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(54) Title: CONTINUOUS PROCESS FOR BIOCATALYTIC DESULFURIZATION OF SULFUR-BEARING HETEROCYCLIC MOLECULES

(57) Abstract

A continuous cyclic process for desulfurizing a petroleum liquid which contains organic sulfur molecules, a significant portion of which are comprised of sulfur-bearing heterocycles. This process involves oxygenating the petroleum liquid and treating it with a biocatalyst capable of catalyzing the sulfur-specific oxidative cleavage of organic carbon-sulfur bonds in sulfur-bearing aromatic heterocyclic molecules such as dibenzothiophene. A particularly preferred biocatalyst is a culture of mutant *Rhodococous rhodocrous* bacteria, ATCC No. 53968. In the present process, the activity of this biocatalyst is regenerated; it can be used for many cycles of treatment. A system for conducting the continuous cyclic process of biocatalytic desulfurization of petroleum liquids is also disclosed.

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CONTINUOUS PROCESS FOR BIOCATALYTIC DESULFURIZATION OF SULFUR-BEARING HETEROCYCLIC MOLECULES

BACKGROUND

Sulfur is an objectionable element which is nearly ubiquitous in fossil fuels, where it occurs both as inorganic (e.g., pyritic) sulfur and as organic sulfur (e.g., a sulfur atom or moiety present in a wide variety of hydrocarbon molecules, including for example, mercaptans, disulfides, sulfones, thiols, thioethers, thiophenes, and other more complex forms). Organic sulfur can account for close to 100% of the total sulfur content of petroleum liquids, such as crude oil and many petroleum distillate fractions. Crude oils can typically range from close to about 5 wt% down to about 0.1 wt% organic sulfur. Those obtained from the Persian Gulf area and from Venezuela (Cerro Negro) can be particularly high in organic sulfur content. Monticello, D.J. and J.J. Kilbane, "Practical Considerations in Biodesulfurization of Petroleum", IGT's 3rd Intl. Symp. on Gas, Oil, Coal, and Env. Biotech., (Dec. 3-5, 1990) New Orleans, LA, and Monticello, D.J. and W.R. Finnerty, (1985) Ann. Rev. Microbiol. 39:371-389.

The presence of sulfur has been correlated with the corrosion of pipeline, pumping, and refining equipment, and with premature breakdown of combustion engines. Sulfur also contaminates or poisons many catalysts which are used in the refining and combustion of fossil fuels. Moreover, the atmospheric emission of sulfur combustion products such as sulfur dioxide leads to the form of acid deposition known as acid rain. Acid rain has lasting deleterious effects on aquatic and forest ecosystems, as well as on agricultural areas

located downwind of combustion facilities. Monticello, D.J. and W.R. Finnerty, (1985) Ann. Rev. Microbiol. 39:371-389. To combat these problems, several methods for desulfurizing fossil fuels, either prior to or immediately after combustion, have been developed.

One technique which is employed for pre-combustion sulfur removal is hydrodesulfurization (HDS). This approach involves reacting the sulfur-containing fossil fuel with hydrogen gas in the presence of a catalyst, commonly a cobalt- or molybdenum-aluminum oxide or a combination thereof, under conditions of elevated temperature and pressure. HDS is more particularly described in Shih, S.S. et al., "Deep Desulfurization of Distillate Components", Abstract No. 264B AIChE Chicago Annual Meeting, presented November 12, 1990, (complete text available upon request from the American Institute of Chemical Engineers; hereinafter Shih et al.), Gary, J.H. and G.E. Handwerk, (1975) Petroleum Refining: Technology and Economics, Marcel Dekker, Inc., New York, pp. 114-120, and Speight, J.G., (1981) The Desulfurization of Heavy Oils and Residue, Marcel Dekker, Inc., New York, pp. 119-127. HDS is based on the reductive conversion of organic sulfur into hydrogen sulfide (H_2S), a corrosive gaseous product which is removed from the fossil fuel by stripping. Elevated or persistent levels of hydrogen sulfide are known to inactivate or poison the chemical HDS catalyst, complicating the desulfurization of high-sulfur fossil fuels.

Moreover, the efficacy of HDS treatment for particular types of fossil fuels varies due to the wide chemical diversity of hydrocarbon molecules which can contain sulfur atoms or moieties. Some classes of organic sulfur molecules are labile and can be readily

desulfurized by HDS; other classes are refractory and resist desulfurization by HDS treatment. The classes of organic molecules which are often labile to HDS treatment include mercaptans, thioethers, and disulfides. Conversely, the aromatic sulfur-bearing heterocycles (i.e., aromatic molecules bearing one or more sulfur atoms in the aromatic ring itself) are the major class of HDS-refractory organic sulfur-containing molecules. Typically, the HDS-mediated desulfurization of these refractory molecules proceeds only at temperatures and pressures so extreme that valuable hydrocarbons in the fossil fuel can be destroyed in the process. Shih et al.

Recognizing these and other shortcomings of HDS, many investigators have pursued the development of commercially viable techniques of microbial desulfurization (MDS). MDS is generally described as the harnessing of metabolic processes of suitable bacteria to the desulfurization of fossil fuels. Thus, MDS typically involves mild (e.g., physiological) conditions, and does not involve the extremes of temperature and pressure required for HDS. Additionally, the ability of a biological desulfurizing agent to renew or replenish itself is viewed as a potentially significant advantage over physicochemical catalysis.

The discovery that certain species of chemolithotrophic bacteria, most notably *Thiobacillus ferrooxidans*, obtain the energy required for their metabolic processes from the oxidation of pyritic (inorganic) sulfur into water-soluble sulfate has stimulated the search for an MDS technique for the desulfurization of coal, in which pyritic sulfur can account for more than half of the total sulfur present.

Recently, Madgavkar, A.M. (1989) U.S. Patent No. 4,861,723, has proposed a continuous *T. ferrooxidans* -based MDS method for desulfurizing coal. However, a commercially viable MDS process for the desulfurization of coal has not yet emerged.

Because of the inherent specificity of biological systems, *T. ferooxidans* MDS is limited to the desulfurization of fossil fuels in which inorganic sulfur, rather than organic sulfur, predominates. Progress in the development of an MDS technique appropriate for the desulfurization of fossil fuels in which organic sulfur predominates has not been as encouraging. Several species of bacteria have been reported to be capable of catabolizing the breakdown of sulfur-containing hydrocarbon molecules into water-soluble sulfur products. One early report describes a cyclic desulfurization process employing *Thiobacillus thiooxidans, Thiophyso volutans,* or *Thiobacillus thioparus* as the microbial agent. Kirshenbaum, I., (1961) U.S. Patent No. 2,975,103. More recently, Monticello, D.J. and W.R. Finnerty, (1985) Ann. Rev. Microbiol. 39:371-389, and Hartdegan, F.J. et al., (May 1984) Chem. Eng. Progress 63-67, have reported that such catabolic desulfurization of organic molecules is, for the most part, merely incident to the utilization of the hydrocarbon portion of these molecules as a carbon source, rather than a sulfur-selective or -specific phenomenon. Moreover, catabolic MDS proceeds most readily on the classes of organic sulfur molecules described above as labile to HDS.

