Journal of Sciences, Islamic Republic of Iran 28(3): 205 - 219 (2017) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Review Article
The Study of Biological Technologies for the Removal of Sulfur Compounds

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Received: 19 November 2016 / Revised: 3 July 2017 / Accepted: 29 July 2017

Abstract

Combustion of sulfur components of fossil fuels such as oil causes the emission of SO_2 in the atmosphere and lead to the formation of acidic rain in the environment. The conventional approach for desulfurization of fossil fuels is the chemical procedure of hydrodesulfurization (HDS). However, this method has low efficiency for desulfurization of ring components of sulfur such as dibenzothiophene (DBT) that include a significant percentage of the total sulfur content of fossil fuel. biodesulfurization (BDS), is a biological method proposed for desulfurization of ring components of sulfur which is a non-destructive pathway to remove sulfur from hydrocarbons of petroleum in the mild conditions which potentially used as complementary with HDS. For industrial application of BDS, the approach needs the new challenge to enhance desulfurization activity by genetic engineering methods and bioreactor development to achieve from a fantasy technique to an industrial and reality method for reduction of sulfur from fossil fuels. In this review, we studied and evaluated the BDS and advances in the two last decades.

Keywords: Biodesulfurization; Hydrodesulfurization; Dibenzothiophene; Fossil fuels.

Introduction

Particulate emissions from the combustion of fossil fuels into the atmosphere have created serious problems for our planet [1]. Particles of carbon dioxide and nitrogen and sulfur oxides play a role in the heating of the earth and the creation of acid rain, destruction of forests, poisoning of lakes and destruction of buildings. Also, due to the high percentage of sulfur in fossil fuels, and a high percentage of sulfur oxides in gases, they can put human health in danger strongly (For example, they can infiltrateinto the respiratory system and disrupt it).

During the combustion of sulfur compounds Sulfur-oxide gases [mostly SO_2] are released and this results in both serious air pollution and poisonous metal catalysts [2]. Benzothiophene [BT], dibenzothiophene [DBT], and their alkylated derivatives make up more than 50% of the total sulfur content of commercial diesel [3,4]. These aromatic thiophenes as recalcitrant organic sulfur compounds have higher resistance to hydrodesulfurization [HDS] treatment than other sulfur compounds such as mercaptans and sulfides [5].

Among sulfur oxides, SO_2 is widely abundant and produced in the lower atmosphere. Moreover, this sulfur

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oxide can cause the formation of sulfate aerosol. Aerosol particles have an average diameter of 2.5 micrometers and can be transferred to the lungs and cause breathing problems (World Bank, 1999 Atlas 2001).

SO_2 can react with atmospheric moisture and cause acid rain or fog with a low pH. The acid formed in this way can cause corrosion of monuments. It can also be transferred to the soil, cause damage to trees or affect the acidity of lakes with low buffering capacity and threaten marine life (EPA, 2006).

If SO_2 is transported by air currents, it can be produced in one region and leave devastating effects in the thousands of kilometers [in a remote location]. So it is necessary to develop international cooperation to control SO_2 emissions.

Also, sulfur oxides emitted from burning of sulfur-containing compounds in fossil fuels can cause unknown effects on the environment and the economy. Therefore, in order to reduce emissions of sulfur oxides governments around the world imposed new laws based on which oil products with low sulfur content must be used [6,7].

In1979, Canada, the United States and Europe in particular, signed a number of agreements to reduce and control emissions of SO_2. Most of the agreements focused on the means of transportation fuels because these means are one of the world's most important sources of SO_2 emission factors.

The highest sulfur content in diesel was set to be 10 ppm in 2010 in America [8]. It is also important to remove sulfur for engines equipped with nitrogen oxide [NOx] storage catalysts because the sulfate produced by the sulfur in the fuel has toxic effects on these catalysts. Sulfate is unusually stable in thermal conditions and saturates the reduction sites on the catalyst. So accessible space for NOx reduction is reduced and their effectiveness is lowered. To overcome this problem, desulfurization processes should be designed or promoted to achieve sulfur content less than 10 ppm [9, 10]. There are generally two different methods to reduce the impact of emissions of sulfur oxides: Pre-combustion and post-combustion.

The pre-combustion method has more advantages than the post-combustion method. For example, in flue gas desulfurization [post-combustion method] applied on hot corrosive exhaust gases is an expensive method. Quality control of treated flue gas in any part of the treatment is virtually impossible. In addition, this method is limited and cannot be extended for everyone. In other words, pre-combustion of treatment method reduces the sulfur dioxide emissions from fossil fuel without being dependant on combustion process type. Also because the desulfurization solutions are not very frequent, their quality control it is much easier [11]. The conventional method for removal of sulfur compounds such as kerosene and diesel oil is the Hydrodesulphurization chemical methods [Hydrogen]. The hydrogenic method is a catalytic process done under high pressure [150-3000 lb/in2] and high temperature [290-455°C] and also uses hydrogen gas in the presence of a metal catalyst, where finally the sulfur of oil compounds is converted to hydrogen sulfide. But as mentioned above, a significant proportion of sulfur in some oil products [especially gasoline and diesel] is in the form of heterocyclic organic compounds [such as Benzothiophenes] that are resistant to this approach [12,13,14,15, 72, 73] and in order to remove them via hydrogenic method, higher temperature, pressure, and rates of catalyst are required, which considerably increase the desulfurization costs.

Biological Removal of Sulfur from Oil

The biological method can be considered as a non-destructive method in which microbes specifically remove sulfur from biodegradable hydrocarbons [16-21]. This method could be used in mild conditions and has presented a proper potential for industrial desulfurization industry.

In the hydrogenic method, in addition, to taking higher cost and energy, sulfur is not separated completely from heterocyclic poly-aromatic compounds. Hence researchers are focused on the use of microbial desulfurization [Biodesulfurization] where sulfur removal reaction [under the very mild condition and with lower costs], is performed by bacteria and enzymes, produced by them. So that they can fix the flaws in the hydrogenic method through microbial desulfurization and in fact replace [full or partial] the hydrogenic method, or complete it as a supplement.

Microorganisms require sulfur [in the structure of some cofactors, amino acids, and proteins] for the growth of and their biological activities [22,23]. In addition, microorganisms are dependent on enzymes and metabolic pathways and have the ability to provide their sulfur requirements by various sources using enzymes.

