Biological Upgrading of Heavy Crude Oil

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Abstract Heavy crudes (bitumen) are extremely viscous and contain high concentrations of asphaltene, resins, nitrogen and sulfur containing heteroaromatics and several metals, particularly nickel and vanadium. These properties of heavy crude oil present serious operational problems in heavy oil production and downstream processing. There are vast deposits of heavy crude oils in many parts of the world. In fact, these reserves are estimated at more than seven times the known remaining reserves of conventional crude oils. It has been proven that reserves of conventional crude oil are being depleted, thus there is a growing interest in the utilization of these vast resources of unconventional oils to produce refined fuels and petrochemicals by upgrading. Presently, the methods used for reducing viscosity and upgradation is cost intensive, less selective and environmentally reactive. Biological processing of heavy crudes may provide an eco-friendly alternative or complementary process with less severe process conditions and higher selectivity to specific reactions to upgrade heavy crude oil. This review describes the prospects and strengths of biological processes for upgrading of heavy crude oil.

Keywords: heavy crude oil, asphaltene, upgradation, bioprocess, viscosity reduction

INTRODUCTION

Heavy crude oils (bitumen) have a density (specific gravity) approaching or even exceeding that of water. They are usually extremely viscous, with a consistency ranging from that of heavy molasses to a solid at room temperature. They also contain high concentrations of asphaltene, resins, nitrogen and sulfur containing heteroaromatics and several metals, particularly nickel and vanadium. Heavy crude oils are known as unconventional crude oils because they cannot be produced, transported, and refined by conventional methods [1-4]. The physico-chemical properties of heavy crude oil present serious operational problems in heavy oil production and downstream processing. Table 1 provides comparative details of the properties of a typical heavy crude oil and light crude oil.

There are vast deposits of heavy crude oils in many parts of the world. In fact, these reserves are estimated at more than seven times the known remaining reserves of conventional crude oils. The largest heavy crude oil reserves are in the Orinoco Oil Belt of Venezuela. Other important reservoirs are the Athabasca Oil Sands in Alberta, Canada, the Olenik Oil Sands in Siberia, Russia, and Maya heavy crude oil in Mexico. Proof exists that reserves of conventional crude oil are being depleted, therefore there is a growing interest in the utilization of these vast resources of unconventional oils to produce refined fuels and petrochemicals by upgrading [5-7]. Presently, the high viscosity of these crudes requires the addition of a solvent in order to allow their production and pipelining over a significant distance. The cost of suitable solvents and expected increased production of heavy crude oil has led to the investigation of new methods to reduce the viscosity of heavy crude oil. In downstream processing, heavy crude requires the conversion of the vacuum residue component into distillable oils. This upgrading has typically been accomplished with either thermal cracking or by catalytic hydroconversion. Thermal processing ranges from mild cracking (to reduce viscosity) to severe cracking (with the formation of coke). These processes are energy and cost intensive, less selective and environmentally reactive and also require supporting infrastructure for the supply of hydrogen and treatment of hydrogen sulfide in cracked off-gases. In Venezuela, naphtha is being used to produce and transport heavy crude oil to a refinery, and an important amount of coke is produced during the process of delay coking [8].

Biological processing of heavy crudes may provide an alternative or complementary process with less severe process conditions and higher selectivity to specific reactions to upgrade heavy crude oil. This review describes the strengths of biological processes for upgrading of heavy crude oil. It is important to point out that so far no biological processes exist for upgrading of heavy crude oil, thus this work is not a conventional review of published information. This review is a prospective analysis of data

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Table 1. Comparison between properties of light and heavy crude oil [1,4,6]

Properties	Light crude oil (Furrial from Venezuela)	Heavy crude oil (Morichal from Venezuela)
API gravity	22.90	8.50
Sulfur (wt %)	1.13	3.96
Nitrogen (wt %)	0.22	0.73
Asphaltene (wt %)	2.00	10.20
Carbon Coradson (wt%)	4.76	15.80
Vanadium (ppm)	49.00	488.00
Nickel (ppm)	11.00	105.00
Viscosity		
50°C	7.40	14257.00
60°C	5.90	5533.00
Pour point°C	less than −30.00	27.00
Yields (vol %)		
350°C +	52.00	88.00
520°C +	21.00	56.00