Although Monticello and Finnerty report that several species of bacteria have been described as capable of desulfurizing the HDS-refractory aromatic sulfur-bearing heterocycles, in particular *Pseudomonas putida* and *P. alcaligenes*, this catabolic pathway is

also merely incident to the utilization of the molecules as a carbon source. Consequently, valuable combustible hydrocarbons are lost, and frequently the water-soluble sulfur products generated from the catabolism of sulfur-bearing heterocycles are small organic molecules rather than inorganic sulfur ions. As a result, the authors conclude that the commercial viability of these MDS processes is limited. Monticello, D.J. and W.R. Finnerty, (1985) Ann. Rev. Microbiol. 39:371-389.

None of the above-described desulfurization technologies provides a viable means for liberating sulfur from refractory organic molecules, such as the sulfur-bearing heterocycles. The interests of those actively engaged in the refining and manufacturing of petroleum fuel products have accordingly become focused on the need to identify such a desulfurization method, in view of the prevalence of these refractory molecules in crude oils derived from such diverse locations as the Middle East (about 40% of the total organic sulfur content present in aromatic sulfur-bearing heterocycles) and West Texas (up to about 70% of the total).

Summary of the Invention

This invention relates to a continuous process for desulfurizing a petroleum liquid which contains organic sulfur molecules, a significant portion of which are comprised of sulfur-bearing heterocycles, comprising the steps of: (a) contacting the petroleum liquid with a source of oxygen under conditions sufficient to increase the oxygen tension in the petroleum liquid to a level at which the biocatalytic oxidative cleavage of carbon-sulfur bonds in sulfur-bearing heterocycles proceeds; (b) introducing the oxygenated petroleum

liquid to a reaction vessel while simultaneously introducing an aqueous, sulfur-depleted biocatalytic agent to the reaction vessel, the agent being capable of inducing the selective oxidative cleavage of carbon-sulfur bonds in sulfur-bearing heterocycles; (c) incubating the oxygenated petroleum liquid with the biocatalytic agent in the reaction vessel under conditions sufficient for biocatalytic oxidative cleavage of said carbon-sulfur bonds, for a period of time sufficient for a significant number of cleavage reactions to occur, whereby the organic sulfur content of the treated petroleum liquid is significantly reduced and a significant amount of water-soluble inorganic sulfate is generated; (d) removing the desulfurized petroleum liquid from the reaction vessel; (e) retrieving the spent aqueous biocatalytic agent from the reaction vessel, the spent agent being significantly enriched in inorganic sulfate; (f) treating the sulfate-enriched spent aqueous biocatalytic agent in a manner sufficient for the removal of a substantial amount of inorganic sulfate from the agent, whereby the biocatalytic activity of the agent is regenerated; and (g) reintroducing the regenerated aqueous biocatalytic agent to the reaction vessel while simultaneously introducing a petroleum liquid in need of biocatalytic desulfurization.

In a preferred embodiment of the invention, the biocatalytic agent comprises a culture of mutant *Rhodococcus rhodocrous* bacteria, ATCC No. 53968. This microbial biocatalyst is particularly advantageous in that it is capable of catalyzing the selective liberation of sulfur from HDS-refractory sulfur-bearing aromatic heterocycles, under mild conditions of temperature and pressure. Therefore, even crude oils or petroleum distillate fractions containing a high

relative abundance of refractory organic sulfur-bearing molecules can be desulfurized without exposure to conditions harsh enough to degrade valuable hydrocarbons. Additionally, the biocatalyst is regenerated and reused in the continuous method described herein; it can be used for many cycles of biocatalytic desulfurization. Moreover, the method and process of the instant invention can be readily integrated into existing petroleum refining or processing facilities.

Brief Description of the Drawings

Figure 1 is a schematic illustration of the structural formula of dibenzothiophene, a model HDS-refractory sulfur-bearing heterocycle.

Figure 2 is a schematic illustration of the cleavage of dibenzothiophene by oxidative and reductive pathways, and the end products thereof.

Figure 3 is a schematic illustration of the stepwise oxidation of dibenzothiophene along the proposed "4S" pathway of microbial catabolism.

Figure 4 is a schematic flow diagram of a preferred embodiment of the instant continuous process for biocatalytic desulfurization (BDS)of this invention.

Detailed Description of the Invention

This invention employs a biocatalytic agent which is capable of selectively liberating sulfur from the classes of organic sulfur molecules which are most refractory to current techniques of desulfurization, such as HDS. The instant biocatalytic agent is used in a continuous process for desulfurizing a petroleum liquid containing organic sulfur molecules, a significant proportion of which are comprised of

sulfur-bearing heterocycles. These HDS-refractory molecules occur in simple one-ring forms (e.g., thiophene), or more complex multiple condensed-ring forms. The difficulty of desulfurization through conventional techniques increases with the complexity of the molecule.

The tripartite condensed-ring sulfur-bearing heterocycle dibenzothiophene (DBT), shown in Figure 1, is particularly refractory to HDS treatment, and therefore can constitute a major fraction of the residual post-HDS sulfur in fuel products. Alkyl-substituted DBT derivatives are even more refractory to HDS treatment, and cannot be removed even by repeated HDS processing under increasingly severe conditions. Shih et al. Moreover, as noted above, DBTs can account for a significant percentage of the total organic sulfur in certain crude oils. Therefore, DBT is viewed as a model refractory sulfur-bearing molecule in the development of new desulfurization methods. Monticello, D.J. and W.R. Finnerty, (1985) Ann. Rev. Microbiol. 39:371-389. No naturally occurring bacteria or other microbial organisms have yet been identified which are capable of effectively degrading or desulfurizing DBT. Thus, when released into the environment, DBT and related complex heterocycles tend to persist for long periods of time and are not significantly biodegraded. Gundlach, E.R. et al., (1983) Science 221:122-129.