Some of these microorganisms are able to consume sulfur compounds in thiophene components by enzymes in the metabolic pathways and reduce sulfur content in fuel. In general, desulfurization by microorganisms has advantages, firstly because it is done in low temperature and gentle pressure and this can be considered as an energy-saving process. Second, biocatalysts are involved in biological activities that can be greatly specific to their substrate.

In addition to structural applications of sulfur in biological macro molecules, some microorganisms take sulfur as an energy source, so the microorganisms

should obtain their sulfur from sulfur compounds such as oil sulfur compounds. The problem is that the bacteria may take hydrocarbon compounds of oil as carbon source that reduces the value of the fuel. Accordingly, the scientist searched for bacteria that do not consume the oil hydrocarbon compounds and only consume isolate and use sulfur from the compounds. In Iran also some strains of of *Gordonia* RIPI [24-26] were separated and characterized by the Department of Biotechnology technology of Research Institute of Petroleum Industry [*Rhodococcous* FMF] [27, 25] and the National Institute for Genetic Engineering and Research Organization for Science and Technology to remove isolated sulfur from benzothiophene compounds model and efforts are made for higher molecular identification for the to modify the microorganisms genetically.

Recent discoveries about microbial desulfurization mechanisms can lead to the commercial application of desulfurization process using novel combination engineered components for high expression of desulfurization genes. Production inhibitors are finally separated or improve biocatalytic processes via connection of related structures in environmental conditions.

Few studies have been conducted in all fields of biodesulfurization, for example, some studies are conducted on biodesulfurization of spent engine oil [26].

The sulfur level in fossil fuel is 1000-3000 ppm. The concentration of sulfur in diesel fuel is often higher than 5000 ppm. In the past decade, the level of sulfur in these fuels is reduced from ppm 2000 - 5000 to less than ppm 500 ppm. Recently, using new mechanisms it becomes much less than 350 ppm. During the period [2005-2007] it was expected that refineries produce low sulfur fuel [sulfur content less than 10-15]. Thus during the last 10 years, there was a significant interest in the development of technologies for removal of sulfur from fossil fuels and their derivative products.

There are many refinery equipments traditionally used for the removal of sulfur. In addition, several new technologies have been developed for the further reduction of the sulfur content of fossil fuels. One solution is to use the ability of enzymes specific for the production of diesel fuel and gasoline with low sulfur content. The pathway known as biocatalytic desulfurization [BDS] has a good potential for the removal of sulfur from petroleum compounds [29,30].

Molecular destructive biodesulfurization

As mentioned, in the first step, by separating the bacteria that have the ability to use DBT, since the isolated microbial species could not specifically separate the sulfur from DBT and in addition some thiophene compounds were used as a source of carbon and sulfur, it was believed that attempts to biodesulfurization were failed [31.32]. In this way, Yamada and colleagues conducted a systematic study on microbial desulfurization. They reported that *Pseudomonas* genus microorganisms, break Dibenzothiophene down into a water-soluble product.

Their Pseudomonas strains were *Pseudomonas abikonasis and Pseudomonas jianii.* On the other hand, Kodama et al. reported that amino acids and other carbon compounds are essential as co-substrate for oxidation of Dibenzothiophene and the growth of microorganisms. Microorganisms with desulfurization activity have a metabolism that breaks c-c links in heterocyclic sulfur compounds such as Dibenzothiophene, thereby decompose benzene ring and finally, sulfate is released in a series of cascade oxidative reactions. The reaction mechanism that attacks carbon skeleton is called “Kodama" pathway that includes hydroxylation of an aromatic ring, splitting the ring and oxidation. Finally, a water-soluble product is obtained. Reactions related to the pathway are briefly

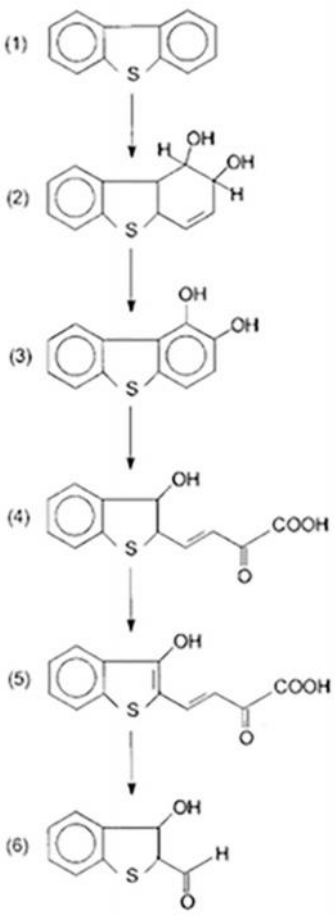

Figure 1. Desulfurization pathway by destroying the molecular structure (34)

described in Figure 1.

In this reaction, other aromatic molecules in the oil are also attacked. And thus, a substantial amount of hydrocarbons enter the aqueous phase [33]. This type of microorganisms generates a component of water-soluble thiophene by oxidative decomposition of the Dibenzothiophene, as the production of oxidation that is difficult to be separated from water [Kodama et al.]. The microorganisms do not target the enzymatic reactions of sulfur and sulfur so it is not practical to separate sulfur organic compounds with high molecular weight in crude oil. The reasons for these limitations can be summarized as follows:

1) The attack on the carbon ring of Dibenzothiophene often is done in positions 2 and 3 of Dibenzothiophene replaced by alkyl or allyl groups as these situations do not apply to a substrate on Kodama pathway. 2) the destruction pathway of the carbon skeleton reduces the amount of fuel energy. 3) The main product of the decomposition pathway of the carbon skeleton is 3-Hydroxy-2-Formyl benzothiophene and plenty of Dibenzothiophene is decomposed to form sulfate and thus sufficient desulfurization does not occur.

Monticello and Denote concluded that the Kodama pathway genes are for decomposition of DBT on a plasmid with 9 open reading frames [open reading frame], and the open reading frames are known as sox and also they found that only genetic pathways control the DBT, naphthalene and phenanthrene metabolize.

The other pathway of destruction that resulted in mineralization of DBT was introduced by Van Afferden et al. in 1993 along the pathway of DBT mineralization, there are three types of metabolic materials including DBT sulfoxide [DBTO], DBT sulfone [$DBTO_2$] and benzoate. This method of desulfurization is possible by destroying the molecular structure and may be valuable for Biodesulfurization of DBT in the environment [34].