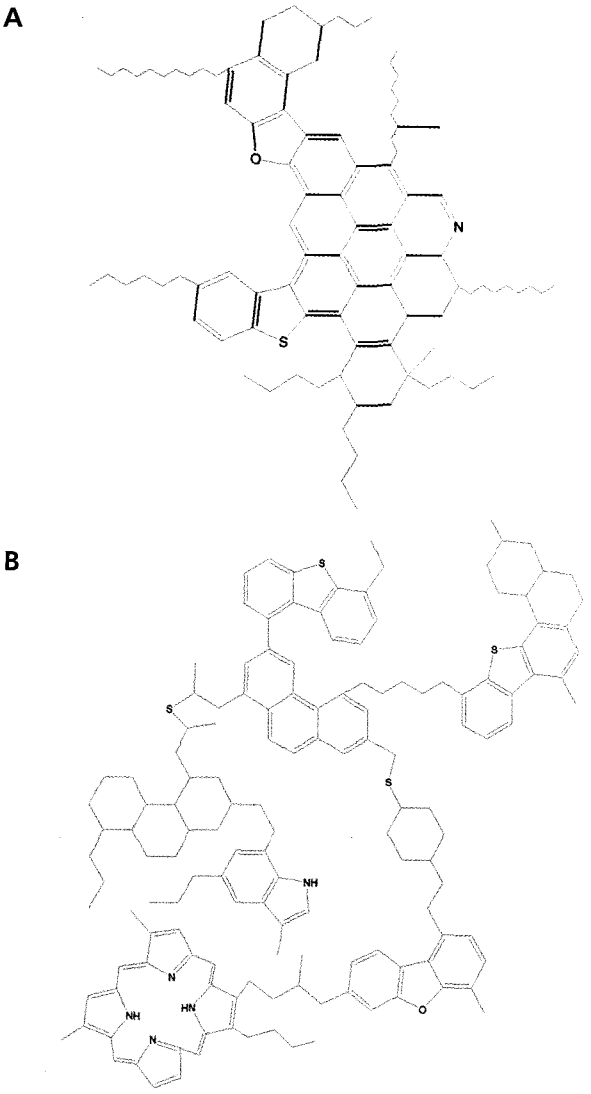

Fig. 1. Representative molecular structures of asphaltene. (A) a single condensed polycyclic aromatic core (B) multiple smaller polycyclic aromatic cores with aliphatic bridges.

on microbial and enzymatic transformations of petroleum products and their derivatives to evaluate the possible impact of biotechnological processes on the upgrading of unconventional crude. Some of the concepts of this review are either hypothetical or based on available research of conventional light crude oil and/or pure hydrocarbons, so they may require further research and validations.

Molecular Structures Responsible for Properties of Heavy Crude Oil

Heavy crude oil can be considered as a continuum of molecules with molecular weights of 1,500~2,000, and most of the molecules have an aromatic part (the trunk) and an aliphatic part (the branches) [8]. Heteroatoms such as nitrogen, sulfur, and oxygen, (NSO) mostly remain associated with aromatics. The molecules of crude oil can be classified in four classes based on their solubility (SARA analysis): Saturates (almost only branches), Aromatics (with branches and trunks), Resins (with more trunks than branches) and Asphaltenes (with many trunks and few branches). The asphaltene fraction and its intermolecular interactions are thought to be largely responsible for undesirable oil properties such as high viscosity and the propensity to form emulsions, polymers, and coke.

Asphaltenes are operationally defined as the part of petroleum that is insoluble in n-alkanes (*i.e.* n-pentane) but soluble in toluene. Conversely, resins are soluble in n-alkanes but polar enough to be retained in a hidroxyapatite column. Waxes are long-chain paraffinic molecules, or alkanes, which typically cause operational problems if longer than 40 carbon atoms. Asphaltenes are the heaviest and most polar fractions found in crude oil. Asphaltene, the highest molecular weight fraction of petroleum, is a dark amorphous solid especially rich in heteroatoms (NSO) and metals (Fe, Ni, V). There are two different views about the molecular structure of the asphaltene. According to the first view asphaltene is having a single large polycyclic aromatic core, with aliphatic chains attached to the periphery (Fig. 1A). The second view as-

Table 2. Biotechnologies in heavy crude oil upgrading

Problem	Molecules responsible	How will microbes improve the property?
Viscosity	Asphaltene Resins	1. Cutting an internal linkage of asphaltene molecules 2. Modification by oxidation of an aromatic ring of a large molecule 3. Inducing lower average molecular weights 4. Untrapping of small molecule-like resins.
Catalysts poisoning corrosion, environmental pollution	1. NSO compounds in the asphaltene structure and in the crude oil matrix 2. Metals as salts, petroporphyrins and other complexes in asphaltene	1. Selective removal of NSO compounds without affecting fuel value. 2. Degradation of metalloporphyrines 3. Deploymerization of asphaltene molecules
Soot formation and poor combustion characteristics of products	Aromatics	Microbes catalyzing cleavage of various aromatic hydrocarbons and heterocycles
Deposition	Asphaltenes, wax	Solubilizing and degradation
Undesirable O/W emulsions	Amphiphilic molecules from the oil, especially the resin fraction, including naphthenic acid	Extra cellular component or whole cell as demulsifier