However, several investigators have reported the genetic modification of naturally-occurring bacteria into mutant strains capable of catabolizing DBT. Kilbane, J.J., (1990) Resour. Cons. Recycl. 3:69-79, Isbister, J.D., and R.C. Doyle, (1985) U.S. Patent No. 4,562,156, and Hartdegan, F.J. et al., (May 1984) Chem. Eng. Progress 63-67. For the most part, these mutants

desulfurize DBT nonspecifically, and release sulfur in the form of small organic sulfur breakdown products. Thus, a portion of the fuel value of DBT is lost through this microbial action. Isbister and Doyle reported the derivation of a mutant strain of *Pseudomonas* which appeared to be capable of selectively liberating sulfur from DBT, but did not elucidate the mechanism responsible for this reactivity. As shown in Figure 2, there are at least two possible pathways which result in the specific release of sulfur from DBT: oxidative and reductive.

Kilbane recently reported the mutagenesis of a mixed bacterial culture, producing one which appeared capable of selectively liberating sulfur from DBT by the oxidative pathway. This culture was composed of bacteria obtained from natural sources such as sewage sludge, petroleum refinery wastewater, garden soil, coal tar-contaminated soil, etc., and maintained in culture under conditions of continuous sulfur deprivation in the presence of DBT. The culture was then exposed to the chemical mutagen 1-methyl-3-nitro-1-nitrosoguanidine. The major catabolic product of DBT metabolism by this mutant culture was hydroxybiphenyl; sulfur was released as inorganic water-soluble sulfate, and the hydrocarbon portion of the molecule remained essentially intact. Based upon these results, Kilbane proposed that the "4S" catabolic pathway summarized in Figure 3 was the mechanism by which these products were generated. The designation "4S" refers to the reactive sulfur intermediates of the proposed pathway: DBT-sulfoxide, DBT-sulfone, DBT-sulfonate, and the liberated product, inorganic sulfate. The hydrocarbon portion of the DBT molecule remains essentially intact; in Figure 3, the theoretical hydrocarbon product, dihydroxybiphenyl is shown. In practice,

monohydroxybiphenyl is also observed. Kilbane, J.J., (1990) Resour. Cons. Recycl. 3:69-79, the teachings of which are incorporated herein by reference.

Subsequently, Kilbane has isolated a mutant strain of *Rhodococcus rhodocrous* from this mixed bacterial culture. This mutant, ATCC No. 53968, is a particularly preferred biocatalytic agent for use with the instant method of continuous biocatalytic desulfurization. The isolation and characteristics of this mutant are described in detail in J.J. Kilbane, U.S. Patent Application Serial No. 07/461,389, filed January 5, 1990, the teachings of which are incorporated herein by reference. In the instant method for biocatalytic desulfurization (BDS), the ATCC No. 53968 biocatalytic agent is employed in a continuous desulfurization process for the treatment of a petroleum liquid in which HDS-refractory organic sulfur molecules, such as the aromatic sulfur-bearing heterocycles, constitute a significant portion of the total organic sulfur content.

Figure 4 is a schematic flow diagram of the continuous process for biocatalytic desulfurization (BDS) of this invention. Petroleum liquid 1, in need of BDS treatment, enters through line 3. As discussed above and shown in Figure 3, oxygen is consumed during biocatalytic desulfurization; accordingly, a source of oxygen (5) is introduced through line 7, and is contacted with petroleum liquid 1 in mixing chamber 9 whereby oxygen tension in petroleum liquid 1 is sufficiently increased to permit biocatalytic desulfurization to proceed. In this manner, the instant process allows the practitioner to capitalize on the greater capacity of petroleum (over aqueous liquids) to carry dissolved oxygen. For example, oxygen is ten times more soluble in octane than in

water. Pollack, G.L., (1991) Science 251:1323-1330. Thus oxygen is more effectively delivered to the biocatalyst than it would be by, for example, sparging air into the reaction mixture during biocatalysis. In fact, direct sparging is to be avoided due to the tendency of such processes to produce explosive mixtures. Source of oxygen 5 can be oxygen-enriched air, pure oxygen, an oxygen-saturated perfluorocarbon liquid, etc. Oxygenated petroleum liquid thereafter passes through line 11 to injection ports 13, through which it enters reaction vessel 15.

An aqueous culture of the microbial biocatalytic agent of the present invention is prepared by fermentation in bioreactor 17, using culture conditions sufficient for the growth and biocatalytic activity of the particular micro-organism used. In order to generate maximal biocatalytic activity, it is important that the biocatalyst culture be maintained in a state of sulfur deprivation. This can be effectively accomplished by using a nutrient medium which lacks a source of inorganic sulfate, but is supplemented with DBT or a liquid petroleum sample with a high relative abundance of sulfur heterocycles. A particularly preferred microbial biocatalyst comrpises a culture of mutant *Rhodoccus rodocrous* bacteria, ATCC No. 53968. This biocatalytic agent can advantageously be prepared by conventional fermentation techniques comprising aerobic conditions and a suitable nutrient medium which contains a carbon source, such as glycerol, benzoate, or glucose. When the culture has attained a sufficient volume and/or density, it is delivered from bioreactor 17 through line 19 to mixing chamber 25, where it is optionally supplemented with fresh, sulfur-free nutrient medium as necessary. This medium is prepared in chamber 21 and delivered to the mixing chamber 25

through line 23. The aqueous biocatalytic agent next passes through mixing chamber 29, and then through line 31, to injection ports 33. It is delivered through these ports into reaction vessel 15, optimally at the same time as the oxygenated petroleum liquid 1 is delivered through ports 13. The ratio of biocatalyst to petroleum liquid (substrate) can be varied widely, depending on the desired rate of reaction, and the levels and types of sulfur-bearing organic molecules present. Suitable ratios of biocatalyst to substrate can be ascertained by those skilled in the art through no more than routine experimentation. Preferably, the volume of biocatalyst will not exceed about one-tenth the total volume in the reaction vessel (i.e., the substrate accounts for at least about 9/10 of the combined volume).

Injection ports 13 and 33 are located at positions on the vessel walls conducive to the creation of a countercurrent flow within reaction vessel 15. In other words, mixing takes place within vessel 15 at central zone 35, as the lighter organic petroleum liquid substrate rises from injection ports 13 and encounters the heavier aqueous biocatalyst falling from injection ports 33. Turbulence and, optimally, an emulsion, are generated in zone 35, maximizing the surface area of the boundary between the aqueous and organic phases. In this manner, the biocatalytic agent is brought into intimate contact with the substrate fossil fuel; desulfurization proceeds relatively rapidly due to the high concentration of dissolved oxygen in the local environment of the aromatic sulfur-bearing heterocyclic molecules on which the ATCC No. 53968 biocatalyst acts. Thus, the only rate-limiting factor will be the availability of the sulfur-bearing heterocycles themselves.