Sulfur-specific desulfurization pathway

In this desulfurization pathway, Dibenzothiophene sulfoxide, Dibenzothiophene sulfonate, 2,2-dihydroxybiphenyls were characterized as intermediates of Dibenzothiophene in desulfurization. Apart from this, strains of *Rhodoccucos rhodochrous* ATCC53968 is isolated that has a pathway to convert Dibenzothiophene to hydroxyphenyl and sulfate.

It has been reported that 70% of the organic sulfur in crude oil and coal is reduced by these microorganisms. Also, in the case of *Corynebacteria* SP, A separate pathway to break Dibenzothiophene is described that

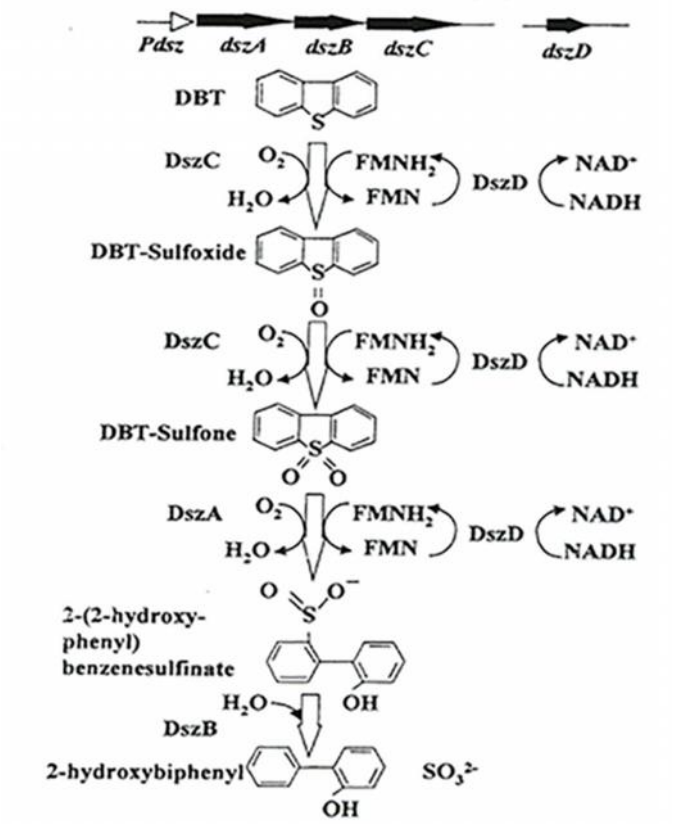

Figure 2. Four step pathway of desulphurization process (37)

through the oxidation of Dibenzothiophene converts it to Dibenzothiophene sulfoxide, then Dibenzothiophene sulfone and finally the 2-Hydroxybiphenyls sulfate [35].

Many researchers have reported that special pathway of desulfurization can be done by aerobic and anaerobic bacteria. However, there are reports that using the tests under controlled anaerobic sulfate reducing conditions, no significant reduction has been observed in the amount of sulfur of vacuum gas oil. Since there is little evidence in terms of the significant potential of anaerobic desulfurization commercially, aerobic desulfurization is widely considered.

The 4s pathway is a special desulfurization pathway where DBT is desulfurized and converted to 2HBP [2-Hydroxybiphenyl] [36, 74,75, 76, 77, 78, 79]. The 4S pathway condition along with factors and involved enzymes, the carbon chain remains unchanged and the fuel does not lose its value in the case of energy and burning value. Also, the sulfate ions formed in the 4S pathway are released in the culture medium or may be adsorbed by other microorganisms. Figure 2. *R.erythropolis* IGTS8 [38,39,40] and *Rhodococcus* sp. X 309 [13] are the first strains of bacteria that researchers obtained lots of information at the molecular level about the 4S desulfurization pathway. In this regard, a gene cluster that could complete the mutant bactericidal activity [IGTS8] were cloned and sequenced, [14] also, it was found that this gene cluster contained three open reading frames on a plasmid identified as ABC dsz.

Desulfurization process in the pathway of development

An investigation carried out after cloning revealed that dsz C product, converts DBT directly to $DBTO_2$ and also converts the gene products of dsz A and dsz B $DBTO_2$ to 2HBP [2-hydroxybiphenyl] [41]. The gene fragment is related to desulfurization phenotype on a large plasmid that contains three genes of B, C dsz A and is about 4kb long. In *Rhodococcus* bacteria, dsz genes of plasmid are close to entering sequences [42,34].

The important point about this genetic desulfurization is that the set of genes related to desulfurization are in the form of one operon with three-genes [dsz A and dsz B and dsz C] organized by a single promoter [43,39].

Due to the weaknesses of *Rhodococcus* IGTS8 and in order to commercialization and optimization of desulfurization, 4S pathway genes of *Pseudomonas* [44-50, 41] have been cloned.

Generally, due to the transfer of genetic material into non-enteric gram-negative bacteria with very low and poor efficiency, the best way is to use *E.coli* bacteria as middle host for transfer of genetic material. For this reason, expression plasmids in *Pseudomonas* have a wide range of host and are more stable in enteric and non-enteric bacteria. Today, the expression vectors used in *Pseudomonas* are mostly plasmids of incompatible groups such as RP4 or IncQ groups that can be transferred to conjugation method [51].

Recombinant bacteria for Commercial Biodesulfurization

Advanced technologies help the development of bio-biodesulfurization for the treatment of oil components, where biocatalysts are activated at 40 to 60 °C leading to the removal of nitrogen and heavy metals, but the most important problem of biocatalysts is their instability because they will die after 2-3 days [31]. So many works have been dedicated to making desulfurization strains commercially advantageous. Unlike, the processes are taken for modification of expression of effective enzymes in this pathway, this process is so slow to be used as an industrial application, both in the case of rate and efficiency of desulfurization. Desulfurization rate higher than 20 micromoles per gram of substrate in a minute per gram of catalyst is required. Successful separation of sulfur from petroleum compounds needs enzymes with high specificity and low Km in *dsz* system for various bacteria [52.53]. Successful separation of sulfur from petroleum compounds needs enzymes with high specificity and low Km in *dsz* system. Recently, some notes about gene shuffling of *dsz* genes have been introduced in different bacteria to create novel genes and more substrate specificity. Results showed that the developed techniques can be used create new hybrid enzymes with required activity in refinery applications and remove the needs of bacteria in removal of sulfur components from petroleum compounds [54]. Use of the developed biocatalysts is essential for commercial application of BDS, however, in most chemical desulfurization processes, oil components are under HP and HT and reaction cooling for microbial processes is not practically limited and they can be stable for a long time. There are a number of very thermophilic enzymes that are active at temperatures higher than 100 °C and can be stable for a long time and on the other hand their stability may be increased by pressure. The main cause of this behavior is not clear and no creation of thermal stability in desulfurization enzymes has been done so far, however Clinical isolates of *Bacillus* have been known recently which desulfurized DBT at 55 °C. In order to optimize and commercialize recombined bacteria, researches aim to use thermophilic enzymes and biosurfactant genes, more than before [51].

