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(54) **METHOD FOR BIO-OXIDATIVE DESULFURIZATION OF LIQUID HYDROCARBON FUELS AND PRODUCT THEREOF**

VERFAHREN ZUR BIOOXIDATIVEN DESULFURISIERUNG VON FLÜSSIGEN KOHLENWASSERSTOFF-BRENNSTOFFEN UND PRODUKT DARAUS

PROCEDE DE DESULFURISATION BIO-OXYDATIVE DE COMBUSTIBLES A HYDROCARBURES LIQUIDES ET PRODUIT AINSI OBTENU

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Description

Field of the Invention

[0001] This invention, in general, relates to the field of hydrocarbon fuel, in particular to the method for desulfurization of the liquid hydrocarbon fuel and product thereof. More specifically but without restriction to the particular embodiments hereinafter described in accordance with the best mode of practice, this invention provides a method for selective biocatalytic oxidation of sulfur containing compounds present in fossil fuels employing enzyme lipase as a biocatalyst.

Background of the Invention

[0002] Sulfur compounds are widely distributed in petroleum distillates of all boiling ranges. In gasoline, the principal sulfur compounds are mercaptans, aliphatic sulfides, disulfides, five and six membered ring cyclic sulfides, while diesel fuel is rich in benzothiophenes and alkyl substituted benzothiophenes. The removal of sulfur from fuels is necessary for both industrial and environmental reasons. Sulfur in fuels poisons catalytic converters, corrodes the parts of internal combustion engines and refinery equipment because of the formation of oxy-acids of sulfur: The combustion of organosulfur compound of petroleum-derived fuels leads to the atmospheric emission of sulfur oxides (SO_x) that contribute to acid rain and air contamination by particulate matter. The demand for low-sulfur fossil fuels has been intensified by the stringent regulatory standards for reduced levels of sulfur-oxides in atmospheric emissions. The introduction of ultra low sulfur fuels (<10 ppmw) has been proposed in various countries of the world.

[0003] Hydrotreating is the most common reductive technology used by refineries to remove sulfur from gasoline and diesel feedstock. In hydro-desulfurization process using supported mixed metal sulfide catalysts, the sulfur in the compounds is removed by hydrogenation as H_2S. Hydrotreating diesel feedstocks for a low-sulfur product require a larger reactor volume, longer processing times, and substantial hydrogen and energy inputs. However, more active catalysts and recent developments in fixed bed hydro treating technology have reduced the time spent in the reactor, thus lowering the required reactor volume and operating costs but it is limited in treating benzothiophene and dibenzothiophene especially DBTs having alkyl substitutions on their 4 and/or 6 positions. The 4,6 disubstituted compounds do not adsorb on the active sites of the catalyst due to steric limitations. An alternative process, capable of being operated under moderate conditions and without the requirements for H_2 and catalysts and capable of desulfurizing substituted DBTs, is therefore urgently required.

[0004] Oxidation of the organosulfur compounds can be done using chemical (chemical oxidative desulfurization) or biological (biological oxidative desulfurization) oxidizing agents. Various studies on the chemical oxidative desulfurization (COD) process have reported the use of different oxidizing agents, such as H_2O_2 in combination with Acetic acid, H_2O_2 with Formic acid, NO_2, Ozone, Phosphotungstic acid/hydrogen peroxide and Tert-butyl-hydroperoxide: But the usage of such oxidants is known to be very non-selective and slow. The use of large amount of hydrogen peroxide make it environment reactive and moreover, safety-related issues are also an area of concern.

[0005] United States Patent 5,958,224 relates to a process for removing hard sulfurs from hydrocarbon streams by selectively oxidizing hard sulfurs in a hydrotreated stream into the corresponding sulfoxides and sulfones, under oxidizing conditions in the presence of an effective amount of an oxidizing agent, wherein the oxidizing agent is a peroxometal complex. The said peroxometal complex is selected from the group consisting of one of the following forms: $LMO(O_2)_2$, $(LL')MO(O_2)_2$, $LMO(O_2)_2.H_2O$, and mixtures thereof, wherein M is selected from the group consisting of Mo, W, Cr and mixtures thereof and wherein L and L' are neutral ligands.