The BDS process is most effective for the desulfurization of crude oils and petroleum distillate fractions which are capable of forming a transient or reversible emulsion with the aqueous biocatalyst in zone 35, as this ensures the production of a very high surface area between the two phases as they flow past each other. However, biocatalysis will proceed satisfactorily even in the absence of an emulsion, as long as an adequate degree of turbulence (mixing) is induced or generated. Optionally, means to produce mechanical or hydrodynamic agitation at zone 35 can be incorporated into the walls of the reaction vessel. Such means can also be used to extend the residence time of the substrate petroleum liquid in zone 35, the region in which it encounters the highest levels of BDS reactivity.

In addition, it is important that the reaction vessel be maintained at temperatures and pressures which are sufficient to maintain a reasonable rate of biocatalytic desulfurization. For example, the temperature of the vessel should be between about 10°C and about 60°C; ambient temperature (about 20°C to about 30°C) is preferred. However, any temperature between the pour point of the petroleum liquid and the temperature at which the biocatalyst is inactivated can be used. The pressure within the vessel should be at least sufficient to maintain an appropriate level of dissolved oxygen in the substrate petroleum liquid. However, the pressure and turbulence within the vessel should not be so high as to cause shearing damage to the biocatalyst.

As a result of biocatalysis taking place in zone 35, the organic sulfur content of the petroleum liquid is reduced and the inorganic sulfate content of the aqueous biocatalyst is correspondingly increased. The

substrate petroleum liquid, having risen from ports 13 through BDS-reactive zone 35, collects at upper zone 37, the region of the reaction vessel located above the points at which aqueous biocatalyst is injected into the vessel (at ports 33). Conversely, the aqueous biocatalyst, being heavier than the petroleum liquid, does not enter zone 37 to any significant extent. As the desulfurized petroleum liquid collects in this region, it is drawn off or decanted from the reaction vessel at decanting port 38 from which it enters line 39. The desulfurized petroleum liquid (41) delivered from line 39 is then subjected to any additional refining or finishing steps which may be required to produce the desired low-sulfur fuel product.

Optionally, any volatile exhaust gasses (45) which form in the headspace of the reaction vessel can be recovered through line 43. These gasses can be condensed, then burned in a manner sufficient to provide any heat which may be necessary to maintain the desired level of BDS-reactivity within the reaction vessel.

Similarly, after passing through injection ports 33 and falling through BDS-reactive zone 35, the aqueous biocatalyst collects in lower zone 47, below injection ports 13. The petroleum liquid substrate entering from these injection ports does not tend to settle into zone 47 to any significant extent; being lighter than the aqueous phase, it rises into zone 35. As noted above, the biocatalyst collecting in zone 47 has acquired a significant level of inorganic sulfate as a result of its reactivity with the substrate petroleum liquid. Biocatalytic activity is depressed by the presence of inorganic sulfate, as this is a more easily assimilable form of sulfur for metabolic use than organic sulfur. Thus, the biocatalyst is said to

be "spent". However, its activity can be regenerated by removing the inorganic sulfate from the biocatalytic agent, thereby restoring the ATCC No. 53968 biocatalyst to its initial sulfur-deprived state.

This is accomplished by retrieving the spent biocatalyst from the reaction vessel through line 49, and treating it in a manner sufficient to remove inorganic sulfate. The spent agent is first introduced into chamber 51, in which solids, sludges, excess hydrocarbons, or excess bacteria (live or dead), are removed from the aqueous biocatalyst and recovered or discarded (53). The aqueous biocatalyst next passes through chamber 55, and optional chamber 57, where it is contacted with an appropriate ion exchange resin or resins, such as an anion exchange resin and a cation exchange resin. Suitable ion exchange resins are commercially available; several of these are highly durable resins, including those linked to a rigid polystyrene support. These durable ion exchange resins are preferred. Two examples of polystyrene-supported resins are Amberlite® IRA-400-OH (Rohm and Haas), and Dowex 1X8-50 (Dow Chemical Co.) Dowex MSA-1 (Dow Chemical Co.) is an example of a suitable non-polystyrene supported resin. The optimal ion exchange resin for use herein can be determined through no more than routine experimentation. Inorganic sulfate ions bind to the resin(s) and are removed from the aqueous biocatalytic agent. As a result, biocatalytic activity is regenerated.

Alternative means to remove aqueous sulfate and thereby regenerate biocatalytic activity can also be employed. Suitable alternatives to treatment with an ion exchange resin include, for example, treatment with an agent capable of removing sulfate ion by

precipitation. Suitable agents include the salts of divalent cations such as barium chloride or calcium hydroxide. Calcium hydroxide is preferred due to the chemical nature of the sulfate-containing reaction product formed: calcium sulfate (gypsum), which can be readily separated from the aqueous biocatalyst. Other examples of suitable regeneration means include treatment with semipermeable ion exchange membranes and electrodialysis.

Any of the above means for regenerating biocatalytic activity can be performed by treating the aqueous culture of the biocatalyst, or by initially separating (e.g., by sieving) the microbial biocatalyst from the aqueous liquid and treating the liquid alone, then recombining the biocatalyst with the sulfate-depleted aqueous liquid.

The regenerated aqueous biocatalyst proceeds to mixing chamber **29**, where it is mixed with any fresh, sulfur-free nutrient medium (prepared in chamber **21**) and/or any fresh ATCC No. 53968 culture (prepared in bioreactor **17**), which may be required to reconstitute or replenish the desired level of biocatalytic activity.

The regenerated biocatalytic agent is delivered through line **31** to injection ports **33**, where it reenters the reaction vessel (**15**) and is contacted with additional petroleum liquid in need of BDS treatment, entering the reaction vessel through injection ports **13** in the manner described previously. It is desirable to monitor and control the rates of reactants entering and products being removed from the reaction vessel, as maintaining substantially equivalent rates of entry and removal will maintain conditions (e.g., of pressure) sufficient for biocatalysis within the vessel. In this manner, a continuous stream of desulfurized petroleum

liquid is generated, without the need to periodically pump the contents of the reaction vessel into a settling chamber where phase separation takes place, as described in Madkavkar, A.M. (1989) U.S. Patent No. 4,861,723, and Kirshenbaum, I. (1961) U.S. Patent No. 2,975,103.