Considerable efforts have been taken to make appropriate strains for commercial use. In 1999, the first patent on the accession of desulfurization genes [*dsz*] was conducted in *Pseudomonas* in the United States. The other work on the accession of flavin reductase in a synthetic operon containing all the genes required for BDS pathway with a single transcript was released. Since both the accession of genes in *Pseudomonas* and use of flavin reductase have been recently released, it seems these patents contain remarkable points for creation of useful business processes. Unlike, the processes for modifying the expression of key enzymes in this pathway, the process is a very slow for commercial use; this is a problem in the rate and the amount of desulfurization. Desulfurization rate more than 20 mM substrate, per minute, per gram of catalyst is also needed [54].

Development of biodesulfurization processes

Recently, a significant development has happened in microbial desulfurization process and its economic discussion. A hypothetical form of industrial Biodesulfurization is shown in Figure 3. 3 sets of reactors are required to obtain a petroleum product with very low sulfur content, due to the high rate of Km of *dsz* system. In this scenario, the cells are grown first and then find resurgence in the process. This was a necessary step for the long-term commercial use of biocatalyst. Isolation stages [boxes] can be used just by hydrocyclone or in combination with other low-cost centrifuge or precipitator systems.

The entire procedure included reactor design, production, recycling and separation of water –oil phase. New hypotheses are significant from several aspects.

First, use of multi-stage reactors to cope with weak kinetic of reactions in low sulfur concentrations, second, keeping the fossil fuel value and ultimately use of the continued growth systems and biocatalysts regeneration in the reaction system and separation of them in external tanks, Figure 4.

Reactor design and implementation of a process for 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.

- 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.

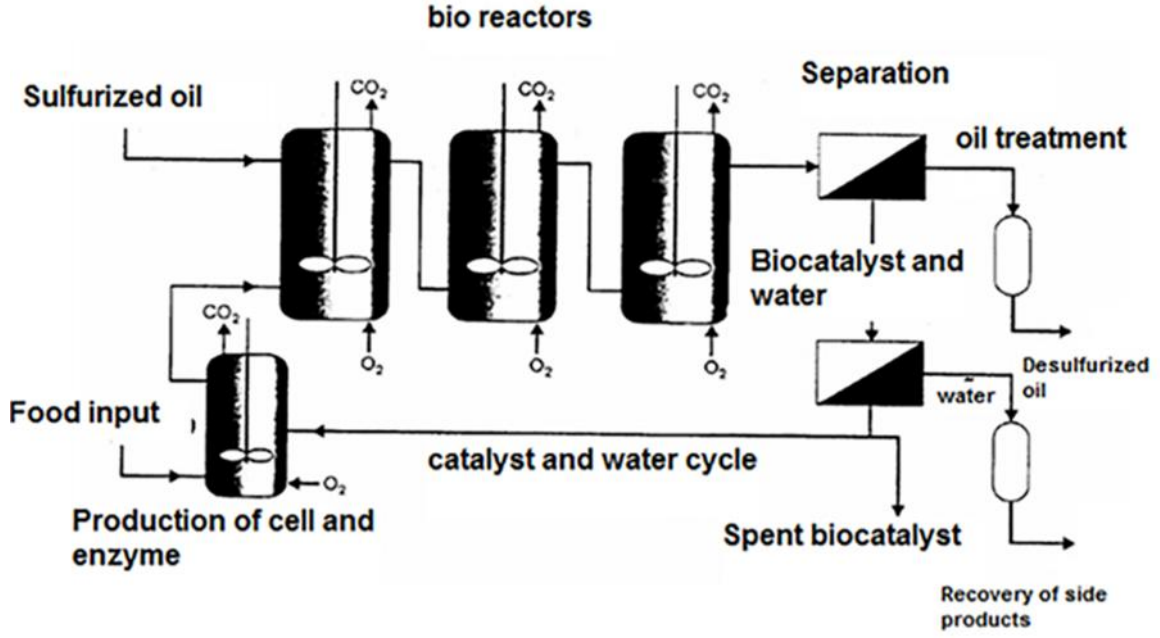

Figure 3. A hypothetical form for microbial desulphurization of oil (55)

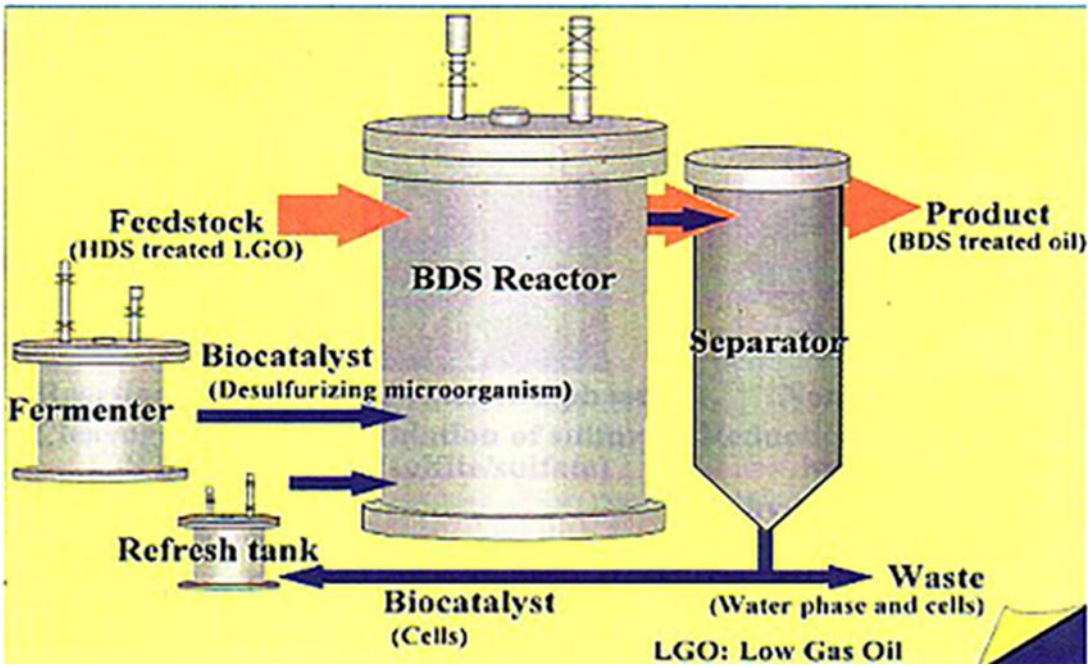

Figure 4. A hypothetical diagram for microbial desulfurization of oil (54)

- 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.

