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The Role of Microorganisms and Productions in Biodesulfurization of Fossiel Fuels

Jamshid Raheb*

Department of Molecular Medicine, National Institute of Genetic Engineering and Biotechnology, Iran

Abstract

Desulfurization of fossil fuels demands a high technology as the crude oil is very complex mixture of molecules with a largest hydrocarbons form in composition.

Hydrogenic method for desulfurization, takes higher cost and energy and also sulfur is not separated completely from heterocyclic poly-aromatic compounds. Researchers are focused on the use of microbial desulfurization [Biodesulfurization] where sulfur removal reaction acts under mild condition and with lower costs by bacteria. The method could be fixed the flaws in the hydrogenic method through microorganisms and their production role in desulfurization and in fact replace, full or partial of the hydrogenic method as a supplement.

Keywords: Microbial desulfurization; Hydrogenic desulfurization method; Dibenzothiophene; Fossiel fuels

Introduction

Small amounts of sulfur compounds, nitrogen and oxygen exist in crude oil composition. After hydrogen and carbon, sulfur is its highest component and the amount of this element in conventional crude oil is from 1000 to 3000 ppm [1]. The results of studying crude oil derivative show that amount of sulfur in crude oil is directly associated with the boiling temperature of this derivatives, so that the light crude oil contains less sulfur content than the heavy crude [2]. Percentage of crude oil compound based on the constituent elements.

There are two main categories of sulfur compounds in the crude oil. One category contains mineral sulfur compounds such as hydrogen sulfide and aliphatic sulfur compounds with low molecular weight such as methyl mercaptan and dimethyl sulfide, which can be removed simply by physical and chemical methods. The second category contains organic sulfur compounds with low molecular weight such as cyclic alkyl, aryl alkyl thioether and thiophene based aromatic heterocyclanes. This group included thiophene, benzothiophene, Dibenzothiophene and their alkalized derivatives that are resistant to conventional chemical methods for the removal of sulfur compounds, and are appropriate substrate for microbial desulfurization of oil compounds [3].

Among the blend stocks, the light cycle oil LCO from the catalytic cracking of the liquid includes the highest amounts of aromatic sulfur. LCO also contains the maximum amount of resistant sulfur compounds, especially 4-methyl Dibenzothiophene and 4,6-dimethyl Dibenzothiophene [4]. There are various 2- and 3-ring sulfur compounds in the Middle distillates of various refining processes that can be used for the production of middle distillate of diesel and jet fuels.

Microbial communities and productions in biodesulfurization process

The most known microorganisms among bacteria on which many studies have been conducted is the bacteria *R. erythropolis* which bacteria uses four genes of dsz [A, B, C, D] from 4S pathway for a special and high activity desulfurization. However these bacteria have series of disadvantages [5-17].The operational cost is significantly reduced by this method and it does not require above ground microbial culture incubation [18]. During the long flooding process the indigenous microbial community is shaped and it involves various microbes with functions that enhance oil recovery. Various dynamic changes have been observed during all exploration periods in the community [19]. Oil reservoir geological conditions or external factors [nutrient injection, water flooding] affect the structure of the microbial community. For example, Lin et al. [20] examined microbial communities in oil reservoirs that were developed by water flooding. The increase of formation temperature remarkably changed the structure of microbial community [21]. As the temperature increased, the species were involved in water formation. Salinity is another factor influencing microbial community structure. It ws found that *in situ* nutrient injection affected MEOR and microbial diversity. The microbial diversity was higher in low salinity level oil reservoir than high salinity level oil reservoir. After nutrient injection, some advantaged microbes, such as Pseudomonas, Ochrobactrum, Alcaligenes and some methane producing archaea, increased significantly [22-24]. Biosurfactant production improves the hydrophobic properties of the cell surface and enhances the hydrocarbons' metabolism. Researchers reported that the sulfur content of crude oil was reduced by 26.38%–71.42% by *P. agglomerans* D23W3 desulfurization [25]. Oleibacter is a hydrocarbon-degrading bacterial species that presents high n-alkane-degrading activity [26,27]. Pseudomonas species including the typical species *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Pseudomonas stutzeri* widely exist in oil reservoirs. These organisms have several crude oil exploitation and the petroleum industry related functions, such as emulsion, rhamnolipid metabolism, hydrocarbon degradation and representation in oil recovery and environmental contamination fields [28,29]. Lenchi et al. reported significant differences between the microbial compositions in the injection and production waters because most of the bacteria in the injection water were not retrieved in the