The progress of BDS treatment of the petroleum liquid within the vessel can be monitored using conventional techniques, which are readily available to those skilled in the art. Baseline samples can be collected from the substrate before it is exposed to the biocatalyst, for example from sampling ports located at mixing chamber 9. Post-BDS samples can be collected from the desulfurized petroleum liquid which collects within the reaction vessel at zone 37, through sampling ports located in the vessel wall, or a sampling valve located at decanting port 38. The disappearance of sulfur from substrate hydrocarbons such as DBT can be monitored using a gas chromatograph coupled with mass spectrophotometric (GC/MS), nuclear magnetic resonance (GC/NMR), infrared spectrometric (GC/IR), or atomic emission spectrometric (GC/AES, or flame spectrometry) detection systems. Flame spectrometry is the preferred detection system, as it allows the operator to directly visualize the disappearance of sulfur atoms from combustible hydrocarbons by monitoring quantitative or relative decreases in flame spectral emissions at 392 nm, the wavelength characteristic of atomic sulfur. It is also possible to measure the decrease in total organic sulfur in the substrate fossil fuel, by subjecting the unchromatographed samples to flame spectrometry. If the extent of desulfurization is insufficient, the desulfurized petroleum liquid collected from line 39 can optionally be reintroduced through line 3 and

subjected to an addional cycle of BDS treatment. Alternatively, it can be subjected to an alternative desulfurization process, such as HDS.

In other preferred embodiments of the present method, an enzyme or array of enzymes sufficient to direct the selective cleavage of carbon-sulfur bonds can be employed as the biocatalyst. Preferably, the enzyme(s) responsible for the "4S" pathway can be used. Most preferably, the enzyme(s) can be obtained from ATCC No. 53968 or a derivative thereof. This enzyme biocatalyst can optionally be used in carrier-bound form. Suitable carriers include killed "4S" bacteria, active fractions of "4S" bacteria (e.g., membranes), insoluble resins, or ceramic, glass, or latex particles.

CLAIMS

1. A continuous process for desulfurizing a petroleum liquid which contains organic sulfur molecules, a significant portion of which are comprised of sulfur-bearing heterocycles, comprising the steps of:

(a) contacting the petroleum liquid with a source of oxygen under conditions sufficient to increase the oxygen tension in the petroleum liquid to a level at which the biocatalytic oxidative cleavage of carbon-sulfur bonds in sulfur-bearing heterocycles proceeds;

(b) introducing the oxygenated petroleum liquid to a vertically elongated reaction vessel having means to collect and decant organic liquid from an upper region and means to remove or retrieve an aqueous liquid from a lower region, while simultaneously introducing an aqueous, sulfur-depleted biocatalytic agent to the reaction vessel, the agent being capable of inducing the selective oxidative cleavage of carbon-sulfur bonds in sulfur-bearing heterocycles;

(c) incubating the oxygenated petroleum liquid with the biocatalytic agent in the reaction vessel under conditions sufficient for biocatalytic oxidative cleavage of said carbon-sulfur bonds, whereby the organic sulfur content of the treated petroleum liquid is significantly reduced and a significant amount of water-soluble inorganic sulfate is generated;

(d) removing the petroleum liquid from the reaction vessel by decanting it from the upper region of the vessel;

(e) removing the spent aqueous biocatalytic agent from the reaction vessel by recovering it from the lower region of the vessel, the spent agent being significantly enriched in inorganic sulfate;

(f) treating the sulfate-enriched aqueous biocatalytic agent in a manner sufficient for the removal of a substantial amount of inorganic sulfate from the agent, whereby the biocatalytic activity of the agent is regenerated; and

(g) introducing regenerated aqueous biocatalytic agent to the reaction vessel while simultaneously introducing a petroleum liquid in need of biocatalytic desulfurization.

2. A method of Claim 1 wherein the biocatalytic agent is introduced to the reaction vessel at a site spatially distinct from the site of introduction of the petroleum liquid, in such a fashion as to create a countercurrent flow system within the reaction vessel.

3. A method of Claim 2 wherein the creation of a countercurrent flow system provides sufficient mixing between the petroleum liquid and the aqueous biocatalyst for a significant number of carbon-sulfur bonds to be biocatalytically cleaved within a reasonable period of time.

4. A method of Claim 3 wherein the petroleum liquid in need of treatment is capable of forming a transient or reversible emulsion with the aqueous biocatalyst, whereby an emulsion zone is produced in the reaction vessel when the petroleum liquid and the biocatalyst are introduced under conditions sufficient to generate a countercurrent flow system, the emulsion zone being bounded above by a zone enriched in treated (desulfurized) petroleum liquid, and bounded below by a zone enriched in spent (inorganic sulfate-enriched) aqueous biocatalyst.

5. A method of Claim 4 wherein the formation or maintenance of the emulsion zone is accomplished with the assistance of mechanical or hydrodynamic agitation.

6. A method of Claim 4 wherein regenerated (inorganic sulfate-depleted) aqueous biocatalyst is introduced to the reaction vessel at or close to the boundary between the treated petroleum liquid zone and the emulsion zone, and petroleum liquid to be treated by the biocatalyst is introduced to the reaction vessel at or close to the boundary between the emulsion zone and the spent aqueous biocatalyst zone.

7. A method of Claim 6 wherein the rates of addition of reactants to and removal of products from the reaction vessel are monitored and controlled such that the rates of removal from and addition to the reaction vessel are substantially equivalent, the reactants comprising petroleum liquid to be treated and a regenerated aqueous biocatalytic agent, and the products comprising desulfurized petroleum liquid and spent aqueous biocatalytic agent.

8. A method of Claim 1 wherein the aqueous biocatalytic agent is a culture of a microbial organism which expresses an enzyme capable of liberating sulfur from sulfur-bearing aromatic heterocycles by catalyzing the sulfur-specific oxidative cleavage of heterocyclic aromatic rings, the products of the cleavage reaction being inorganic sulfate ions and desulfurized organic molecules.

9. A method of Claim 8 wherein the aqueous biocatalytic agent is a culture of mutant *Rhodococcus rhodocrous* bacteria, ATCC No. 53968.

10. A method of Claim 8 wherein the regeneration of the aqueous biocatalytic agent comprises both
(a) the removal of a significant number of inorganic sulfate ions; and
(b) the replenishment of nutrients and/or bacterial culture as required to maintain sufficient biocatalytic activity in the regenerated agent.

11. A method of Claim 10 wherein the removal of sulfate ions is accomplished by contacting the spent aqueous biocatalyst with a resin capable of binding inorganic sulfate ions, under conditions sufficient for the binding of inorganic sulfate ions to the resin.

12. A method of Claim 1 wherein the aqueous biocatalytic agent is an enzyme capable of liberating sulfur from sulfur-bearing aromatic heterocycles by catalyzing the sulfur-specific oxidative cleavage of heterocyclic aromatic rings, the products of the cleavage reaction being inorganic sulfate ions and desulfurized organic molecules.

13. A method of Claim 12 wherein the enzyme is derived from mutant *Rhodococcus rhodorous* bacteria ATCC No. 53968.

14. A method of Claim 12 wherein the enzyme is bound to a carrier.

15. A method of Claim 12 wherein the removal of sulfate ions is accomplished by contacting the spent aqueous biocatalyst with a resin capable of binding inorganic sulfate ions, under conditions sufficient for the binding of inorganic sulfate ions to the resin.