- The lack of precision in the use of proper hosts [bacteria], could lead to the creation of substrate resistant biocatalysts.

- Biocatalysts should not be under Feedback effect (Effect [positive or negative] that the product can put on the expression of genes) of the product. It was observed that using ion exchange resins and removal of reaction products, oxidation of DBT has been increased 10 times. In addition, by selection of suitable strains, genetic engineering or mutagenesis of biocatalyst cells, it is possible to prevent the feedback effect of the product.

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 [55].

In new studies Desulfurization of a model compound [e.g, DBT], often was carried out in aqueous systems, resulting in a 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. It has been found that emulsions of the oil phase and the upper layer contain significant amounts of bacterial particles that form droplets with a diameter of 1 to 10 micrometers, 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% of 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 a a reduction of 24 to 40% of sulfur, nitrogen, oxygen and metallic elements together with hydrocarbon fractions [56].

Economic benefits of BDS

BDS is more economical compared to the existing HDS method and other desulfurization technologies. Advanced technologies help to develop a BDS method for the treatment of oil components, where bio-catalysts are active at 40 to 65 °C and lead to the removal of nitrogen and metals from the oil. Another consideration

for economic acceptability of BDS, includes safety issues, transportation, storage and use of living bacteria on the large scale for the refinery to use. Of course, BDS process is not accepted in this view, as the cells remain alive for 1 to 2 days maximally. A common way to solve this problem is to produce and regenerate biocatalysts within the BDS process. In this way, the lifetime of biocatalysts reaches the 200 to 400 hours [8 to 17 days].

Studies to design a reactor led to the creation of modified condition, which reduces the mass transfer limitations and increases the volumetric reaction rate. The design of reactors required for the BDS process included designing tanks, appropriate aeration and optimal environment with the low water-to-oil ratio. This process reduces the size of the reactor, but at the same time, other processes are required to break the resulting emulsion. Increased concentration of biocatalysts [up to 50 g dry cell weight per liter] leads to stable emulsion formation and causes problems in separation. The information about the products obtained by BDS is low and scattered and there is generally little information in this regard. Pacheco et al. produced a type of diesel oil by BDS that had more fluidity than oil in the HDS method [31].

Biological Removal of Sulfur Compounds From Gas

Natural gas extracted from wells is different from what is known as regular gas. In underground reservoirs, gas is available in the free form [isolated] and dissolved in an oil or in contact with oil and water. So, the gas extracted from the ground is in the form of associated gas or dry gas and wet gas, respectively. Natural gas with a high percentage of methane, from any source, contains hydrocarbons such as ethane, propane, butane, and pentane after oil extraction and separation. In addition, the gas usually contains other substances such as water vapor, hydrogen sulfide [H_2S], carbon dioxide, helium, nitrogen and other elements. Natural gas before the transfer and use should be separated from these compounds so that some isolated compounds are very precious and have other expenses. Natural gas is transported to the refineries via pipeline network. The main natural gas processing involves the separation of dense gas, water, gas liquids, carbon dioxide and hydrogen sulfide, Figure 5, [58].

The production and use of biogas have recently increased as this fuel represents a valuable renewable energy source. Biogas utilization produces an indirect reduction of greenhouse gas emissions through the replacement of fossil fuel [59]. However, the use of biogas is limited by the presence of hydrogen sulfide [H_2S] at high concentrations [0.1-2%]. H_2S is a

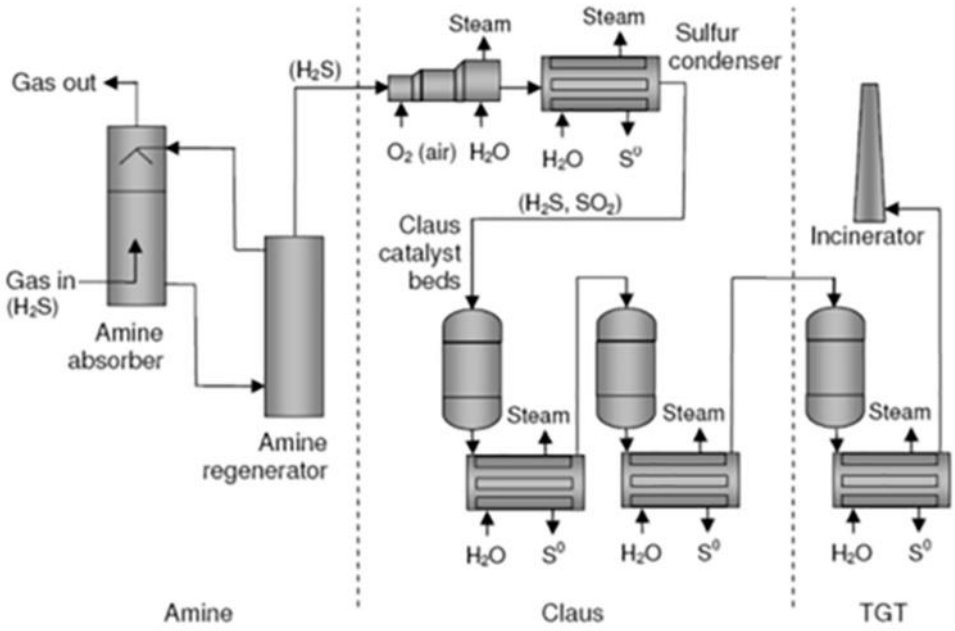

Figure 5. Simplified scheme of the Claus process for chemical desulfurization under pressure of sour gas (57)

corrosive and toxic compound that has an adverse environmental effect due to the sulfur oxides generated during combustion.