phaltene is having multiple smaller polycyclic cores linked by aliphatic bridges (sulfides, esters *etc.*) (Fig. 1B). The second view is more acceptable among the researcher as this structure accounts for most of observed physical and chemical properties of asphaltene molecule [9-11]. It is possible to imagine aggregates of three or more asphaltenes with smaller molecules of resins or aromatics trapped within. Asphaltenes that are mainly natural or unprocessed are composed of several aromatic regions bonded together by aliphatic links instead of a huge aromatic center with aliphatic branches. They have the important capacity of adapting or folding, which minimizes the interaction energy with other molecules. They also retain metal bridging aromatic regions in the aggregates.

Heavy crudes also have characteristic molecular interactions (basically the van der Waals interactions), which are small for small molecules but large for asphaltenes. These forces keep the asphaltene molecules together (>100 atoms) with good fitting. Of importance, at low temperatures (below 60°C) these close contacts are more common and result in increased crude viscosity. Other molecular interactions responsible for increasing viscosity are at free radical sites, which are associated with condensed polycyclic aromatic structures with highly reactive unpaired electrons. These sites are involved in complexion of metals, inter- and intra-molecular reactions, molecular rearrangements and hydrogen bonding. The combined effects of the mentioned functionalities play decisive roles in the behavior of crude oils in reservoirs as well as their behavior in upstream and downstream processing.

Biological Upgrading of Heavy Crude Oil

The biological upgrading goal is to include all activities that make the material easier to produce and transport, as well as the chemical changes that increase the value of the oil. Different reactions that could change the physical properties of heavy crude oil and improve its viscosity include the following: (i) cut an internal linkage of the asphaltene molecules to lower the average molecular weight, (ii) oxidize the aromatic ring of a large molecule to modify the interactions with other molecules and to induce a rearrangement of aggregates to liberate the smaller trapped molecules, (iii) chelate the metals present in asphaltene aggregates, (iv) cut the originating two resins or aromatics molecules, thus inducing lower average molecular weights, and (v) cut the large aliphatic chains. Biocatalytic upgrading of heavy crudes includes asphaltene upgrading, compositional improvement and various other activities that are mainly helpful in downstream processing. Table 2 provides details of possible areas where biological processes can help in upgrading crude oil and targeted molecules. The main areas where biological processes can be applied to upgrade heavy crude include the following.

Biological Asphaltene Upgrading

Asphaltene molecules and their interactions, which form a continuous structure throughout the oil, are thought to be responsible for the viscosity of heavy crude oil [12-14]. Breaking the asphaltenes into smaller molecules and cutting an internal aliphatic linkage (sulfides, esters and ethers) of an asphaltene molecule can lead to a reduction in viscosity [15]. These large molecules are problematic for biological transformation and the transformation rates are limited by the mass transfer of target molecules to the biocatalyst and, in the case of whole cells, across the cell membrane [16]. Moreover, these molecules are highly hydrophobic, thus mass transfer limitations are expected in aqueous reactions [17]. Despite these difficulties, there is evidence in the literature for bacterial transformation of these complex, high molecular weight substrates. This is possible because these compounds contain carbon, hydrogen, sulfur, nitrogen and oxygen, which are necessary elements for the devel-

opment of any organism. Recently, Pineda-Flores *et al.* [18] reported on the utilization of asphaltenes as a sole carbon and energy source by a consortium isolated from Maya crude oil. The isolates belong to the bacterial genus reported for the degradation of other hydrocarbons and they were identified as *Corynebacterium* sp., *Bacillus* sp., *Brevibacillus* sp., and *Staphylococcus* sp. The respiration of the microbial consortium using asphaltenes as a sole carbon source (800 micromoles of CO_2 in 13 days) was significantly higher than those of the samples containing only the microbial consortium (200 micromoles of CO_2) or only asphaltenes (300 micromoles of CO_2). Ferrari *et al.* [19] reported on the effective biodegradation of a hydrocarbon class resistant to biodegradation (aromatics, resins and asphaltenes) using microorganisms having a greater metabolic capacity. Pendrys [20] isolated seven gram-negative, aerobic asphalt-degrading bacteria by an enrichment technique. The predominant genera of these isolates were *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Flavimonas*, and *Flavobacterium*. A mixed culture preferentially degraded the saturate and naphthene aromatic fractions of asphalt cement-20 and also utilized asphalt as the sole carbon and energy source. Rontani *et al.* [21] studied asphaltenic fraction degradation of Asthart crude, which was partially degraded by a marine mixed bacterial population with saturated hydrocarbons as a cosubstrate. Rojas-Avelizapa *et al.* [26] reported on the degradation of aromatic and asphaltenic fractions by *Serratia liquefasciens* and *Bacillus* sp. There are a number of reports on crude oil biodegradation of the asphaltenic fraction by mixed bacteria; however, none of these reports describe the mechanism or the end product of the degradation.