16. A method of Claim 1 including the additional step of trapping and condensing any volatile, flammable exhaust gasses escaping from the reaction vessel during the removal of the desulfurized petroleum liquid, and burning the same in a manner sufficient to provide any heat necessary to promote biocatalytic activity within the reaction vessel.

17. A continuous, cyclic process for desulfurizing a petroleum liquid which contains organic sulfur molecules, a significant proportion of which are comprised of sulfur-bearing aromatic heterocycles, the petroleum liquid being capable of forming a reversible emulsion with an aqueous phase, comprising the steps of:

(a) contacting the petroleum liquid with a source of oxygen under conditions sufficient to increase the oxygen tension in the petroleum liquid to a level at which the biocatalytic oxidative cleavage of carbon-sulfur bonds in sulfur-bearing heterocycles proceeds;

(b) introducing the oxygenated petroleum liquid to a reaction vessel while simultaneously introducing an aqueous, sulfur-depleted biocatalytic agent to the reaction vessel at a site spatially distinct from the site of introduction of the petroleum liquid, in such a fashion as to create a countercurrent flow system within the reaction vessel, the biocatalytic agent being capable of inducing the selective oxidative cleavage of carbon-sulfur bonds in sulfur-bearing heterocycles;

(c) incubating the oxygenated petroleum liquid with the biocatalytic agent in the reaction vessel under sufficient conditions for a significant number of biocatalytic cleavages to occur, said conditions comprising the formation of a zone of reversible emulsion between the oxygenated petroleum liquid and the aqueous biocatalyst, bounded above by a zone enriched in biocatalytically desulfurized petroleum liquid and bounded below by a zone enriched in spent (inorganic sulfate-enriched) aqueous biocatalyst;

(d) removing the desulfurized petroleum liquid from the vessel through a decanting port located at a site of the vessel wall corresponding to the region occupied by the zone enriched in biocatalytically desulfurized petroleum liquid, while retrieving spent biocatalytic agent from the vessel through a recovery port located at a site of the vessel wall corresponding to the region occupied by the zone enriched in spent aqueous biocatalyst;

(e) regenerating spent biocatalyst by

(i) treating it with an agent capable of substantially decreasing the concentration of inorganic sulfate ions in an aqueous liquid in such a manner and for such a period of time that the aqueous biocatalytic agent becomes sulfur-depleted, and

(ii) replenishing such components of the aqueous phase as may be required to maintain sufficient biocatalytic activity in the regenerated agent; and

(f) introducing regenerated aqueous biocatalytic agent to the reaction vessel while simultaneously introducing a petroleum liquid in need of biocatalytic treatment, in such a fashion as to maintain countercurrent flow and a zone of emulsion within the reaction vessel.

18. A method of Claim 17 including the additional steps of:

(a) trapping and condensing any volatile, flammable exhaust gasses excaping from the reaction vessel during the decanting of the biocatalytically desulfurized liquid petroleum; and

(b) burning the condensed exhaust gasses in a manner sufficient to provide any heat which may be necessary to promote a sufficient level of biocatalytic activity in the reaction vessel.

19. A method of Claim 17 wherein the aqueous biocatalytic agent is a culture of a microbial organism which expresses an enzyme capable of liberating sulfur from sulfur-bearing aromatic heterocycles by catalyzing the sulfur-specific oxidative cleavage of heterocyclic aromatic rings, the products of the cleavage reaction being inorganic sulfate ions and desulfurized organic molecules.

20. A method of Claim 19 wherein the aqueous biocatalytic agent is a culture of mutant *Rhodococcus rhodocrous* bacteria, ATCC No. 53968.

21. A method of Claim 17 wherein the aqueous biocatalytic agent is an enzyme capable of liberating sulfur from sulfur-bearing aromatic heterocycles by catalyzing the sulfur-specific oxidative cleavage of heterocyclic aromatic rings, the products of the cleavage reaction being inorganic sulfate ions and desulfurized organic molecules.

22. A method of Claim 21 wherein the enzyme is derived from mutant *Rhodococcus rhodorous* bacteria ATCC No. 53968.

23. A method of Claim 21 wherein the enzyme is bound to a carrier.

24. A continuous, cyclic system for the desulfurization of a petroleum liquid which contains organic sulfur molecules, a significant proportion of which are comprised of sulfur-bearing aromatic heterocycles, the petroleum liquid being capable of forming a reversible emulsion with an aqueous phase, comprising:

(a) means for contacting the petroleum liquid with a source of oxygen under conditions sufficient to increase the oxygen tension in the petroleum liquid to a level at which the biocatalytic oxidative cleavage of carbon-sulfur bonds in sulfur-bearing heterocycles proceeds;

(b) means for introducing the oxygenated petroleum liquid to a reaction vessel while simultaneously introducing an aqueous, sulfur-depleted biocatalytic agent to the reaction vessel at a site spatially distinct from the site of introduction of the petroleum liquid, in such a fashion as to create a countercurrent flow system within the reaction vessel, the biocatalytic agent being capable of inducing the selective oxidative cleavage of carbon-sulfur bonds in sulfur-bearing heterocycles;

(c) means for incubating the oxygenated petroleum liquid with the biocatalytic agent in the reaction vessel under sufficient conditions for a significant number of biocatalytic cleavages to occur, said conditions comprising the formation of a zone of reversible emulsion between the oxygenated petroleum liquid and the aqueous biocatalyst, bounded above by a zone enriched in biocatalytically desulfurized petroleum liquid and bounded below by a zone enriched in spent (inorganic sulfate-enriched) aqueous biocatalyst;

(d) means for removing the desulfurized petroleum liquid from the vessel through a decanting port located at a site of the vessel wall corresponding to the region occupied by the zone enriched in biocatalytically desulfurized petroleum liquid, while retrieving spent biocatalytic agent from the vessel through a recovery port located at a site of the vessel wall corresponding to the region occupied by the zone enriched in spent aqueous biocatalyst;

(e) means for regenerating spent biocatalyst by
(i) treating it with an agent capable of substantially decreasing the concentration of inorganic sulfate ions in an aqueous liquid in such a manner and for such a period of time that the aqueous biocatalytic agent becomes sulfur-depleted, and
(ii) replenishing such components of the aqueous phase as may be required to maintain sufficient biocatalytic activity in the regenerated agent; and

(f) means for introducing regenerated aqueous biocatalytic agent to the reaction vessel while simultaneously introducing a petroleum liquid in need of biocatalytic treatment, in such a fashion as to maintain countercurrent flow and a zone of emulsion within the reaction vessel.