The main approaches employed for gas desulfurization are physicochemical methods. However, physicochemical methods are characterized by high consumption of energy and/or chemicals, and these methods can lead to other pollution problems such as the generation of large amounts of carbon dioxide [CO_2], nitrogen oxides or exhausted adsorbents that require disposal [60]. One of the most widely used biological methods for the purification or treatment of gas streams is biofiltration. Biofiltration is a safer and cleaner technology. The development of biofiltration has been rapid in recent years because it is less expensive than other technologies, has good performance at the pilot scale and in industrial applications, and is feasible for the treatment of a wide variety of gaseous effluents [61,62]. A biotrickling filter [BTF] is a packed bed bioreactor with biomass immobilized. The gas flows through a fixed bed usually counter-currently to a mobile liquid phase. Synthetic carriers are usually used such plastic, ceramic, lava rocks, polyurethane foam, etc. First of all, the pollutant compound must be transfer from the gas to the liquid phase and finally, the degradation is carried out in the biofilm. Fresh medium is fed to provide nutrients and remove the oxidation products [60].The biological removal of H_2S from biogas has been mainly studied under aerobic conditions [63,64, 69-71].

Biodesulfurization of gas varies depending on different sources. In order to separate H_2S from biogas, anaerobic methods via phototroph bacteria are used. In this way, no environmental problems are caused. In this method, the bacteria [cholorobium limicola] is used which is very convenient to use. Due to the use of the non-organic environment for bacterial growth, it has high efficiency in converting the sulfide to elemental sulfur [57].

The method because of being used to separate the biomass and being used in high-volume, fixed reactors are economically suitable to convert sulfide to elemental sulfur. If the light is fully under control, the product of oxidation will be non-toxic to a large amount of sulfur. The light source plays an important and valuable role in the process. It is important to use an economic light source in this method so that it can be a good alternative to chemical methods [58]. In the case of chemotrophic bacteria, there is no need to more control over the use of oxygen to produce elemental sulfur rather than sulfate. Even in the best circumstances, conversion of sulfide to sulfur, still relatively unknown sulfate contents are produced. *Thiobacillus* species that can grow in different conditions of environmental stress such as lack of oxygen, acidic conditions and other factors, are increasingly being used to convert H_2S and sulfur compounds in a biological process. Separation of hydrogen sulfide using a system of biological gas treatment was conducted in two stages: first, adsorption of hydrogen sulfide followed by the oxidation of hydrogen sulfide by the absorbent solution.

Biological filters are used for separation of H_2S in wastewater treatment. The process is basically conducted by aeration and oxidation product is often sulfate rather than elemental sulfur. The disadvantage of this technology is to reduce the pH of environment that has a negative impact on the process. The H_2S anaerobic bio-filters, as well as aerobic biofilters are effective, with the advantage of using cheaper raw materials. The removal means the removal of safety risks often associated with aerobic processes which are another advantage of anaerobic methods [65].

Improving biological removal of sulfur from natural gas

A technology was designed and developed in Wageningen University's environmental technology sector, in collaboration with Delft University Department of Biotechnology. The process is designed in alkaline pH and high concentration of sodium which has high performance for desulphurization of natural gas. First, the biological oxidation of sulfide was used to study sulfide separation in anaerobic form, then Wageningen University used the process for the separation of H_2S from biogases produced in anaerobic form by wastewater. Then, by collaboration with Shell Co the process was developed for desulfurization of natural gas, refinery gas and other types of gases [Figure 6] [57].

The mechanism of the process

In this process, the H_2S carrier gas is passed through an alkaline solution and this solution absorbs H_2S which form disulfide later. Absorbed carrier solution is then pumped to a bioreactor such that sulfide is oxidized to elemental sulfur in the bioreactor. This is done by bacteria oxidizing sulfur components [Chemical autotrophics]. Insoluble sulfur components were separated from the water flow and immediately used as sulfur fertilizers [57]. Biological oxidation of sulfide to disulfide is conducted in by hypoxic conditions while the formation of sulfate is done in extra oxygen condition.

1) $H_2O+OH^- \longrightarrow HS^-+H_2O$
2) $HS^-+½O_2 \longrightarrow S+OH^-$

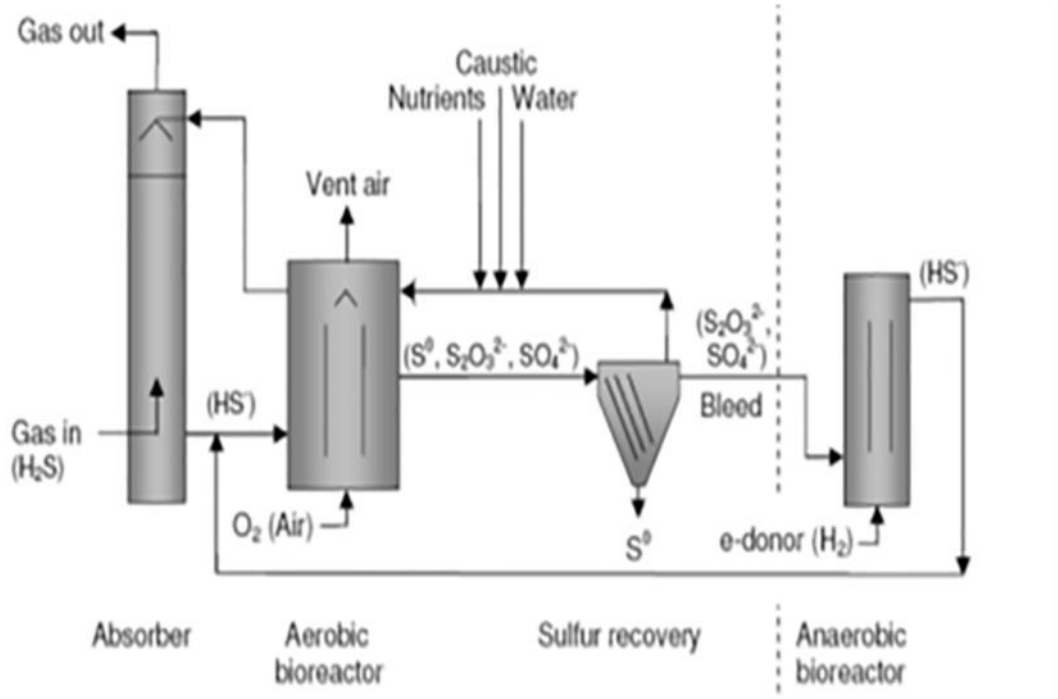

Figure 6. Gas desulphurization biological process to remove H_2S: the right side after the cross lines is the sign of the decline of thiosulfate (57)

3) $HS^-+2O_2 \quad SO_4^{2-}+H$

Thiosulfate [$S_2O_3^{2-}$] is generated in hypoxic conditions as low-sulfur oxidative compounds.

