

**BIODESULFURIZATION OF FOSSIL FUELS- AN OVERVIEW****K. SRINIVAS RAJU¹, V.SRIDEVI *², M.V.V.CHANDANA LAKSHMI³
AND K.K.THOMAS⁴**¹*M.tech, Dept. of Chemical Engineering, Andhra University, A.P, INDIA*²*Associate Professor, Dept. of Chemical Engineering, Andhra University, A.P, INDIA***ABSTRACT**

Since the industrial revolution mankind has been dependent on fossil hydrocarbon fuel for a very large proportion of total energy needs. Many of the fluid forms also had lower sulphur content than coal and were therefore more attractive from the environmental impact standpoint. Combustion of sulfur-containing compounds in fossil fuels emits sulfur oxides, which can cause adverse effects on health, environment and economy. Therefore, there is a need to develop technologies that can remove sulphur pollutants. The stringent new regulations to lower sulfur content in fossil fuels require new economic and efficient methods for desulfurization of recalcitrant organic sulfur. Hydro desulfurization of such compounds is very costly and requires high operating temperature and pressure. Biotechnology offers an alternative way to process fossil fuels. There have been several important advances in the elucidation of the mechanisms of biodesulfurization. The low sulphur fuels, however, are limited in supply and future energy needs indicate that, until alternate non-hydrocarbon energy sources can be fully developed, there will be an increasing reliance on heavier hydrocarbons, including coal, heavy oils, bitumens, etc., all of which tend to be relatively high in sulphur. This article highlighting the great importance of the biodesulfurization process like how biotechnology may provide a 'greener' way to avoid acid rain, Molecular biology and metabolic engineering.

KEYWORDS: Biodesulfurization, sulphur, Protein, organic cofactors and Environmental Fate.**V.SRIDEVI**

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INTRODUCTION

Increased population, industrialization and urbanization are responsible for environmental contamination. Environmental decontamination is an enigma. Sulfur, Nitrogen, and Oxygen heterocycles are most potent environmental pollutants. The pollution in the air is a complex mixture of chemical substances of varying toxicity of which the sulfur oxides are a principal component. Those components which pose the primary hazards to human health have not yet been fully identified, nor have their respective contributions to human disease been fully determined. Consequently, in recent years, a number of studies have been undertaken for developing a better understanding of the effects of sulfur dioxide on humans, vegetation, animals and materials. Microbial degradation of these pollutants is attracting more and more attention because such bioprocesses are environmentally friendly. Sulfur dioxide and other sulfur compounds are generated as primary pollutants from the major industries such as sulfuric acid plants, copper smelters, catalytic cracking units, etc. and cause acid rain. The biotechnological potential of these processes is being investigated. To react to the continual changes in energetic demand, it is necessary to convert petroleum residues into high value fractions. To upgrade this heaviest part of crude oil, the removal of impurities as sulfur or metals, is a necessary step. Gaseous pollutants like sulfur dioxide that are once emitted into the atmosphere have no boundaries and become uncontrollable and cause acid deposition. This paper represents the desulfurization using the biological methods. The most recent developments in the field involve the use of directed evolution techniques to improve biocatalysis and new reactor and separation technologies to lower the cost of the process and produce valuable chemical byproducts. Hydrodesulfurization is carried using hydrogen in the presence of cobalt, nickel and molybdenum as catalyst at moderate temperature ($>400^{\circ}\text{C}$) and high pressure (4-10 MPa). But it is very difficult to remove polyaromatic sulfur compounds. To reduce sulfur to an ultra low-level [10ppm(mg/dm^3) or below] to meet global

specifications, Biotechnology offers an alternative way to process fossil fuels. Microbial desulfurization can be used to transform sulfur compounds efficiently¹. Microbial desulfurization technology shows great potential in energy industry and environmental protection. This review introduces the isolated strains for removing sulfur from sulfur-containing compounds in oil and coal, and the desulfurization mechanism and kinetics. The status of the development and application of the technologies, economic evaluation, existing problems and trend of development for further study are deliberated meanwhile the importance of developing the biodesulfurization (The biochemical process of using biological organisms to remove sulfur from a substance.) technology.