25. A system of Claim 24 including additional means for:

(a) trapping and condensing any volatile, flammable exhaust gasses excaping from the reaction vessel during the decanting of the biocatalytically desulfurized liquid petroleum; and

(b) burning the condensed exhaust gasses in a manner sufficient to provide any heat which may be necessary to promote a sufficient level of biocatalytic activity in the reaction vessel.

Fig. 3

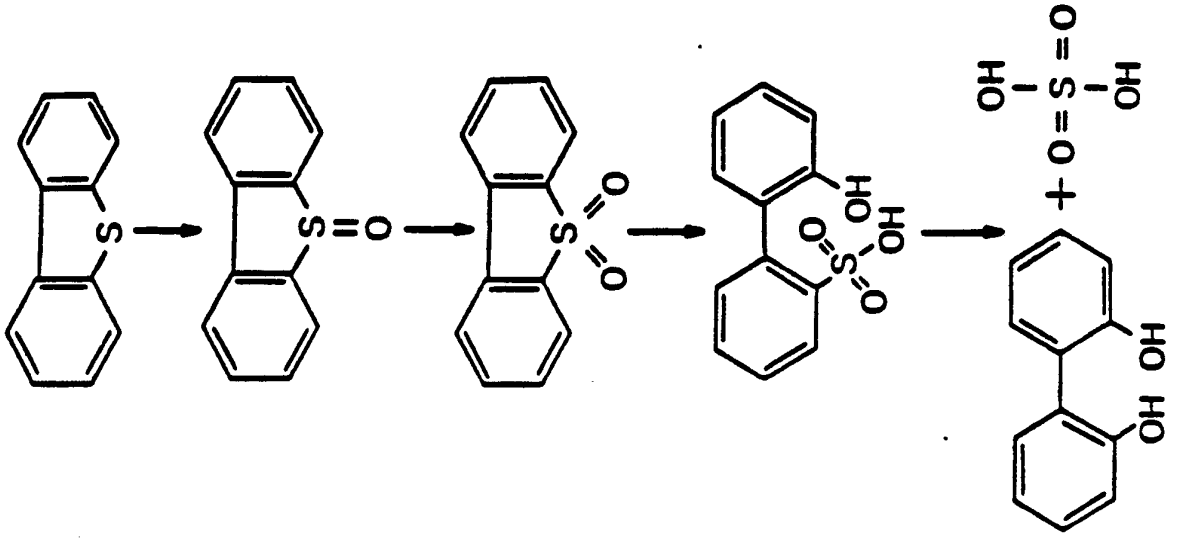




Fig. 1

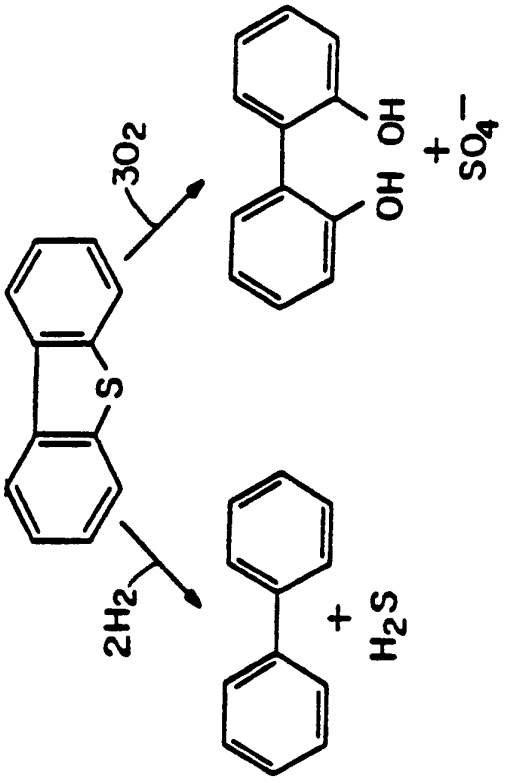


Fig. 2

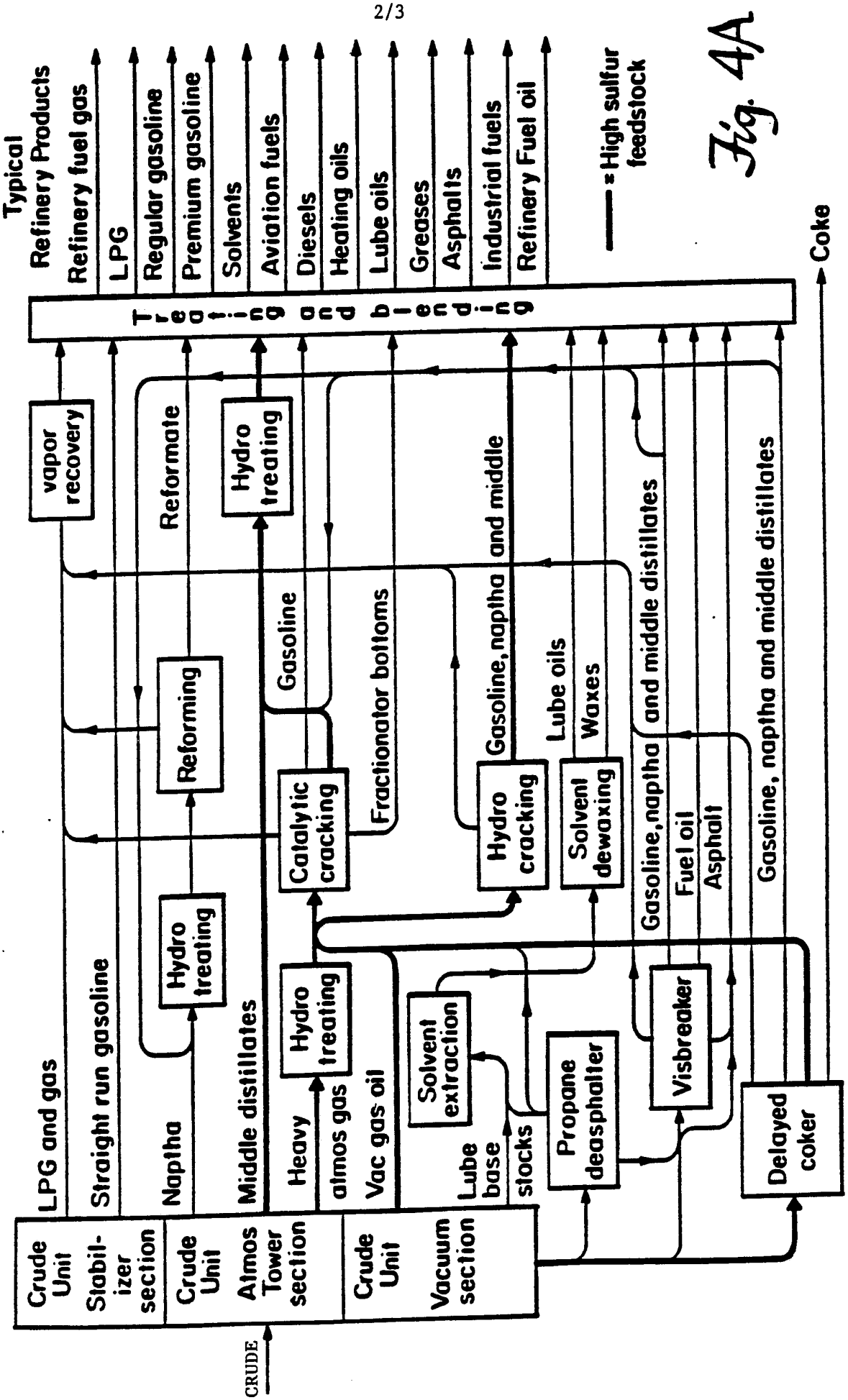

Typical Refinery Products
Refinery fuel gas
LPG
Regular gasoline
Premium gasoline
Solvents
Aviation fuels
Diesels
Heating oils
Lube oils
Greases
Asphalts
Industrial fuels
Refinery Fuel oil
Treating and blending
= High sulfur feedstock
Coke
CRUDE
Crude Unit Stabil-izer section
Crude Unit Atmos Tower section
Crude Unit Vacuum section
LPG and gas
Straight run gasoline