[0006] United States Patent 6,160,193 describes a two-step process for the removal of sulfur and nitrogen containing compounds from petroleum distillates. The first step of the process is to oxidize the sulfur-containing compounds of the fuel. The oxidization process converts sulfur compounds to highly polar sulfones. Nitrogen compounds are likewise converted to polar oxidized species. An example of an oxidizing agent that can be successfully used in this process is peroxysulfuric acid, often called Caro's acid. Oxidations are typically carried out at about 30 to 100°C, and preferably at 60 to 95°C. Low pressures are used, typically less than about 150 psig (pounds per square inch, gauge), and preferably less than about 30 psig, the autogenous pressures created by the vapors of the fuel and the various reactants and solvents. Sulfur- and nitrogen-containing compounds are oxidized using a selective oxidant to create compounds that can be preferentially extracted from a petroleum distillate due to their increased relative polarity. The second step of the process uses a solvent to extract the sulfones from the fuel oil.

[0007] United States Patent 6,596,177 describes a method of improving the quality of diesel fuel. In this invention, sulfur in diesel fuel is oxidized to a sulfoxide or a sulfone utilizing an oxidizing gas. Once in the sulfoxide or sulfone form, the sulfur-containing molecule can be removed from the diesel fuel by distillation or extraction. The oxidizing gas, preferably ozone which is utilized immediately upon its manufacture, is formed into sub-micron size bubbles which are dispersed into diesel fuel, after which the treated fuel is recovered. Due to the sub-micron size of the bubbles, the surface area of the oxidizing gas is greatly increased, thereby greatly increasing the efficiency of the oxidation reaction.

[0008] United States Patent 6,638,419 relates to a method for obtaining oil products such as diesel fuel with improved quality, from a gas oil fraction containing organosulphur compounds such as dibenzenethiophenes and/or their derivatives. The method includes at least two steps which consist in: (a) liquid-liquid extraction wherein the diesel fuel fraction is contacted with a solvent chosen from the group consisting of methanol, acetonitrile, monomethyl formamide, dimethyl formamide, dimethyl acetamide, N-methyl pyrrolidone, dimethyl sulfoxide and furfural, so as to obtain a gas oil-type raffinate with low content of sulphur and aromatic compounds and an extract rich in solvent and with high content of sulphur and aromatic compounds; (b) oxidation of the extract sulphur compounds by biological or chemical route , so as to obtain, after separation, a heavy gas oil-type hydrocarbon effluent with low sulphur content, and a residue comprising oxidized organosulphur compounds.

[0009] Chemical oxidation route as described above are known to be very non-selective and slow. The use of large amount of hydrogen peroxide makes it environment reactive and moreover, safety-related issues are also an area of concern.

[0010] Organic sulfur compounds can also be removed from a fossil fuel by a process that combines oxidative desulfurization with the use of ultrasound. The oxidative desulfurization is achieved by combining the fossil fuel with a hydroperoxide-oxidizing agent in the presence of an aqueous fluid, and the ultrasound is applied to the resulting mixture to increase the reactivity of the species in the mixture. Although this process is effective, it is energy-intensive and requires aqueous medium for reaction.

[0011] An Environmentally benign and less energy intensive option of the oxidative desulfurization is bio-assisted system. This approach utilizes either whole microbial cell (microbial desulfurization) or microbial enzymes for oxidation of organosulfur compounds. The microbial desulfurization (BDS) utilizes the versatility of certain microorganisms to selectively degrade the organic sulfur compounds. In this process the microbes utilize the organosulfur compound as sulfur source for their growth and oxidize DBT to DBT sulfones and subsequently to 2-hydroxybiphenyl (2-HBP) and sulfate. Various microbes like Corynebacterium sp., *Pseudomonas* sp., *Rhodococcus erythropolis,* and *Rhodococcus* sp. *strain IGTS8* etc., are reported to desulfurize diesel fuel (Microbiol Mol Bio Rev, 67, p 503-549, 2003). Biodesulfurization has the potential benefits of low capital and operating costs and will produce fewer amounts of greenhouse gases. Microbial desulfurization, however, requires a vast amount of water during the desulfurization process to sustain microbial activity (intrinsic mass transfer issue). Beside this, microorganisms for desulfurization also degrade some aliphatic or aromatic fractions of fuels causing the decrease of fuel yield. On the other hand, the microbial biocatalyst must have broad substrate specificity for the various organosulfur compounds present in oil.

[0012] Several enzymes are reported to oxidize thiophenes and organosulfur compounds in vitro; e.g. cytochromes P450, lignin peroxidase from the white rot fungus *Phanerochaete chrysosporium* lactoperoxidase chloroperoxidase from *Caldariomyces fumago* and horseradish peroxidase. Some of these enzymes are also reported to oxidize organosulfur compounds of diesel matrix. Some non-enzymatic hemoproteins are also able to perform the DBT oxidation in vitro, such as hemoglobin, cytochrome c and microperoxidase as summarized below. All the proteins mentioned above are hemoproteins, and in all cases the product of the biocatalytic oxidations are the respective sulfoxides.