The main disadvantage of sulfate and thiosulfate are:

1) Less consumable elemental Sulfur is produced.

2) Production of protons [H +] causes acidification of alkaline solution.

3) In order to prevent the accumulation of sulfate and thiosulfate, these ions must be removed by a fluid flow. By the flow, bicarbonate ions are also eliminated and this loss should be compensated [57]. The existing process is activated in an alkaline medium of pH [8.2-8.5] and 5.0 M of salt. However, in high pH and high salt concentration, following benefits are obtained:

1) Removing sulfur from gases with high CO_2 content such as natural gas is performed easier.

2) Cumulative capacity of sulfide alkaline solution significantly increases at high pH and high concentration of carbonates. This reduces energy used in absorbing columns.

3) In this case there is no need for energy consumption to compensate for lost bicarbonate and in terms of energy consumption, it is more economic because reactor solution contains a higher concentration of sulfate and thiosulfate ions [57].

If only sodium ions are used to be combined with bicarbonate, sulfate, and thiosulfate, due to sediment accumulation of sodium bicarbonate higher scales problems will appear. This problem is due to acidification of the alkaline solution. Therefore, because the solubility of potassium salt is more than three times of sodium salt, a mixture of NaOH or KOH solution is used to prevent acidification. The flow used to drain sulfate and thiosulfate can be removed by a direct route of biological sulfate reduction.

In the second anaerobic bioreactor, sulfate reductant bacteria cause conversion of sulfate and thiosulfate to sulfide which can again be directed straight back to the manufacturer aerobic sulfate bioreactor. As a result, the water cycle of the process can be eliminated by reducing water consumption. In order to form sulfide in the step of reduction of thiosulfate, a donor electron such as ethanol or hydrogen is required. In terms of using high pH and high concentrations of sodium and potassium, just natran-alkaliphilic bacteria are able to grow. In recent decades, different types of bacteria have been isolated from alkaline lakes that have high performance.

Biological Removal of Sulfur Compounds From Coal

Coal is an inhomogeneous fossil fuel that includes varying amounts of oxygen, sulfur, nitrogen and minerals in addition to carbon and hydrogen. Sulfur content of coal deposits depends on the formation place and associated sediments, which varie between 0.5 and 10% and sometimes more. Sulfur in coal rocks is in two

Organic and inorganic forms.

An economic analysis of desulfurization of coal rocks

Investigating the cost of removing sulfur and comparison with the rising price of coal due to reductions in the sulfur content is economically interesting. Generally, the value of coal depends on these factors some of which can be improved by desulfurization.

1) Thermal power of coal [by increased thermal power, its value will be enhanced]

2) The ash content [coal value has a reverse relationship with the ash content. By increasing the ash, its value decreases]

3) The sulfur content [sulfur and coal costs are also correlated inversely, by reducing sulfur content, its value will enhance]

4) Coal dimensions [overall value of coal has a direct relationship with a particle size and by the larger dimensions, its value also rises]

5) Moisture content [moisture content decreases its value]

6) Coke dealer capability [the value of coal with Coking capability is several times greater than thermal coal value] [31].

Microbial desulfurization method is new and by the application of bacteria, almost all inorganic sulfur [at least 90 percent] can be removed in a relatively short time [a few days]. Organic sulfur removal is more difficult because its elimination needs special type of bacteria mostly self-sufficient in terms of food [autotrophic bacteria which obtain their required oxygen, nitrogen and carbon from carbon dioxide and nitrogen in the air] and time to remove is very long [several days] and is also very costly. Furthermore, nowadays the microorganisms used for this purpose are not able to remove organic sulfur completely and at the best condition, they can remove sulfur at 50 to 60 percent. Today, using genetic engineering, developed researches have been conducted to cultivate bacteria, which are self-sufficient for providing food, apply all organic sulfur components and have the maximum rate for removal of sulfur. But still no applicable result has been presented in the industry. Since the sulfur removal bacteria produce sulfuric acid which affects coal ash more or less and solves part of it, they decrease the ash content to some extent, too [31].

Types of bacteria used to remove coal sulfur

Thiobacillus ferrooxidans [TF] bacteria are the most common microorganisms used for the removal of sulfur from coal. These bacteria are easily capable of oxidizing pyrite and sulfuric acid production, but they are actually not able to remove organic sulfur. The self-sufficient [in terms of food] and aerobic bacteria require relatively thick oxygen, and if the amount of oxygen in the ambient air is less than 5% their activity is reduced and they are destroyed. Suitable pH range for their activity is 1.5-3.5 and outside the range diminishes their activity. The bacteria are naturally activated in temperatures of 25 to 40 degrees Celsius, abundant in the coal mines and output of many mine waters. The other category of desulfurization bacteria are *Sulfolobus*. The bacteria are found in the warm waters and adapted to temperatures higher than 50 °C and are active. The bacteria are not self-sufficient in terms of food and nutrition is needed. These bacteria are capable of removing organic sulfur and from this point of view *Thiobacillus* has an advantage over *Thiobacillus ferrooxidans* [31].

Laptev bacteria are also ideally suited for desulfurization. These bacteria are free from iron oxidation effect, but in the presence of sulfur oxidative bacteria such as *Thiobacillus* they quickly oxidize pyrite and remove its sulfur content. The thermophilic bacteria remove sulfur from coal more quickly which may be due to the activity of this type of bacteria at a higher temperature.

In general, most bacteria quickly remove mineral sulfur. But in the case of organic sulfur they are less active and don not have the potential for its removal or perform it slowly and incomplete. In practice, the active bacteria on organic sulfur require feeding. So the main factors in choosing the type of bacteria include replication rate and self-nutrition should be considered [31].