Natural occurrence

^{32}S is created inside massive stars, at a depth where the temperature exceeds $2.5 \times 10^9 \text{ K}$, by the fusion of one nucleus of silicon plus one nucleus of helium. As this is part of the alpha process that produces elements in abundance, The distinctive colors of Jupiter's volcanic moon Io are attributed to various forms of molten, solid and gaseous sulfur.

Characteristics

Physical properties

Sulphur usually occurs as a pale yellow, brittle, crystalline solid. It has a faint characteristic smell, but no taste. Sulphur is insoluble in water; it is not ever wetted by it. If a pinch of powdered sulphur is dropped into water, the particles will not sink but spread on the surface to form a yellow film. Sulphur is highly soluble in carbon disulphide and sparingly soluble alcohol and ether. It has a low melting point (114°C). It occurs in a number of allotropic forms a two of which, the rhombic and monoclinic, are crystalline. Sulfur forms polyatomic molecules with different chemical formulas, with the best-known allotrope being octasulfur, cyclo-S₈. Octasulfur is a soft, bright-yellow solid with only a faint odor, similar to that of matches. It melts at 115.21°C , boils at 444.6°C and sublimates easily². At 95.2°C , below its melting temperature, cyclo-octasulfur

changes from α -octasulfur to the β -polymorph. The structure of the S₈ ring is virtually unchanged by this phase change, which affects the intermolecular interactions. Between its melting and boiling temperatures, octasulfur changes its allotrope again, turning from β -octasulfur to γ -sulfur, again accompanied by a lower density but increased viscosity due to the formation of polymers³.

Chemical properties

Sulphur is a multivalent non-metal, abundant, tasteless and odorless. In its native form sulphur is a yellow crystalline solid. In nature it occurs as the pure element or as sulfide and sulfate minerals. Although sulphur is infamous for its smell, frequently compare to rotten eggs, that odor is actually characteristic of hydrogen sulphide (H₂S). The crystallography of sulphur is complex. Depending on the specific conditions, sulphur allotropes form several distinct crystal structures.

Allotropes

Sulfur forms over 30 solid allotropes, more than any other element⁴. Besides S₈, several other rings are known⁵. Removing one atom from the crown gives S₇, which is more deeply yellow than S₈. HPLC analysis of "elemental sulfur" reveals an equilibrium mixture of mainly S₈, but with S₇ and small amounts of S₆⁶. Larger rings have been prepared, including S₁₂ and S₁₈^{7,8}.

Isotopes

Sulfur has 25 known isotopes, four of which are stable: ³²S (95.02%), ³³S (0.75%), ³⁴S (4.21%), and ³⁶S (0.02%). Other than ³⁵S, with a half-life of 87 days and formed in cosmic ray spallation of ⁴⁰Ar, the radioactive isotopes of sulfur have half-lives less than 170 minutes.

Production

Sulfur is referred to in the Bible as *brimstone* (burn stone) in English, with this name still used in several nonscientific tomes. It was needed to make the best quality of black gunpowder. In 1777, Antoine Lavoisier helped convince the scientific community that sulfur was a basic element, rather than a compound.. Today, almost all elemental sulfur is produced

as a byproduct of removing sulfur-containing contaminants from natural gas and petroleum.