[0013] United States Patent 5,985,650 describes a method for enhancing the rate of desulfurizing a fossil fuel containing organic sulfur compounds, comprising the steps of: a) contacting the fossil fuel with an aqueous phase containing a biocatalyst capable of cleaving carbon-sulfur bonds and a rate-enhancing amount of a flavoprotein, thereby forming a fossil fuel and aqueous phase mixture; b) maintaining the mixture of step (a) under conditions sufficient for cleavage of the carbon-sulfur bonds of the organic sulfur molecules by the biocatalyst, thereby resulting in a fossil fuel having a reduced organic sulfur content; and c) separating the fossil fuel having a reduced organic sulfur content from the resulting aqueous phase.

[0014] US Patent 6,071,738 relates to a method for the desulfurization of a fossil fuel containing one or more organosulfur compounds. This method comprises the steps of (1) contacting the fossil fuel with a biocatalyst capable of converting the organosulfur compound to an oxyorganosulfur compound which is separable from the fossil fuel; and (2) separating the oxyorganosulfur compound from the fossil fuel. Biocatalytic enzyme preparations that are useful in the present invention include microbial lysates, extracts, fractions, subfractions, or purified products obtained by conventional means and capable of carrying out the desired biocatalytic function. Generally, such enzyme preparations are substantially free of intact microbial cells, i.e., the enzyme preparations are cell-free fractions.

[0015] US Patent 6,461,859 relates to a method of removing thiophenic and organosulfide compounds from a fossil fuel comprising the steps of contacting the fossil fuel with hemoproteins, which oxidize the sulfur containing compounds to sulfoxides and sulfones in a reaction system containing organic solvent or not, and followed by a distillation step in which sulfoxides and sulfones are removed from the fuel. Used biocatalysts include hemoproteins such as chloroperoxidase from *Caldariomyces fumago,* and peroxidases and cytochromes from animal, plant or microbial cells in free or immobilized forms. The reaction is carried out in the presence of the fuel alone or with addition of any organic solvent. The biocatalytically oxidized fuel is then distilled in order to eliminate the heavy fraction which contains most of the oxidized organosulfur compounds. The light distillate contains significantly lower concentrations of sulfur when compared with the starting fossil fuel.

[0016] In a study, Ayala et al. (Fuel Processing Technology, 57 p 101-111, 1998) has enzymically oxidized Straight-run diesel fuel containing 1.6% of sulfur utilizing chloroperoxidase from *Caldariomyces fumago.* Most organosulfides and thiophenes were transformed to form sulfoxides and sulfones. The oxidized organosulfur compounds can be effectively removed by distillation. The resulting fraction after distillation contained only 0.27% sulfur, while the untreated straight-run diesel fuel after the same distillation process still showed 1.27% sulfur. When nine organosulfur compounds were transformed using choloroperoxidase, all organosulfur compounds tested were oxidized to form sulfoxides and sulfones.

[0017] The method reported in prior art for biocatalytic oxidation of organosulfur compounds require considerable amount of water in diesel mixture for reaction leading to stable emulsion formation and poor recovery of water soluble products. Moreover, some of the enzyme requires costly co-factors (co-enzymes) and are pH sensitive. The major drawback of these co-factor dependent enzymatic desulfurization reactions is that co-factors are gradually destroyed due to undesired side reactions. These co-factors (co-enzymes) are very expensive.

[0018] Therefore, there is a need for an industrially feasible method for the desulfurization of fossil fuel, which involves minimum steps, is less time consuming, is economical by use of inexpensive route to perform the method and the other drawbacks associated with the prior art. The present invention addresses these needs.

[0019] The invention described herein directly addresses the problems posed by the limitations of prior art techniques for desulfurizing fossil fuels. The instant invention provides for the removal of a significant amount of sulfur from the fossil fuel employing a lipase enzyme as a biocatalyst.

Summary of the Invention

[0020] It is a principal object of the present invention to provide a method for production of ultra-low-sulfur fuel oils employing bio-oxidative desulfurization of fossil fuel using a selective catalyst, wherein the catalyst used in the method is lipase enzyme.

[0021] Further object of the present invention is to provide a method for bio-oxidative desulfurization of fossil fuel to remove sulfur content from said fuel by selective activity on carbon-sulfur-carbon bonds in said sulfur-containing compounds without apparently changing non-sulfur-bearing components of the fuel.

[0022] Furthermore, the instant invention provides a pH independent enzyme based approach for desulfurization of liquid hydrocarbon fuels, wherein fuel itself can act as a solvent, hence does not require water and/or co-factors) for reactivity.