***Corresponding author:** Jamshid Raheb, Department of Molecular Medicine, National Institute of Genetic Engineering and Biotechnology, Iran, Tel: 21 44787387; Fax: 21 44787399; Email: jamshid@nigeb.ac.ir

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production water [30]. So, the surface water introduction could improve the microbial diversity of the formation water but fails to change the major bacterial community structure or the bacterial composition in the reservoir environment. In other words, as foreign species invaded the oil reservoir, they adapted to the reservoir environment. However, the extreme environment condition [such as high salinity, relatively high temperature and high pressure] declined the growth of these foreign species and selected the well survived species, which results in a relatively stable indigenous bacterial ecosystem. Temperature mineralization, ionic type and hydrocarbon content affected the microbial community structure. Many researchers have reported the effect of temperature. Zhang et al. reported that the microorganisms' effect in the injected water on microbial community diversity in the production water was reduced by increasing temperature [21]. Wang et al. showed that low temperature reservoirs grouped together in the PCoA analysis, but high temperature petroleum reservoirs did not group together due to large differences in mineralization and the Cl concentration [31]. The salinity distribution range affects the phylogenetic diversity of the bacterial community, even in the same area. As the range becomes larger, there are more genetic differences among the production water. Previous studies also illustrated that the microbial diversity of hypersaline environment was low and it was reduced as the salinity increased [28]. Wang et al. analyzed the relationship between salinity [ranging from 0.2 mg/L to 280 mg/L] and microbial community diversity and indicated that the bacterial community of Tibetan lakes with higher salinity had higher diversity than the communities of freshwater lakes, whereas the phylogenetic diversity in the hypersaline lake was lower than the freshwater lake [32].

Biodesulfurization capabilities of a mixed culture

The increasing global human population and extensive fossil energy consumption have caused serious threats to the environment and human health [33]. Organosulfur [thiophenes] compounds found in crude oil and diesel have particular importance due to their hazardous impact on the ecosystem and human health [34]. Most studies conducted on microbial desulfurization have adopted axenic cultures of selected microorganisms. However, it is important to invetigate biodesulfurization capabilities of microbial consortia to enjoy the cooperative or synergistic microbe-microbe interactions [35,36]. Engineered synthetic bacterial consortia have shown enhanced desulfurization and revalorization of oil sulfur compounds [37].

The selected enrichment procedure produced a microbial culture that an oolied DBT as sole sulfur source in the presence of glucose as a carbon source. Consistent with these results, many authors reported the isolation of biodesulfurization-competent microorganisms from hydrocarbons or crude oil contaminated soil Several morphologically distinct colonies' observation on spread plates confirmed that AK6 is a mixed culture [38]. Deployment of mixed cultures and engineered consortia in bio desulfurization research has been reported by some authors limitedly and studied biodesulfurization of heavy oil and model thiophenic compounds by mixed cultures enriched with oil sludge [39,40]. Most recently reported enhanced desulfurization of oil sulfur compounds by engineered synthetic bacterial consortia [37].

AK6 culture growth on several organosulfur compounds in the presence of glucose and HPLC analysis confirmed the AK6's ability to apply those substrates as sulfur sources, except DBS. Moreover, lack of growth on the tested organosulfur compounds in the absence of glucose suggests that AK6 cannot utilize them as a carbon source. The moderate growth observed on DBT [as a carbon and sulfur source] is most likely due to the application of ethanol in which DBT was dissolved. Different bacteria were reported to utilize ethanol as an efficient carbon source for enhanced desulfurization of DBT [41]. The genetic determinants of the 4S pathway have been the target of enormous genetic boosting strategies. Nevertheless, none of the genetically improved axenic cultures has shown biodesulfurization rate proportional with the industrial requirements for the development of a commercial biodesulfurization process [38].