Applications

Sulfuric acid

Elemental sulfur is mainly used as a precursor to other chemicals. Approximately 85% (1989) is converted to sulfuric acid (H₂SO₄):



With sulfuric acid being of central importance to the world's economies, its production and consumption is an indicator of a nation's industrial development. The principal use for the acid is the extraction of phosphate ores for the production of fertilizer manufacturing. Other applications of sulfuric acid include oil refining, wastewater processing, and mineral extraction. One of the direct uses of sulfur is in vulcanization of rubber, where polysulfides crosslink organic polymers. Sulfites are heavily used to bleach paper and as preservatives in dried fruit. Many surfactants and detergents, e.g. sodium lauryl sulfate, are produced are sulfate derivatives. Calcium sulfate, gypsum, (CaSO₄ · 2H₂O) is mined on the scale of 100 million tons each year for use in Portland cement and fertilizers. When silver-based photography was widespread, sodium and ammonium thiosulfate were widely used as "fixing agents." Sulfur is a component of gunpowder. Sulfur is increasingly used as a component of fertilizers. Sulfur improves the use efficiency of other essential plant nutrients, particularly nitrogen and phosphorus. Biologically produced sulfur particles are naturally hydrophilic due to a biopolymer coating. Organosulfur compounds are used in pharmaceuticals, dyestuffs, and agrochemicals. Many drugs contain sulfur, early examples being antibacterial sulfonamides, known as *sulfa drugs*. Sulfur is a part of many bacterial defense molecules. Most β -lactam antibiotics, including the penicillins, cephalosporins and monolactams contain sulfur. Elemental sulfur is one of the oldest fungicides and pesticides. "Dusting sulfur," elemental sulfur in powdered form, is a common fungicide for grapes, strawberry, many vegetables and several other crops.

Biological role

Protein and organic cofactors

Sulfur is an essential component of all living cells. It is the seventh or eighth most abundant element in the human body by weight, being about as common as potassium, and a little more common than sodium or chlorine. A 70 kg human body contains about 140 grams of sulfur. In plants and animals, the amino acids cysteine and methionine contain most of the sulfur. The element is thus present in all polypeptides, proteins, and enzymes that contain these amino acids. In humans, methionine is an essential amino acid that must be ingested. However, save for the vitamins biotin and thiamine, cysteine and all sulfur-containing compounds in the human body can be synthesized from methionine. Disulfide bonds (S-S bonds) formed between cysteine residues in peptide chains are very important in protein assembly and structure. These covalent bonds between peptide chains confer extra toughness and rigidity. For example, the high strength of feathers and hair is in part due to their high content of S-S bonds and their high content of cysteine and sulfur. Eggs are high in sulfur because large amounts of the element are necessary for feather formation, and the characteristic odor of rotting eggs is due to hydrogen sulfide. The high disulfide bond content of hair and feathers contributes to their indigestibility and to their characteristic disagreeable odor when burned.

Toxicological Effects

Acute Toxicity

Sulfur is known to be of low toxicity, and poses very little if any risk to human and animal health. Short-term studies show that sulfur is of very low acute oral toxicity and does not irritate the skin (it has been placed in EPA Toxicity Category IV, the least toxic category, for these effects). Sulfur also is not a skin sensitizer. However, it can cause some eye irritation, dermal toxicity and inhalation hazards. When taken orally, it has a mild laxative action. It may cause irritation of skin and the mucous membranes. Sulfur is considered a skin and eye irritant. Acute exposure inhalation of large amounts of the dust may cause catarrhal inflammation of the nasal mucosa which may lead to hyperplasia with abundant nasal

secretions. Trachibronchitis is a frequent occurrence, with dyspnea, persistent cough and expectoration which may sometimes be streaked with blood.

Chronic Toxicity

Exposure to sulphur dioxide of concentration 1ppm leads to the constriction of air passage in the respiratory system. Higher concentrations can cause irritation of the nose and throat, coughing and eye irritation. Long exposure can lead to fatigue and shortness of breath. Chronic exposure to elemental sulfur at low levels is generally recognized as safe. Epidemiological studies show that mine workers exposed to sulfur dioxide throughout their lives often had eye and respiratory disturbances, chronic bronchitis and chronic sinus effects. However, no known risks of oncogenic, teratogenic, or reproductive effects are associated with the use of sulfur. Also, sulfur has been shown to be non-mutagenic in microorganisms. An average SO₂ threshold concentration for odour is 0.8-1.0 ppm. Repeated or prolonged exposure to dust may cause irritation to the mucous membranes. Bronchopulmonary disease may occur which, after several years, may be complicated by emphysema and bronchiectasis. Early symptoms in sulfur miners often include upper respiratory tract catarrh, with cough and expectoration which is mucoid and may even contain granules of sulfur. Asthma is a frequent complication. The maxillary and frontal sinuses may be affected; involvement is usually bilateral and pansinusitis may occur.