[0023] In particular, the objects are achieved by a method according to claim 1. Preferred embodiments are subject of dependent claims. The process for producing ultra-low-sulfur fuel oils according to the present invention comprises the step of contacting the fossil fuel with lipase enzyme in presence of incremental amount of hydrogen peroxide and carboxylic acid to oxidize thiophenes and organosulfides to their respective sulfoxides and sulfones, further, removing the resultant oxidized polar compounds from fuel by any suitable process such as extraction by appropriate solvents, by distillation or any other physiochemical process.

[0024] In accordance with the invention, there is provided a method to product ultra-low-sulfur fuel oils, wherein hydrogen peroxide is added in controlled and incremental amounts in the reaction mixtuxe under proper mixing conditions to avoid excess concentration of hydrogen peroxide in the fuel.

[0025] The enzyme used in the method is obtained from microbial source and is recoverable and reusable.

[0026] The high effectiveness of the present process is the observation that dibenzothiophene and related sulfur-bearing organic sulfides, which are the most refractory organic sulfur compounds in fossil fuels, are readily converted by this process to the corresponding sulfoxides and sulfones under ambient conditions, however, this catalyst is active upto 70°C, preferably 35 to 60°C.

[0027] An industrial and environmental friendly hydrocarbon fuel having ultra-low-sulfur content is effectively produced by the method described above.

Detailed Description of the Invention

[0028] The present invention deals with an improved method for the production of ultra-low-sulfur fuel by desulfurization of said fuel using lipase enzyme as a biocatalyst. The disclosed biodesulfurization offers the potential for a more selective and cost-effective method for lowering the sulfur content of said fossil fuel.

[0029] The removal of sulfur compounds, which are widely distributed in petroleum distillates of all boiling ranges, is necessary for both industrial and environmental reasons. The demand for low-sulfur fossil fuels has been intensified by the stringent regulatory standards for reduced levels of oxides of sulfur in atmospheric emissions. The introduction of ultra sulfur diesel fuel (sulfur <10 ppmw) has been proposed in various countries. Hydrodesulfurization (HDS) is the most common reductive technology used by refineries to remove sulfur from gasoline and diesel feedstock. But HDS is limited in treating benzothiophene and dibenzothiophene (DBT), especially DBTs having alkyl substitutions on their 4 and/or 6 positions. An alternative way of removing the sulfur compounds (without using hydrogen) is to oxidize them

into sulfones and removing oxidized polar species by extraction or adsorption. The advantage of oxidative route is that the sterically hindered DBTs are relatively more prone for oxidation than the unsubstituted compounds. Several chemical oxidizing agents like H_2O_2, formic acid, NO_2, ozone, phosphotungstic acid, tert-butyl-hydroperoxide etc., have been reported in the literature, but reactions are non-selective and slow. Bio-assisted oxidative desulfurization using microbial enzyme and whole cell microbial systems has also been reported. These systems are considered to be environmentally benign and offer less energy -intensive option for removal of organosulfur compounds from the diesel fuels. However, such enzymatic systems work under aqueous phase and inevitably require co-factors. This leads to practical difficulty of getting rid of hydrocarbon - water phase emulsions and regeneration of co-factors.

[0030] The process of production of ultra-low-sulfur fuel of the present invention comprises the step of contacting the fossil fuel with lipase enzyme in presence of incremental amount of hydrogen peroxide and carboxylic acid to oxidize thiophenes and organosulfides to their respective sulfoxides and sulfones, further, removing the resultant oxidized polar compounds from fuel by any suitable process such as extraction by appropriate solvents, by distillation or any other physiochemical process.

[0031] An advantage of the process of this invention is that oxidative enzymatic system employed has greater selectivity towards the conversion of alkylated aromatic sulfur-bearing compounds, which are relatively unattacked under normal HDS process conditions, with no apparent change in the non-sulfur-bearing components of the diesel fuel.