A biodesulfurization-competent strains consortium such as AK6 would be a rich pool of these host factors for more efficient and robust biodesulfurization process. The AK6 mixed culture seems to contain both desulfurizing and non-desulfurizing bacteria adapted to co-metabolize the 4S pathway intermediates. This mosaic nature of AK6 is expected to fit better than axenic monocultures for the development of biotechnological processes targeting desulfurization of a broad range of sulfur compounds present in crude oil and diesel. Furthermore, deployment of mixed cultures may prevent the decay of the biodesulfurization activity resulting from the accumulation of the inhibitory intermediates of the 4S pathway [37,42].In fact, all detected 16S rRNA gene sequences are related to those of bacterial genera that are known as biodesulfurization-competent, hydrocarbon degraders, or inhabitants of hydrocarbons-polluted environments [43,44].

Applying mixed microbial cultures for hydrocarbons' biotransformation and biodegradation is advantageous. This is due to intraspecific and interspecific interactions enabling microbial consortia to out-perform pure cultures [45].The microbial interactions in mixed populations or consortia are not understood well, some types of interactions such as collaborative degradation or transformation of the substrate, removal, or sequestration of toxic intermediates and provision of essential metabolites [46]. Studying biosurfactants effect on desulfurization activity of cells in stationary phase in single-phase and two-phase systems. The results of single-phase system showed that in the presence of bio-surfactant, the ability to remove DBT is 64%. While in the absence of bio-surfactants in the DBT environment, it is about 6%. In the two-phase system both in the presence of bio-surfactants the ability to remove DBT was 37% and in the absence of bio-surfactants in the DBT environment it is of 53% [47-49].

The overall result of the effect of adjuvant [surfactants and immobilizing

It has been proven that surfactant can cause solubility of hydrophobic materials in water. On the other hand, among the diverse biocatalytic conversion methods, biodesulfurization is a reaction taking place in the water/oil two-phase system. Polyethylene glycol, sorbitol mono-oleate [twin 80] is non-ionic surfactants and also an emulsifier of petroleum compounds in the water. Tween 80 Surfactant can increase the biodesulfurization activity in both two-phase and liquid system by decreasing concentration of product around the cell. Twin 80 can also reduce the concentration of hydrophobic substrates associated with cells [50].

In addition, measures were performed on pseudomonas delafieldii R8 bacteria in the calcium alginate grains to improve desulfurization activity in the two-phase system of oil/water. Desulfurization specific rate in grain with diameter of 1.5 mm was higher than those with a diameter of 4 mm. Also a number of surfactants can significantly increase the activity of immobilized cells. Desulphurization rate was increased 1.8 times by adding 0.5% of span 80 compared to non-exposed grains. Therefore, activity of desulfurization was dramatically raised by decreasing grain size of Resting cells and addition of span 80 surfactant which is the result of increasing the mass transfer from the substrate to

the matrix gel. The maximal rate of desulfurization in immobilized cells was lower than that of non-immobilized cells. The main advantages of immobilized cells could be repeated and convenient operations [51]. However, the adsorption process increases the bioavailability of sulphur substrates for bacterial cells; caused the biomodification of inorganic supports improving BDS activity [52].

Improvement and development of biodesulfurization process

Accelerating desulfurization: Removal of sulfur per gram of dry cell weight is consistent with the amount of sulfur required for bacterial growth. Desulfurization rate for non-genetic engineered strains of Rhodococcus was about 1-5 mg of 2HBP production for dry cell weight an hour. In this case, the released amount of 2HBP is about 75-55% of DBT used that is independent of the applied equipment. Desulfurization rate rises by increasing the cell density, which shows that reaction rate-limiting step is higher in the intermediate phase [aqueous-organic]. If aeration is done properly, use of older cultures creates no limit on the amount of DBT consumption. On the other hand, rate of desulfurization for new engineered recombinant strains is rapidly increasing and is promising to use this route for desulfurization compounds of oil and refinery products.

In order to conduct commercial and industrial applications and also for the desulfurization of petroleum products containing a range of sulfur compounds, a mixture of engineered catalysts or biocatalysts with enhanced desulfurization activity may be required [53,54].

Biological conditions: In addition to the genetic engineering, several other conditions should be considered for industrial use of a biocatalyst. First, the already grown cells should be considered as none- grown biocatalysts. This reduces the probability of producing agents that are active in their surface area, in response to cell growth in oil. These agents make it difficult to isolate production Pathways. Biocatalyst cells should not need the presence of expensive induction agents, such as naphthalene or salicylate to achieve maximum activity [53-55].