Some studies on upgrading heavy crude oils used extremophilic microorganisms, which are capable of desulfurizing, denitrogenating and demetalizing low grade oil by selectively cleaving molecular structures at organic carbon-carbon, carbon-sulfur and other heteroatom sites (including nitrogen, oxygen and trace metals). These reports have shown a significant reduction in the asphaltene content of heavy crude with a decrease in viscosity. It is supposed that these microbes depolymerize the asphaltene molecule and free the small molecule [23-31]. Studies also show that the addition of co-metabolic substrates can favor asphaltene degradation, especially if adding related compounds, such as alkanes, alkylbiphenyles, propane and methane, and those not related like succinate and salicilate, since they have been shown to favor biodegradation of complex molecular structure hydrocarbons like fluorantene and benzopyrene [32]. An ideal condition for asphaltene degradation is in a complex matrix of crude oil. Recently, Van Hamme *et al.* [33] isolated bacteria capable of cleaving subterminal C-S bonds within alkyl chains using the novel fluorinated organosulfur compound, bis-(3-pentafluorophenylpropyl)-sulfide, as a substrate. This may not only help in desulphurization of heavy crudes but also in viscosity reduction as these bacteria can cleave alkyl C-S bonds of an asphaltene molecule without reducing the carbon value of the substrate.

Various experimental evidences show that enzymes are able to modify asphaltene molecules. Chloroperoxidase from the fungus *Caldariomyces fumago* can transform petroporphyrins and asphaltenes, leading to the removal of Ni and V from asphaltene molecules (as in the case of synthetic nickel and vanadium porphyrins) [34]. Studies also show the feasibility of the biocatalytic oxidation of the asphaltene fraction of heavy oils by chemically modified cytochrome c. Sulfur- and carbon-based oxygenated compounds are the main products from the biocatalytic reaction with modified cytochrome. The cytochrome capacity to catalyze peroxidase-like reactions on thiophenes and organic sulfides is also well known [35-39]. However, the above results should be taken cautiously because the oxidation process, which introduces polar groups in the molecules, may affect asphaltenes aggregation.

There are only a few studies on asphaltene degradation/modification in the heavy crude oil matrix (if bioremediation studies are not included); nevertheless, the available literature suggests that there is the possibility of asphaltenes cracking by a biological process. The mass transfer problem of this type of big molecules for transformation can probably be improved through emulsification and increasing the interfacial contact area. However, emulsification is of limited value in overcoming the barrier of transport into biological cells unless appropriate uptake mechanisms are available. Surface-engineered (a bacterial display system) bacteria can probably be useful in this application [40]. Most of the studies cited here are laboratory based sterile conditions experiments, it is still to study the how these microbes will behave when introduced in non-sterile conditions during bioprocesing of heavy crude oils. Beside that metabolic diversity of non-cultivable microbes yet to be studied for bioprocessing of hydrocarbon.

Biological Compositional Upgrading

A compositional improvement includes the removal of sulfur, nitrogen, and metals, aromatic ring cleavage, and hydrogenation. After asphaltene breakdown, NSO compounds need to be reduced in order to upgrade the heavy crude oil as these contaminants not only contribute to environmental pollution, resulting from the combustion of petroleum, but also interfere with the processing of petroleum by poisoning catalysts and contributing to corrosion. These molecules are supposed to be responsible for increased viscosity. Selectively removing contaminants from petroleum while retaining the fuel value is a difficult technical challenge. Biological processes may be highly effective in this area. Many examples are available on compositional up-gradation of petroleum by biological methods; indeed, some of these methods may be applicable for heavy crude oil.