Ecological Effects

Effects on Birds

Sulfur is considered non-toxic to birds. The 8-day dietary LC50 for bobwhite quail is reported to be greater than 5,620 ppm in a study using a 95% sulfur wettable powder formulation. In studies on ecological effects involving bobwhite quail, sulfur has been shown to be practically non-toxic to the species tested.

Effects on Aquatic Organisms

The 96-hour LC50 values for two fish species, bluegill sunfish and rainbow trout, are greater than 180 ppm in a study using a 99.5% sulfur

dust formulation. The 48-hour LC50 for daphnia and the 96-hour LC50 for mysid shrimp is reported to be greater than 5,000 and 736 ppm, respectively, in a study using 90% sulfur. In studies on ecological effects involving two fish species, daphnia, and mysid shrimp, sulfur has been shown to be practically non-toxic to the species tested.

Effects on Other Animals (Nontarget species)

Sulfur is considered non-toxic to bees. In studies on ecological effects involving honeybees, sulfur has been shown to be practically non-toxic to the species tested. Thus, although there is potential for non-target organisms to be exposed to sulfur, little hazard to these species is expected to result. Two beneficial insect studies demonstrated that sulfur (98% dust and 92% wettable powder) is low in toxicity to the honeybee through contact and ingestion.

Environmental Fate

Breakdown of Chemical in Soil and Groundwater

Sulfur is a component of the environment, and there is a natural cycle of oxidation and reduction reactions which transforms sulfur into both organic and inorganic products. Sulfur in the form of sulfate constitutes about 0.1% of U. S. soils. Elemental sulfur is slowly converted to sulfate in soil by the action of autotrophic bacteria. Elemental sulfur leaches in soil as sulfate at a slow rate. About 3-6% of the sulfur (formulation and purity unspecified) applied at 56 kg/Ha leached through lysimeters of loam soil (soil depth unspecified) as a result of 40 inches of rain over a six-month period. After two years, 23-29% of the applied sulfur had leached.

Remedial Methods For removal of sulfur

Physical methods

Two different approaches can be applied to lower the impact of SO_x emissions: pre-combustion and post-combustion treatment methods. Pre-combustion treatment method has more advantages compared to the post-combustion method. For instance, in the case of flue gas treatment, post combustion method can be applicable. However, this method deals

with hot corrosive effluents and it is expensive. Monitoring of the treated flue gas at every treatment location is almost impossible. Liquid-phase adsorption is another interesting methodology explored to remove refractory organic sulfur compounds. This alternative offers also some advantages, such as mild operation conditions (temperatures close to ambient, atmospheric pressure) and no need of hydrogen or oxygen. Many studies have been undertaken to develop adsorbents for the desulfurization of transportation fuels using zeolites⁹, mesoporous materials^{10,11} and activated carbons¹²⁻¹⁴. In the case of activated carbons it was concluded that the micropore volume, the diameter of which is similar to the critical dimension of sulfur compounds, plays a crucial role in sulfur compound adsorption¹⁵.

Chemical Methods

Chemical precipitation can be effectively executed with the use of Ba(OH)₂. In practice because of the toxicity of barium salts that technique is not used. Most often lime is used with however, limited effects and large quantities of sludge produced. As much as several thousand of mg- SO₄/l can be present in such wastewaters. Precipitation of sulfates with lime results in production of gypsum according to the reactions given below.