[0032] The invention employs enzyme Lipase as biocatalyst for oxidation of organosulfur compounds. The enzymes that can be used in the process according to the invention can be selected from the group consisting of a lipase derived from Candida cylindracea, Candida lipolytica, Candida rugosa, Candida antarctica, Candida utilis, Chromobacterium viscosum, Geotrichum viscosum, Geotrichum candidum, Mucor javanicus, Mucor miehei, Porcine pancreas, Pseudomonas species, specifically Pseudomonas fluorescens, Pseudomonas cepacia, Pseudomonas pseudoalkaligenes, Pseudomonas alkaligenes, Thermomyces species, Rhizopus arrhizus, Rhizopus delemar, Rhizopus niveus, Rhizopus oryzae, Rhizopus javanicus, Aspergillus niger, Penicillium roquefortii, Penicillium camembertii or an esterase derived from Bacillus species, specifically Bacillus thermoglucosidasius; Mucor miehei, Horse liver, Saccharomyces cerevisiae, Pigs liver. Any one or a combination of such enzymes can be used. Preferred enzymes include the lipase derived from Rhizomucor miehei, Candida antarctica B, Mucor miehei, Penicillium camembertii, Rhizopus niveus, Rhizopus javanicus, Pseudomanas species and Aspergillus niger. Especially preferred enzymes are NOVOZYM™ 388 L (Rhizomucor miehei lipase, free), LIPOZYM™ IM (Rhizomucor miehei lipase, immobilized), NOVOZYM™ 735 L (Candida antarctica A Lipase, free), NOVOZYM™ 525 L (Candida antarctica B Lipase, free) NOVOZYM™ 435 (Candida antarctica B Lipase, immobilized), each of which is a trademark product of Novo Nordisk, Denmark. Also preferred are Lipase G (Penicillium camembertii, Amano), Lipase PS and AK (Pseudomonas sp., Amano), Lipase N (Rhizopus niveus, Amano) and Lipase FAP (Rhizopus javanicus, Amano). The most preferred enzymes are Candida antarctica B lipase, immobilized, available commercially as NOVOZYM™ 435 and NOVOZYM™ LC, and Rhizomucor miehei lipase, commercially available as LIPOZYM™ IM.

[0033] The lipases used in the process do not require any cofactor and can perform catalytic reactions in organic solvents. Lipases accept hydrogen peroxide as a nucleophile in the catalytic formation of peroxycarboxylic acid. The peroxycarboxylic acids are unstable and break into acid and reactive oxygen molecule. The reactive oxygen molecule cause in situ oxidation of organosulfur compounds present in liquid hydrocarbon fuels.

[0034] The lipases used in the method is immobilized or free, improving the stability and recovery and can be used repeatedly in the recycled form.

[0035] The oxidation of organosulfur compounds in liquid hydrocarbon fuels in the process according to the invention is carried out in presence of aliphatic (C3 to C22) carboxylic acid and hydrogen peroxide. The preferred carboxylic acids are valeric acid, decanoic acid, myristic acid and palmitic acid.

[0036] The bio-oxidative reaction can be performed under ambient conditions of temperature and pressure, however process can be carried out at temperatures upto 70°C under the preferred embodiment of the invention and enzyme can be re-used upto several cycles. The reaction can be carried out in open or closed vessel.

[0037] According to the disclosed process, the dibenzothiophene and related sulfur-bearing organic sulfides present in liquid hydrocarbon fuels, which are the most refractory organic sulfur compounds in fossil fuels, are readily converted to the corresponding sulfoxides and sulfones, leaving majority of other hydrocarbons in their original form. Therefore, an advantage of the process of this invention is that the oxidation is selective towards the conversion of alkylated aromatic sulfur-bearing compounds, which are relatively unattacked under normal HDS process conditions, with no apparent change in the non-sulfur-bearing components of the fuel.

[0038] After bio-oxidative reaction, the oxidized organo-sulfur compounds in reaction mixture with unaffected hydrocarbons are removed preferably by solvent extraction using solvents like methanol, DMSO, acetonitrile, furfural, DMF and NMP etc., or any other physicochemical method that is or will became available in the art.

[0039] Said liquid hydrocarbon fuels according to the present invention could be naphtha, gasoline, kerosene and diesel or the like.

[0040] Details of the present invention will be described in conjunction with Examples. It is to be noted that the present

invention is limited in no way by Examples set forth below.

Example 1

Biocatalytic oxidation of model organosulfur compounds:

[0041] Myristic acid (0.5 mM) and dibenzothiophene DBT (2 mM) were dissolved in n-hexane (10 ml) in presence of Lipase NOVOZYM™ 435 and NOVOZYM™ LC (210 mg). To this, 0.8 ml hydrogen peroxide (50%) was added in six equal increments, over 4.5 hours. The reaction mixture was agitated with magnetic stirring at room temperature for 24 h. Reactions were also carried out for oxidation of benzothiophene (BT), phenyl sulfide (PS), 4,6 dimethyl benzothiophene (DMDBT). Oxidation was also attempted in combination, i.e., DBT+46 DMDBT, DBT+BT.

[0042] To study the effect of acid chain length on oxidation of organosulfur compounds, Myristic acid (0.5 mM) was replaced with 0.5 mM of either Behanic acid, Palmitic acid, Valeric acid, propionic acid, or decanoic acid. TABLE 1 depicts the effect of chain length on extent of oxidation.