Sulfur and Nitrogen Removal

Heavy oil and bitumen contain 3 to 6% sulfur, which must be removed before being acceptable as refinery feedstock. As regulations for sulfur in fuels become more stringent, existing chemical desulphurization methods become inadequate for "deeper desulfurization", which produces lower-sulfur fuels. Bio-based approaches are

thus attractive and innovative alternatives as they can selectively desulfurize the alkylated DBTs (recalcitrant to hydrodesulphurization) under less stringent reaction conditions in an energy intensive manner. Several microorganisms have been found to desulfurize dibenzothiophene (DBT), a representative of the organic sulfur compounds in petroleum, forming the sulfur-free compound, 2-hydroxybiphenyl. These microorganisms are promising as biocatalysts in the microbial desulfurization of petroleum because without assimilation of the carbon content, they remove only sulfur from the heterocyclic compounds, which is refractory to conventional chemical desulfurization. The current paper does not discuss details of the biodesulfurization concept, notable advances in the microbial desulfurization of crude oil or middle distillate as they have previously been extensively reviewed [41-43]. This paper does, however, offer few interesting examples that may be more relevant. Surprisingly, a *Sphingomonas* strain was reported to desulfurize sterically hindered substituted DBTs more efficiently than DBT. A thermophilic bacterial strain, identified as *Paenibacillus*, is able to selectively desulfurize DBT without degrading its hydrocarbon matrix. These strains follow the same metabolic pathway of *Rhodococcus erythropolis* IGTS8. *Paenibacillus* enzymes are homologous to *Rhodococcus* enzymes, however they are active at higher temperatures: from 50 to 60°C [44]. These strains were proposed for the development of a biodesulfurization process for crude oil at high temperatures where crude oil viscosity is lower and mass transfer limitations are reduced. The application of a new gene shuffling method allowed the isolation of an evolved Dsz monooxigenase more active toward substituted DBTs. Bladi *et al.* [45] isolated a yeast strain that, can grow in a variety of sulfur compounds and can desulfurize orimulsion (a bitumen amended with an emulsifying agent and water) by 68% in 15 days. This yeast is reported to produce exoploysaccharides. Biological desulfurization is rising as one of the effective candidates for sulfur removal. Bhadra *et al.* [46] reviewed the potential of biological desulphurization of heavy oil and bitumen. According to their study, conventional hydrodesulphurization processes are uneconomic in the case of high sulphur oils due to high utility and catalyst costs. Microbial desulphurization, on the other hand, appears to be promising due to the inherent low energy requirement. This process may become more attractive upon the application of genetically modified bacteria and improvements in bioreactor designs for heavy crude oil and bitumen.

Like sulfur, nitrogen is known to contribute to acid rain and atmospheric contamination, and it is found to be in heterocyles such as quinolines and carbazole. Nitrogen containing compounds are a refinery catalyst inhibitor, presumably by binding to the acid site of the catalyst. The microbial products from the degradation of these compounds are expected to possess a reduced affinity for the catalyst-active sites. Therefore, microbial transformation of nitrogen heteroaromatics will also be useful to alleviate refining catalyst inhibition. The concept of biodenitrogenation has been recently reviewed by Van Hamme *et al.* [41] and Salvie *et al.* 2002 [47]. Many aerobic and anaerobic microbial cultures that can degrade organonitrogen compounds have been found [48]. The majority, if not the entirety, of microbial cultures described in the literature that metabolize organonitrogen do so by fully degrading it. These cultures can therefore utilize quinoline as a sole source of carbon, energy, and nitrogen. However, for use in a petroleum upgradation application, it would be preferable if nitrogen was selectively removed from quinoline, thus leaving the carbon and the calorific value of the molecule intact. There is very little information concerning the use of organonitrogen degrading microorganisms to remove nitrogen from petroleum [49,50]. The removal of 20 to 45% of nitrogen from heavy crude oil by unspecified mixed cultures has been reported but the ability of these cultures to metabolize organonitrogen is unknown [23]. To date, few microorganisms have been reported that can selectively remove nitrogen from heterocyles without degrading the carbon skeleton of the molecules. Recently Riddle *et al.* [51] reported on the biotransformation of carbazole in a liquid two-phase system by recombinant *Pseudomonas putida*, when solubilized in either 1-methylnaphthalene or in diesel fuel. These bacteria partially convert carbazole to a non-aromatic species and allow almost complete retention of the carbon content and fuel value.

An enzymatic conversion of carbazole using laccase from *Coriolopsis gallica* was totally accomplished in a reaction medium containing 15% acetonitrile. This type of microbe/biocatalyst was probably envisaged for upgrading heavy crude oil. The major barrier to using a microbial process to remove nitrogen from crude oil is the same as that for desulfurization [53]. To make economic sense, denitrogenation processes need to be integrated with crude oil desulfurization steps. It has been reported that carbazole enrichment cultures are capable of degrading a wide range of alkylcarbazoles present in crude oil, generally yielding water-soluble nontoxic metabolites [48,49].