Calcium sulfate is however, relatively well soluble. The solubility is 1990 mg /l, what means 1404 mg SO₄/l at 20°C. In practice due to the presence of other impurities (other ions) the amount of remaining sulfates is even higher. Usually the pH should be above 8, and relatively long reaction time is required. Biological desulfurization has many more advantages over conventional physico-chemical methods due to low operational cost and no production of secondary pollutants.

Biological Methods

Biotechnology may provide a 'greener' way to avoid acid rain

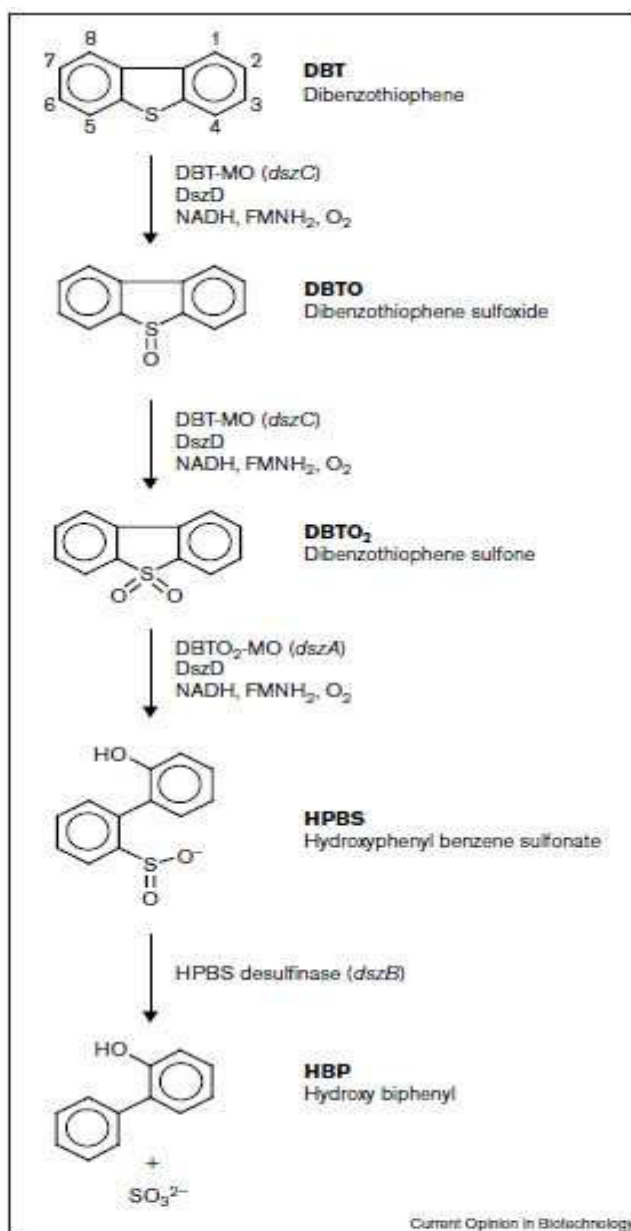
Sulfur levels in crude oil range from about 1000 ppm to over 30,000 ppm. Typical sulfur

concentrations in 'straight run' diesel often exceed 5000 ppm. In the past decade, 'acceptable' levels of sulfur in this fuel have gone from 2000–5000 ppm to less than 500 ppm. Recent and impending regulations will drive levels to below 350 ppm. In the not too distant future (2005–2007), refiners expect to be faced with a 'no sulfur' specification (less than 10–15 ppm sulfur). As a result, over the past 10 years there has been considerable interest in developing technologies to remove sulfur from transportation fuels and the feed stocks that are used to make them. There are many conventional refinery operations that can be used to remove sulfur and a number of new technologies that have been developed to address the need for additional low-cost desulfurization capacity. One possible approach is to take advantage of the specificity of enzymes and the relatively low costs of biotransformations to produce low sulfur diesels and gasolines. This approach, biocatalytic desulfurization (BDS), has the potential benefits of lower capital and operating costs and will produce substantially less greenhouse gases¹⁶. This review will focus on the developments in BDS in the past 18 months. There have been a number of relatively recent reviews written on the progress made over the past decade in biodesulfurization¹⁷⁻¹⁹. These are useful in that they have discussed a great deal of both old and more recent work. The past two years have seen the publication of a number of process patents and papers that reflect the slow maturation of this idea into an actual technology. The most recent developments in the field involve the use of directed evolution techniques to improve biocatalysis and new reactor and separation technologies to lower