Table 1

Acid Chain length	Extent of conversion of organosulfur compound (DBT and 4.6.6 DMDBT to corresponding sulfoxides/sulfones (%) in hexane
Behenic acid C22	37.9
Palmitic acid C16	96.7
Myristic acid C14	92.3
Decanoic acid C10	95.3
Valeric acid C5	97.2
Propionic acid C3	78.8

[0043] Biocatalytic oxidation of model organosulfur compounds was also attempted in hexadecane and low sulfur diesel, kerosene, naphtha blend (DKN) containing 10 ppm sulfur. Typically, DBT (10 mg) and myristic acid (114 mg) were dissolved in 10 ml DKN or hexadecane and 210 mg of Novozym 435 was added. Hydrogen peroxide (50% w/v, 1 ml) was added in six equal increments, each at an interval of 45 minutes. The reaction was carried out at room temperature while stirring, for 24 h. In a separate experiment, low sulfur diesel enriched with DBT (2%) diesel was treated with lipase.

[0044] In work up protocol, 10 ml dichloromethane (DCM) was added to the reaction mixture and filtered through Whatman NO. 41 paper to separate enzyme. The filtrate was then passed through anhydrous Na_2SO_4. Solvents were evaporated under N_2, residue dissolved in known amount of Dichloromethane and analyzed by GC-FID, and GC-PFPD & FD-MS.

[0045] More than 90% conversion of organosulfur compounds to their oxidized product was achieved. DMDBT was completely oxidized to its sulfoxide/sulfone under the reaction conditions. The reaction products were identified by GC-PFPD and GC-FID using commercially available sulfone/sulfoxide as standard.

Example 2

Biocatalytic oxidations of diesel:

[0046] Biocatalytic oxidations of diesel containing 6400 ppm to 100 ppm sulfur were carried out in 20 ml reaction volume containing 114 mg myristic acid and 210 mg of lipase NOVOZYM™ LC. Reaction was started by adding H_2O_2 (50% w/v, 1.0 ml). H_2O_2 was added in increments over 4.5 hrs. Reaction mixture was incubated at room temperature. At the end of reaction 10 ml dichloromethane (DCM) was added to the reaction mixture and filtered through Whatman NO. 41 paper to separate enzyme. The filtrate was then passed through anhydrous Na_2SO_4. Solvents were evaporated under nitrogen atmosphere.

[0047] Solvent extraction of sulfoxide and sulfones from oxidized diesel was carried out by several solvents like DMF, DMSO, Methanol, NMP, Furfural and Acetonitrile. The tables 2a and 2b below show the extent of desulfurization of diesel before and after bio-oxidation process when extracted with furfural and DMF solvents.

Table 2a

Sulfur content after extraction at ambient temperature with furfural		
Sulfur content in original diesel (ppm)	Sulfur content in un-oxidized and furfural extracted diesel (ppm)	Sulfur content in oxidized and furfural extracted diesel (ppm)
6400	5500	2300
1000	820	115
500	385	29

Table 2b

Sulfur content after extraction under heating condition with DMF	
Sulfur content in original diesel	709 ppm
Sulfur content in un-oxidized and DMF extracted sample	~530 ppm
Sulfur content in bio-oxidized and DMF extracted sample	~12 ppm
Sulfur content in second stage of 12 ppm bioxidative desulfurized diesel bio-oxidation after extraction with DMF	~9 ppm

[0048] The enzyme separated from first set of reaction was weighed and successively used for further set of reaction to establish its recyclability and reusability. Recyclability was checked upto six cycles with model compounds and in diesel as well and no loss of desulfurization propensity was observed.

[0049] Certain modifications and improvements of the disclosed invention will occur to those skilled in the art without departing from the scope of invention, which is limited only by the appended claims.

Claims

1. A method for desulfurizing liquid hydrocarbon fuel, **characterized in** comprising selective biocatalytic oxidation of sulfur containing compounds present in the liquid hydrocarbon fuels, wherein the fuel itself acts as a solvent, employing enzyme lipase as biocatalyst in presence of hydrogen peroxide and aliphatic carboxylic acid having carbon number from C_3 to C_{22} by contacting the fossil fuel with the lipase enzyme in presence of incremental amount of hydrogen peroxide and carboxylic acid, wherein hydrogen peroxide is added in controlled and incremental amounts in the reaction mixture under proper mixing conditions to avoid excess concentration of hydrogen peroxide in the fuel, and wherein the biocatalytic oxidation is performed at a temperature up to 70°C to oxidize thiophenes and organosulfides to their respective sulfoxides and sulfones, and removing the resultant oxidized polar compounds from the fuel.