Biological Demetalization

Crude oil contains metals in the form of salts, petroporphyrins and other complexes in the asphaltene. The removal of metals trapped in petrophyrins and complexes is more problematic because porphyrins are embedded in the extremely complex asphalteneic structure. Among the organometallic compounds, vanadium and nickel containing compounds are the most prevalent and are found almost exclusively in the resin and asphaltene fraction of crude oil. Metals in petroleum lead to two major problems for the industry. Combustion of these fuels leads to the formation of ash with high concentrations of metal oxides, leading to undesirable waste disposal issues. When crude oil is refined, metals stay with the residual fraction and are concentrated as other fractions are boiled off. The residual fraction is often subjected to catalytic cracking, a thermal process to decompose the large molecules in residual oil to smaller, lower boiling point molecules. During catalytic cracking, metals in the oil deposit on the cracking catalysts, resulting in poisoning of the catalysts and decreasing their selectivity and

activity.

Although microorganisms have been shown to be associated with the degradation of metalloporphyrines, there is little clear evidence that demetalization of crude oil can be achieved by biotechnological approaches. *C. fumago* chloroperoxidase enzymes have been used to remove metals contained in petroporphyrins and asphaltenes and reductions of 93% and 53% for nickel and vanadium respectively were reported [54]. However, this system requires chloride and the resulting products are chlorinated. Chlorinated products pose a substantial and undesirable environmental impact from the combustion of fuels demetalized with this enzyme-catalyzed reaction. The oxidations of petrophyrins and crude using cytochrome c reductases from *Bacillus megaterium* and *Catharanthus roseus* have also been reported in literature. Hemoproteins present the advantage of liberating metals by oxidation of porphyrinic rings instead of chlorination, thus avoiding the formation of chlorinated products that are undesirable due to environmental concerns [54].

Biological Dearomatization

Aromatic hydrocarbons have adverse effects on the production and processing of petroleum, and the combustion of fuels rich in aromatic hydrocarbons contributes to soot formation and poor combustion characteristics. Aromatics are commonly cracked during conventional upgrading by high-temperature and high-pressure catalytic hydrogenation to saturate and break the aromatic rings. In contrast to chemical catalysis, biological dearomatization would be more substrate specific and occur at ambient temperature and pressure. A research group at the University of Alberta, Canada has proposed a biological alternative that employs whole cell biocatalysts and two-phase (oil-water) reactions to specifically oxidize one or more rings of the aromatic substrates present in crude oil or middle distillate fractions. Enzymatic ring cleavage without carbon loss would produce polar compounds soluble in the water phase. These would be recovered for chemical hydrogenation under mild conditions to yield alkylaromatics with improved combustion characteristics compared to the parent compounds. This has been named as biological aromatic ring cleavage [55,56]. Exxon Research and Engineering Company in their patented process called biodearomatization as the biological activation of aromatics. The first step in this process is the hydroxylation of the material using microorganisms or other biocatalysts. The hydroxylated material is then transformed by hydrogenation and/or hydrogenolysis. Hydroxylation of the aromatic rings in a variety of hydrocarbon and heteroaromatic compounds activates them for hydrogenation and cleavage under, for example, aqueous/CO conditions [57].

The researchers of this review isolated various bacteria from Guanaco asphalt lake, Venezuela that can accumulate the ring-opened compounds upon DBT degradation. With time and upon further incubations, these compounds start to degrade to HFBT and then to unknown compounds. These microbes have an ability to grow in various PAHs and yield saturated growth within 24 h in Minimum Basal medium containing PAH as the sole source of carbon and energy. These bacteria can grow in bitumen and reduce the asphaltene, resin, sulfur and nitrogen contents of heavy crude oil, resulting in decreased viscosity [58]. Molecular studies suggest that most bacteria metabolic machineries are different from the prototype naphthalene degrader *P. putida* NCIB 9816-4. In southern blotting we could not get any hybridization using the *P. putida* NCIB 9618-4 gene as the probe [our unpublished results].

Miscellaneous Biological Processes

Hydrogenation is required to increase the H/C ratio of these feeds to a level suitable for transportation fuels. The primary target is the aromatics, including the heterocyclic sulfur and nitrogen species. The use of microorganisms specifically for aromatic ring hydrogenation has not been explored, although ring hydrogenation has been observed in the biodegradation pathways of some aromatic compounds. In addition, ring saturation is also observed in the biodegradation of aromatic hydrocarbons. There are some initial reports on the use of the enzyme hydrogenase for hydrogenation of dibenzothiophene [59-65].