- In order to have a low-cost biocatalyst production, cells must grow on a low cost carbon source and produce a large amount of active biomass for each substrate.
- Biocatalysts' substrates should be non-toxic. Fortunately inappropriate ring sulfur toxicity is decreased by dissolving in these compounds in oil, as a result, these components have lower toxicity during the desulfurization of oil, compared to when used in pure form.

Some of unresolved metabolic issues

Although reports about the isolation of various DBT decomposing microorganisms and enzymes with different substrate specificity, continues, still several important issues remain to be resolved. The most important thing is how these highly hydrophobic molecules find their way onto the primary enzyme.

Basic DBTs by 7 free carbons and solubility in water at nanomolar scale, is oxidized by DBT monooxygenase enzymes. This problem is proved by a genetic structure that only contains dszC, dszD genes. Cells that contain this structure have a high level of DBT monooxygenase and NADH: FMN oxidoreductase activity. When these cells are incubated by a sample of diesel oil containing Cx-DBTs, the Cx-DBTo2 molecule which has 8 free carbons is produced. These molecules are not soluble in water and cannot be available for the first enzyme in the pathway [56].

The question is that how cells can transfer their products out of the cell. Engineered Rhodococcus can produce a large amount of 2HBP outside the cell. Another question is how the cells cannot analyze this compound. In one strain containing dszB, the 2HBP product produced in this reaction could be produced with a high concentration outside the cell. The answer could be the subject of future research of scholars.

Development of new systems for microbial desulfurization of crude oil

In new studies Desulfurization of a model compound [e.g. DBT], often was carried out in aqueous systems, resulting in little resemblance to conditions of biocatalysts is used in commercial affairs. Recently, two solution phases are used. In aqueous systems in order to increase the rate of desulfurization, DBT was used in the presence of 40 to 50% of normal Tetradecane or kerosene and 50% of diesel or 96% hexadecane [57].

DBT desulfurization in Rhodococcus shows that DBT removal of the oil phase is done in inter-cell form and after transfer of absorbable materials, it occurs into the cell. In the *E. coli* strains and recombinant *Pseudomonas putida*, before DBT absorption by cells, it partially appears in the aqueous phase.

It has been found that emulsions of oil phase and the upper layer contain significant amounts of bacterial particles that form droplets with a diameter of 1 to 10 mm, which accumulate in high concentrations of hexadecane during desulfurization of DBT. This requires the use of bio-surfactants for separation of parts effective in a commercial process.

Result of microbial desulfurization of petroleum compounds was about removal of 30 to 70% of oil sulfur content for middle oil, 40 to 90% for diesel oil, 65 to 70% for diesel oil treated with HDS, 20 to 60% for light gas-oil, 75 to 90% for refined oil and 25 to 60% crude oil. Although this is a significant amount of separation, according to the sulfur content the amount of desulfurization of fuels is not effective. For example, in the case of crude oil a chemical mechanism used for separation of heteroatoms from asphalt and polar fractions lead to reduction of 24 to 40% of sulfur, nitrogen, oxygen and metallic elements together with hydrocarbon fractions.

Future of commercialization of oil microbial desulfurization

Amount of investment in BDS method is about half the amount of HDS [between 40 and 50 million dollars] with about 15% lower functional value. However, HDS removes nitrogen and metals as well. To improve the economic conditions, it is recommended that chemical products produced by BDS process converted to economically valuable products.

2HBP can be converted to Hydroxy phenyl benzene which is a producer for surfactants such as linear benzene sulfinate and sulfune or used for conversion of sulfoxides to hydrotrops, phenolic resins, adhesives and insecticides.

In recent studies, it has been found that the amount of CO_2, greenhouse gas emissions and energy requirements is reduced, while using of BDS instead of HDS. Commercial applications of BDS depend on our knowledge of BDS mechanisms and scientific and engineering technologies associated with it. Correction of specific activity, stability, longevity and new techniques for regeneration of biocatalysts are useful and are leading to increased efficiency. The most important new activities include suitable reactor design, separation [oil-water biocatalysts], production of byproducts and increase of the quality of the product. The most important factors in successful commercial application of this process involve the application of biological processes to be used as a supplement available with chemical processes [58].

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