Naptha
Middle distillates
Heavy atmos gas
Vac gas oil
Lube base stocks
vapor recovery
Hydro treating
Reformate
Reforming
Hydro treating
Gasoline
Catalytic cracking
Fractionator bottoms
Gasoline, naptha and middle
Hydro cracking
Lube oils
Waxes
Solvent dewaxing
Solvent extraction
Propane deasphalter
Gasoline, naptha and middle distillates
Fuel oil
Asphalt
Visbreaker
Gasoline, naptha and middle distillates
Delayed coker

Fig. 4A

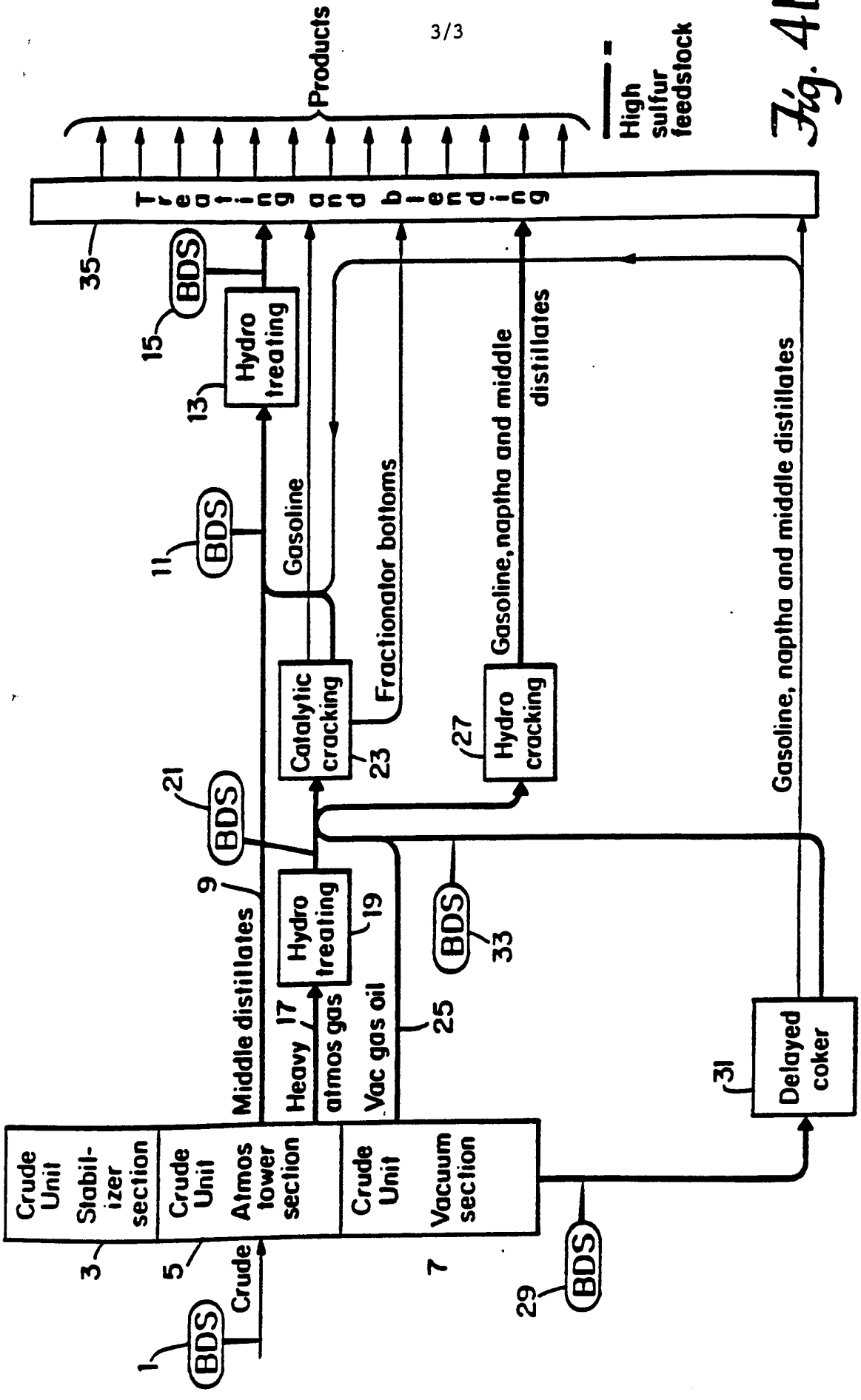

Products
High sulfur feedstock
Treating and blending
35
BDS
15
Hydro treating
13
BDS
11
Gasoline
Fractionator bottoms
Gasoline, naptha and middle distillates
Catalytic cracking
23
Hydro cracking
27
21
BDS
9
Middle distillates
Hydro treating
19
Heavy atmos gas
17
Vac gas oil
25
BDS
33
Gasoline, naptha and middle distillates
Delayed coker
31
Crude Unit Stabil-izer section
3
Crude Unit Atmos tower section
5
Crude Unit Vacuum section
7
BDS
29
Crude
BDS
1

Fig. 4B