Molecular weight reduction in the residue fraction of heavy oils by a biological agent has also been reported [66-69]. There are few bacterial strains reported that act on paraffines and functionalize them. British Petroleum coined the concept of biological dewaxing in 1970 with some value added as a by product [70]. Microbes can help in deposition control by producing metabolites (from carbon sources other than the oil) that improve the solubility of either waxes or asphaltenes, biotransform waxes and asphaltenes to more soluble products (through molecular weight reduction or functionalization), and biodegrade to remove the problematic compounds either from the oil or from existing deposits [71]. Rocha *et al.* [72] disclosed a method for preparing biosurfactants for use in making emulsions of high viscosity hydrocarbons such as high viscosity crude oil wherein the biosurfactant is a metabolite of *Pseudomonas aeruginosa* (USB-CS1). The resulting biosurfactant can be used to produce emulsion having a viscosity below about 500 centipoise and, more preferably, below about 100 centipoise at ambient temperatures. The production of biosurfactants *in situ* by microbial organisms grown in the presence of crude oil has also been reported in literature [73-76]. These biosurfactants assist in the dispersal of crude oil in seawater, thus facilitating the bioremediation of oil spills and chronic petroleum pollution. Microorganisms used for bioremediation purposes, however, are not generally compatible with petroleum extraction and refining processes because they also attack and catabolize (destroy) combustible hydrocarbons.

Undesirable water in oil (W/O) emulsions occur throughout oil production, transportation, and processing, and represent a major problem in heavy crude oil. Crude oil emulsions are complex and the emulsifying agents may be amphiphilic molecules from the oil, especially the resin fraction, including naphthenic acids, asphaltenes, fine solids, including clays, scale, wax crystals

Table 3. Constraints in biological processing of heavy crude oil

Reaction-specific constraints	Biocatalyst-specific constraints
1. A crude oil composition is complex. 2. Composition of crude oil varies from location to location. 3. Water insoluble reactants. 4. High volume of reaction.	1. Interaction of biocatalyst with crude oil components. 2. Lack of rigorous controls for the bacterial conversion of crude oil components. 3. No natural "Ideal biocatalyst" 4. Optimum activity and stability biocatalyst in a range of temperatures, pressures and salinities etc. 5. Specificity and broad substrate activity 6. Tolerance of various concentration ranges of the product(s)/reactant(s) by the biocatalyst without inhibition or saturation effects

Table 4. Characteristics of an ideal biocatalyst

Whole cell biocatalyst
- Should not utilize hydrocarbon as a C-source
- Constitutive biocatalytic activity
- Stability, specificity, and selectivity
- Broad substrate specificity
- Extremophile but with the ability to grow under normal growth conditions
- Higher activity in a two phase (aqueous-oil) reaction system
- Non pathogenic
- Recyclable and reusable
- Blocked mutant/truncated enzymatic pathway
- Able to work in a resting cell condition

Enzyme biocatalyst
- Recyclable and reusable
- No requirement of co-factor (s)
- Broad substrate specificity
- No product or substrate inhibition
- High activity
- Stability, specificity, and selectivity

or by microorganisms. De-emulsification in the oil industry is challenging due to the variety of possible emulsion properties, and treatments are currently tailored to each site and adapted over time. Various microbes including *Nocardia amarare, Pseudomonas* sp., *Corynebacterium petrophilum, Rhodococcus auranticus, Bacillus subtilis,* and *Micrococcus* sp. are known to exhibit demulsification activity. Some biologically produced agents like glycolipids, polysaccharide, glycolipids, glycoproteins, phospholipids and rhamnolipids destabilize petroleum emulsions. The surface of bacterial cells is also responsible for major demulsifying activity of some microorganisms [77-82]. The applicability of biotechnology to asphaltene- or solids-stabilized emulsions has not been studied. Biologically produced molecules may be effective in removing or dispersing asphaltenes or wax crystals, particularly in combination with suitable cell-surface properties to aid in dispersion of the heavy crude oil or in aiding flocculation.

Biological Upgradation: *In Situ* or *Ex Situ*

Biological upgrading can be applied to *in situ* (reaction occurring underground) or *ex situ* situations. There are few limitations with both of these possibilities. An *in situ* treatment can avoid excessive use of costly diluents, economy of scale, and it can be used in giant reservoirs and reduce the contaminants. Downstream upgrading (*ex situ*) can be useful and successful due to the ability to control operational conditions. The later method will probably be more effective and useful with regard to the flexibility of finished products. *In situ* oil upgrading has several advantages over surface upgrading technologies concerning the viscosity problem of extraction and transportation. Because *in situ* upgrading can be implemented on a well-by-well basis, there is no need for large capital-intensive projects. Rather, the size of an *in situ* project for a particular field can be tailored to available production rates. Cooper [82] screened several organisms for their ability to release bitumen from tar sands and demonstrated the ability of bacteria to produce biosurfactant and release of bitumen from tar sand. Bryant and Douglas [80] have demonstrated the oil recovery efficiency of several different bacterial strains in Berea sandstone cores. They found that treatment with microorganisms can recover an average of 32% more residual light crude oil than water flood recovery. There were some spore-forming bacteria that brought about a recovery of 60 and 50% more crude oil. Berea sandstone core experiments showed that selected microbial strains can recover up to 72% of the heavy oil left after water flooding. Hayes *et al.* [84] demonstrated that when Boscan, Venezuelan heavy crude oil was treated with emulsan, oil viscosity was reduced from 200,000 to 100 cP; thus, it was feasible to pump heavy oil miles in a commercial pipeline after this treatment. Premuzic demonstrated the capability of microbes to upgrade crude oil in an extreme tempreture, pH, salt concentration and pressure with enhanced oil recovery [29]. But despite numerous laboratory and reservoir studies, the *in situ* upgradation has some inherent problems: control over microbial growth, manipulation required in the process to promote microbial growth, variability from reservoir to reservoir *etc.* We propose the use of some microbes (probably a blend) *in situ* for upgrading crude oil particularly to reduce the viscosity and make