the cost of the process and produce valuable chemical byproducts. It is clear from these publications that the successful application of BDS will require a sophisticated combination of molecular biology, directed evolution and metabolic and bioengineering, and creative integration of the BDS technology with existing refinery operations²⁰.

Molecular biology and metabolic engineering

Most of the work over the past 10 years, especially in the past two years, has focused on the metabolism of sulfur heterocycles, particularly dibenzothiophenes (DBTs), by *Rhodococcus* strains and other relatively closely related species. *Rhodococcus* seems particularly well suited for hydrocarbon metabolism²². Applied aspects of the genetics of this genus have been recently reviewed²¹. A recent publication by Folsom *et al.*²² consolidates much of the recent work on the characterization of the metabolism of the target molecules for BDS, the alkylated benzo- and dibenzothiophenes (Cx-BT and Cx-DBT). The main pathway for the liberation of sulfur from Cx-DBT is shown in Figure 1. Folsom *et al.*²² illustrate that the enzymes involved in the metabolism of Cx-DBTs have more or less preferred substitution patterns. In general, DBT and C1-DBT (methylated somewhere on the rings) are attacked preferentially, followed by the more highly alkylated molecules. The position of the methyl group also influences the reaction rate. In most cases, alkylation near the sulfur leads to slower rates, although the opposite is true with the enzymes from a recently patented *Sphingomonas* strain. This variability has been used to advantage in recent directed evolution work.



The '4S' pathway for the biological desulfurization of DBT and its derivatives. The key enzymes in the pathway are dibenzothiophene monooxygenase (DBT-MO), a tetramer encoded by the *dszC* gene, dibenzothiophene sulfone monooxygenase (DBTO₂-MO), a dimer encoded by the *dszA* gene, HPBS desulfinase, encoded by the *dszB* gene, and the NADH:FMN oxidoreductase encoded by the *dszD* gene.

Figure 1
The '4S' pathway for the biological desulfurization of DBT

Better enzymes are needed for a commercial process

Considerable work has been done to construct optimized strains for commercial use. This work has only recently begun to surface as patents are issued. In 1999, the first patent on the incorporation of the desulfurization (*dsz*) genes into *Pseudomonas* issued in the United

States. Another on the incorporation of a flavin reductase into an artificial operon to collect all the genes required for BDS into a single transcript was also issued. While both the incorporation of the genes in *Pseudomonas* and the use of the flavin reductase were preceded by earlier publications, the patents contain considerably more information and are

more relevant (because their filing dates proceed the publications) to creating a commercial process.

CONCLUSION

Biological sulfate removal process has a high potential for practical application. The fate of produced sulfides are an open question, and more research is required. We need a better understanding of the critical issues in order to turn this interesting microbial pathway into a commercial process. We've learned a lot about how *Rhodococcus* deals with these sulfur heterocycles dissolved in oil and how this can be turned into a technology. Recent work has yielded the following knowledge. for dsz-like

enzymes are found in Gram positive and negative species. Although they are only ~70% homologous, the proteins catalyze similar reactions. The specificity and substrate preferences vary between even closely related strains. Genes can be 'shuffled' together to create hybrid genes with better rates and extents of desulfurization. Secondly, the physical properties of the cells are important for good mass transfer (hydrophobic surfaces that let them 'drink from the oil'), effective separations and product recovery. Finally, the pathway can be truncated to produce value-added molecules. This application is cost effective now and may be the first application of the technology.

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