-aqueous biocatalytic system for selective BDS using microorganisms or enzymes in complex organic solvents, such as fuels, may be interesting. Immobilization of enzymes or microorganisms may overcome the limitation of the ratio between the organic/aqueous phases and the cost of the biocatalysts. In addition, the search should continue for new microorganisms with different substrate specificity for efficient desulfurization of other organic sulfur compounds.

Economic Feasibility

From a cost perspective, BDS has favorable features:

1) operation at low temperature and pressure,

2) cost-effective, For BDS, the capital and the operating costs are 50% and 10% to 15% less than HDS, respectively.

3) flexible nature. The process can be applied to many process streams: crude feeds, FCC gasoline, and middle distillates. In addition, there is the potential to apply BDS to heavy oils which are not generally treated in the refinery (personal communication to Monticello, 1995).

Fields of Development

Improvements in some areas will increase the economic feasibility of BDS. These include improvements in the biocatalyst in terms of higher specific activity, stability for extended use, and increased productivity in the fermentation process for biocatalyst production. This development is necessary to produce enough materials economically for the petroleum refining industry.

Another important area of development in a BDS process is resolution of the bioreactor/process engineering issues. This includes the development of new bioreactor designs, mixing technologies, separation technologies and byproduct disposition strategies. These will enable engineers to create reactor designs that maximize the petroleum desulfurization rate while preserving biocatalyst desulfurization capacity. Fortunately, there is a wealth of information on this subject. Two-phase bioreactors have been studied for several years (76). Other issues include separating the biocatalyst from the oil after desulfurization and disposing of sulfur byproducts. Biotechnology and process engineering issues related to this development effort are common to many scale up efforts.

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