its extraction easier. Then the extracted oil can be further treated at the well head for better upgrading.

Biological Upgradation: Whole Cell or Enzymes?

The key to *ex situ* or *in situ* biological approaches is the complete recovery of the carbon skeleton without loss as either carbon dioxide or biomass. This can be achieved by using a pre-grown biocatalyst with the truncated enzymatic pathway so that the biocatalyst cells do not use petroleum hydrocarbon for biomass or to oxidize it to CO_2. This type of biocatalyst does not exist in reality, but can be achieved through genetic engineering. The characteristics of an ideal biocatalyst are given in Table 4.

Whole cell upgradation of crude oil has two main problems: microbial activity is carried out in the aqueous phase and under mild conditions, thus a two phase system reactor with intrinsic mass transfer limitations would be needed to metabolize the hydrophobic substrate. Ideally, all upgrading reactions should be performed in the absence of water or in a very low water medium. This limitation might be addressed by using enzymes instead of whole microorganisms [85,86]. Enzymes require less water than microorganisms to be active and stable in organic solvents and, theoretically, only a film of water covering their surface should be sufficient for catalysis to occur. The fuel itself could act as a reaction matrix, thus avoiding or minimizing the addition of water. Moreover, the use of reaction media with low water content increases the solubility and the bioavailability of hydrophobic substrates. Few reports are available with an interest on the use of enzymes for upgradation of asphaltene, biodesulfurization, biodenitrogenation and, oxidation of PAH. Therefore, it is desirable to develop a biotechnological process that can upgrade fossil fuels in a one-phase, non-aqueous system with a broad substrate and a well-adapted biocatalyst to "harsh" environments. It is expected that an enzyme from extremophilic microorganisms may have the desired characteristics [87-89]. The directed evolution methods and methods like gene shuffling can help in obtaining improved biocatalysts. Any enhancement of the thermal stability of an enzyme would confer significant operational advantages such as higher reaction rates, increased substrate solubility in lower viscosity media, productive shifts in thermodynamic equilibrium, and reduced risks of microbial contamination. We believe the use of enzymes (may be crude extract) will be useful for the processing of heavy crude oil *ex situ* while whole cell upgrading would be useful *in situ*.

Concluding Remarks and Future Prospects

The available research shows that biological upgrading of crude oil, at least to some extent, is a technically feasible process. The biocatalytic upgrading process may be at least a feasible adjunct process, if not a replacement for conventional upgrading processes. But it requires a lot of research particularly for better understanding the interaction between the biocatalyst and the heavy crude oil component. Table 3 provides details of the various possible constraints in biocatalytic upgrading of heavy crude oil. Moreover, the reality of an "Ideal Biocatalyst" still needs a lot of research. Another area for exploration in biological upgradation is the use of enzymes (probably crude enzyme) to catalyze biotransformations of molecules. Further research is required for new enzymatic activities upon petroleum products, especially in extreme environments; and for improvement of the enzymatic activities in very low water systems to increase the transformation rates using petroleum fractions without further addition of water. This would be done for activity enhancement and protein stabilization under the actual conditions found in the petroleum industry. Biocatalyst regeneration and recyclability need to be studied for economic and technical feasibility of any biological process related to upgrading. Advances in genetic engineering techniques will most likely play an important role in the development of such a biocatalyst. The economic and technical feasibility studies of any proposed methodology for upgrading will also be very important for sustainability of such a process in industry. Ideally the proposed upgrading treatment methodology should be able to handle viscous petroleum liquids at any desired stage of the extraction and/or transporting refining processes without a requirement for any specialized equipment or safety procedures. The proposed treatment should also fail to, degrade the caloric (fuel) value of the treated petroleum.

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