2. The process according to claim 1, wherein the biocatalyst used in the method is immobilized or free.

3. The process according to claim 1, wherein the biocatalyst used in the method is immobilized.

4. The process according to claim 1, wherein said lipase enzyme is derived from an organism selected from the group consisting of Candida cylindracea, Candida lipolytica, Candida rugosa, Candida antarctica, Candida utilis, Chromobacterium viscosum, Geotrichum viscosum, Geotrichum candidum, Mucor javanicus, Mucor miehei, Porcine pancreas, Pseudomonas species, specifically Pseudomonas fluorescens, Pseudomonas cepacia, Pseudomonas pseudoalkaligenes, Pseudomonas alkaligenes, Thermomyces species, Rhizopus arrhizus, Rhizopus delemar, Rhizopus niveus, Rhizopus oryzae, Rhizopus javanicus, Aspergillus niger, Penicillium roquefortii, Penicillium camembertii, Bacillus species, Horse liver, Saccharomyces cerevisiae, Pigs liver or a combination thereof.

5. The process according to claim 4, wherein said lipase enzyme is derived from an organism preferably selected from the group consisting of Rhizomucor miehei, Candida antarctica B, Mucor miehei, Penicillium camembertii, Rhizopus niveus, Rhizopus javanicus, Pseudomanas species and Aspergillus niger or a combination thereof.

6. The process according to claim 5, wherein said lipase enzyme is derived from an organism most preferably selected from Candida antarctica B lipase or Rhizomucor miehei lipase or a combination thereof.

7. The process according to claim 1, wherein said aliphatic carboxylic acid is preferably selected from valeric acid, decanoic acid, myristic acid and palmitic acid.

8. The process according to claim 1, wherein said removing step is carried out by extraction or distillation.

9. The process according to claim 1, wherein said biocatalyst is recoverable and reusable in the reaction.

Patentansprüche

1. Verfahren zur Entschwefelung von flüssigem Kohlenwasserstoff-Treibstoff,
dadurch gekennzeichnet, dass
es eine selektive biokatalytische Oxidation von schwefelhaltigen Verbindungen enthaltend in flüssigen Kohlenwasserstoff-Treibstoffen umfasst, worin der Treibstoff selbst als Lösungsmittel dient,
Verwendung von Lipase-Enzym als Biokatalysator in Gegenwart von Wasserstoffperoxid und aliphatischer Carbonsäure mit einer Kohlenstoffzahl von C_3 bis C_{22} durch Zusammenführen des fossilen Brennstoffs mit dem Lipase-Enzym in Gegenwart eines inkrementellen Wasserstoffperoxid-Gehalts und Carbonsäure, worin das Wasserstoffperoxid in kontrollierten und inkrementellen Mengen zum Reaktionsgemisch unter geeigneten Mischungsbedingungen hinzugefügt wird, um eine Überschusskonzentration des Wasserstoffperoxids in dem Brennstoff zu vermeiden, und worin die biokatalytische Oxidation bei einer Temperatur von bis zu 70°C durchgeführt wird, um Thiophene und Orgnanosulfide zu ihren jeweiligen Sulfoxiden und Sulfonen zu oxidieren, und Entfernen der resultierenden oxidierten polaren Verbindungen aus dem Brennstoff.

2. Verfahren nach Anspruch 1, worin der in dem Verfahren verwendete Biokatalysator immobilisiert oder frei ist.

3. Verfahren nach Anspruch 1, worin der in dem Verfahren verwendete Biokatalysator immobilisiert ist.

4. Verfahren nach Anspruch 1, worin das Lipase-Enzym aus einem Organismus stammt ausgewählt aus der Gruppe bestehend aus Candida cylindracea, Candida lipolytica, Candida rugosa, Candida antarctica, Candida utilis, Chromobacterium viscosum, Geotrichum viscosum, Geotrichum candidum, Mucor javanicus, Mucor miehei, Porcine pancreas, Pseudomonas Spezies, im speziellen Pseudomonas fluorescens, Pseudomonas cepacia, Pseudomonas pseudoalcaligenes, Pseudomonas alcaligenes, Thermomyces Spezies, Rhizopus arrhizus, Rhizopus delemar, Rhizopus niveus, Rhizopus oryzae, Rhizopus javanicus, Aspergillus niger, Penicillium roquefortii, Penicillium camembertii, Bacillus Spezies, Pferdeleber, Saccharomyces cerevisiae, Schweinsleber oder eine Kombination davon.