Application of different methods to remove sulfur from coal depends on the type of fuel, form of the sulfur, sulfur percentage, environmental standards and so on. Therefore, evaluating the advantages and disadvantages of microbial method, performance of bacteria and bacterial factors should be examined in designing applications. [31]

Advantages and disadvantages of using bacteria to remove sulfur from coal

The use of microorganisms to remove sulfur from coal, like any other method, has advantages and disadvantages that must be considered in the course of their use. The advantages of using bacteria to remove sulfur from coal can be summarized as follows:

1. Working at low temperature
2. Low power consumption
3. High efficiency of coal recovery
4. The relatively simple technology
5. Lower investment
6. Reducing transport cost

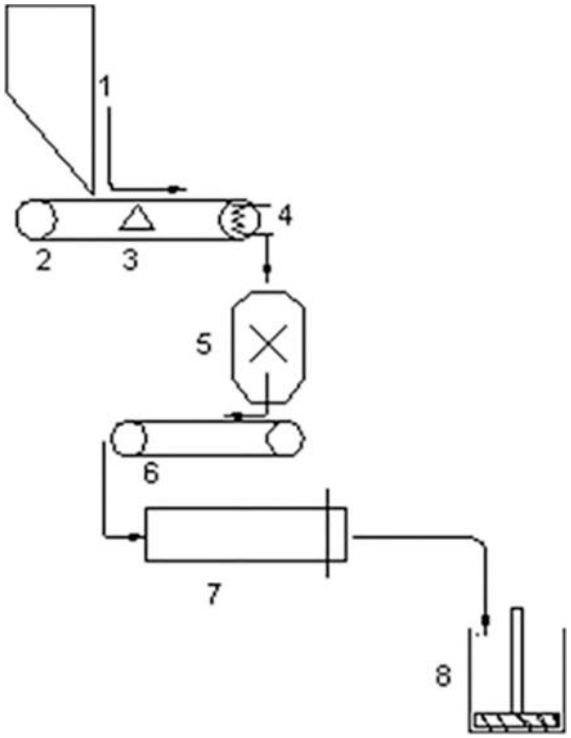

Figure 7. Porto Torres biodepyritization pilot plant: Coal comminution bay. 1 head bin, 2 belt feeder, 3 automatic scale, 4 magnetic separator, 5 hammer mill, 6 belt conveyor, 7 wet ball mill, 8 preparation tank (Modified and redrawn from Rossi (66), and Loi et al. (67)

7. Lower side-products

8. Conversion of coals with high sulfur content to sale coals

Advantages and disadvantages of using bacteria:

1. Sulfur removal microorganisms are aerobic and need oxygen. Supply of oxygen in the desulfurization environment is only feasible for chemical reactors and can be done by injecting air, but in other cases the supply of oxygen for the bacteria is associate withproblems and increase the cost of desulphurization because of mass transfer limitations and effectively low solubility of oxygen in the bioreactor.

2. Some of the microorganisms used for this purpose are not self-sufficient in terms of food and nutrition, which increases the price of desulfurization by bacteria.

3. Microorganisms are sensitive to the presence of heavy metals in coal and if the amount of these metals exceeds the limit, they are a not able to function and are destroyed. Therefore, the bacteria cannot be used on all types of coal.

The first semi-commercial continuous bio-depyritization operation was designed With a capacity of 50 kg raw coal per hour and consisted of three main sections: a comminution bay, a bioreactor bay, and a reject water purification [66, 67] and disposal system [Figures 7 and 8].

Results and Discussion

Natural energy resources include fossil fuels [oil, coal and gas], uranium [nuclear energy], water [source of electrical power], and other cases such as wind, solar and etc. Due to limited energy resources on the one hand and heavy losses [such as environmental pollution or corrosion] of energy consuming on the other hand, researches have been performed in different areas on the efficient use and reduction of the damage. According to current and emerging issues especially in the field of environmental concerns, it is predicted that more researches would be conducted in this context in future.

A significant part of energy is generated through fossil fuels. About 80 million barrels of crude oil are extracted from the ground daily, including oil derivatives such as natural gas, crude oil, coal, tar and etc. Almost 90% of the oil extracted from the ground is used as an energy source. Because much of the oil contains significant amounts of sulfur, its derived fuels' combustion causes a release of high amounts of sulfur oxides into the atmosphere.

Now most sulfur in fossil fuels can be removed easily. However, there is a section known as biodegradable organic sulfur that it is very difficult to

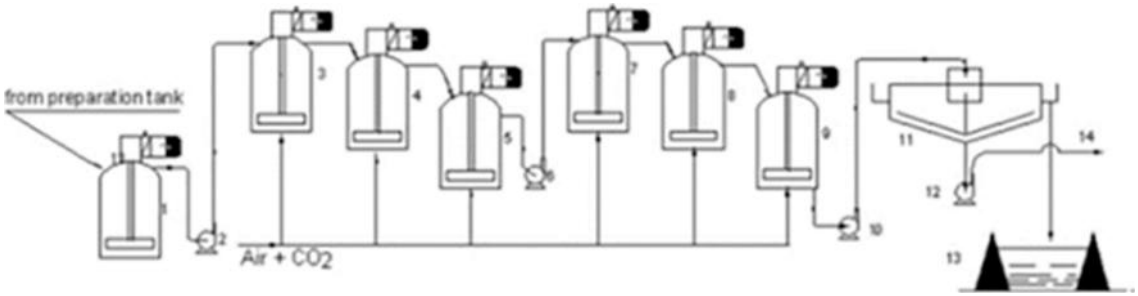

Figure 8. Porto Torres biodepyritization pilot plant: bioreactor and coal dewatering bay. 1 propagator, 2 pump, 3–9 stirred tank bioreactors, 10 pump, 11 rake thickener, 12 diaphragm pump,13 settling pond, 14 to stock-pile (Modified and redrawn from Rossi (66), and Loi et al. (67)

remove. Current methods that can be industrially used to remove sulfur in this section is in an invasive conditions [structure of a molecule]. They are so costly and produce significant amounts of carbon dioxide. New stringent laws and regulations [regarding the reduction of sulfur content in fossil fuels], require innovation in efficient and economical methods for desulfurization of biodegradable organic compounds [sulfur]. But because of the decline in oil reserves in the past decade and the increasing demand for distillates sectors [which usually have a high sulfur content] with low sulfur content, the oil companies took all their efforts for desulfurization of fossil fuels in the refining process. The new conditions led to high investments focused on construction and establishment of refining desulfurization chemical conversion processes.

Microbial desulfurization is an environmentally friendly method that could remove sulfur from late biodegradable organic compounds [under ambient temperature and pressure] without reduction of caloric value of the fuel. So, microbial method is an appropriate method to remove late biodegradable sulfur under mild conditions [ambient temperature and pressure]. However, this method has its advantages and disadvantages. Extensive researches are performed on microbiology and molecular biology of proper species to increase the desulfurization activity. Although even the highest activity is obtained, it is still not enough to satisfy industrial requirements. For the removal of microbial desulfurization efficiency, it is needed to work more in the areas such as increasing the desulfurization specific activity, increasing hydrocarbon phase tolerance, removing sulfur at higher temperatures and isolating new species for desulfurization in vast areas of sulfur compounds.

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