5. Verfahren nach Anspruch 4, worin das Lipase-Enzym aus einem Organismus stammt bevorzugt ausgewählt aus der Gruppe bestehend aus Rhizomucor miehei, Candida antarctica B, Mucor miehei, Penicillium camembertii, Rhizopus niveus, Rhizopus javanicus, Pseudomonas Spezies und Aspergillus niger oder eine Kombination davon.

6. Verfahren nach Anspruch 5, worin das Lipase-Enzym aus einem Organismus stammt besonders bevorzugt ausgewählt aus Lipase B aus Candida antarctica oder Lipase aus Rhizomucor miehei oder eine Kombination davon.

7. Verfahren nach Anspruch 1, worin die aliphatische Carbonsäure bevorzugt ausgewählt wird aus Valeriansäure, Decansäure, Myristinsäure und Palmitinsäure.

8. Verfahren nach Anspruch 1, worin der Schritt des Entfernens mittels Extraktion oder Destillation ausgeführt wird.

9. Verfahren nach Anspruch 1, worin der Biokatalysator in der Reaktion wiedergewinnbar und wiederverwendbar ist.

Revendications

1. Procédé de désulfurisation d'un combustible à base d'hydrocarbures liquides, **caractérisé en ce qu'**il comprend l'oxydation biocatalytique sélective de composés contenant du soufre présents dans les combustibles à base d'hydrocarbures liquides, où le combustible lui-même agit en tant que solvant, en employant une enzyme lipase en tant

que biocatalyseur en présence de peroxyde d'hydrogène et d'un acide carboxylique aliphatique comportant un nombre d'atomes de carbone de C_3 à C_{22}, par la mise en contact du combustible fossile avec l'enzyme lipase en présence d'une quantité graduelle de peroxyde d'hydrogène et d'acide carboxylique, où le peroxyde d'hydrogène est ajouté dans des quantités contrôlées et graduelles dans le mélange réactionnel dans des conditions correctes de mélange pour éviter une concentration en excès de peroxyde d'hydrogène dans le combustible, et où l'oxydation biocatalytique est réalisée à une température allant jusqu'à 70 °C pour oxyder les thiophènes et les composés organosulfurés en leurs sulfoxydes et sulfones respectifs, et l'élimination des composés polaires oxydés résultants à partir du combustible.

2. Procédé selon la revendication 1, dans lequel le biocatalyseur utilisé dans le procédé est immobilisé ou libre.

3. Procédé selon la revendication 1, dans lequel le biocatalyseur utilisé dans le procédé est immobilisé.

4. Procédé selon la revendication 1, dans lequel ladite enzyme lipase est dérivée d'un organisme choisi dans le groupe constitué par *Candida cylindracea, Candida lipolytica, Candida rugosa, Candida antarctica, Candida utilis, Chromobacterium viscosum, Geotrichum viscosum, Geotrichum candidum, Mucor javanicus, Mucor miehei, Porcine pancreas,* une espèce de *Pseudomonas,* spécifiquement *Pseudomonas fluorescens, Pseudomonas cepacia, Pseudomonas pseudoalkaligenes, Pseudomonas alkaligenes,* une espèce de *Thermomyces, Rhizopus arrhizus, Rhizopus delemar, Rhizopus niveus, Rhizopus oryzae, Rhizopus javanicus, Aspergillus niger, Penicillium roquefortii, Penicillium camembertii,* une espèce de *Bacillus,* le foie de cheval, *Saccharomyces cerevisiae,* le foie de porc ou une combinaison de ceux-ci.

5. Procédé selon la revendication 4, dans lequel ladite enzyme lipase est dérivée d'un organisme choisi de préférence dans le groupe constitué par *Rhizomucor miehei, Candida antarctica B, Mucor miehei, Penicillium camembertii, Rhizopus niveus, Rhizopus javanicus,* une espèce de *Pseudomonas* et *Aspergillus niger* ou une combinaison de ceux-ci.

6. Procédé selon la revendication 5, dans lequel ladite enzyme lipase est dérivée d'un organisme choisi de manière préférée entre toutes parmi la lipase de *Candida antarctica B* ou *Rhizomucor miehei* ou une combinaison de ceux-ci.

7. Procédé selon la revendication 1, dans lequel ledit acide carboxylique aliphatique est choisi de préférence parmi l'acide valérique, l'acide décanoïque, l'acide myristique et l'acide palmitique.

8. Procédé selon la revendication 1, dans lequel ladite étape d'élimination est réalisée par extraction ou distillation.

9. Procédé selon la revendication 1, dans lequel ledit biocatalyseur est récupérable et réutilisable dans la réaction.

REFERENCES CITED IN THE DESCRIPTION

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