




Biotechnology-driven applications for advancing sustainability in petroleum industry

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Abstract

The petroleum industry faces increasing pressure to enhance sustainability while addressing environmental challenges. This review explores the transformative potential of biological innovations, highlighting microbial enhanced oil recovery (MEOR) as a cost-effective, eco-friendly alternative to conventional methods, with operational cost reductions of up to 83% and oil recovery enhancements exceeding 44%. MEOR mechanisms, including biosurfactant production and oil viscosity reduction, show promise even under extreme reservoir conditions. This review summarizes the significant investments that aim to integrate cost-effective, less energy-intensive, ecofriendly bioprocesses that transform heavy crude oils (HCO) into lighter forms (LCO) via biodearomatization (BDA), alongside methods like biodepolymerization of asphaltenes (BDPA), biometallization (BDM), biodesulfurization (BDS), and biodenitrogenation (BDN). These biological approaches optimize oil recovery and upgrade petroleum, achieving up to 96% sulfur and nitrogen removal, while mitigating petroleum hydrocarbon pollution and reducing the carbon footprint of the industry. Bioremediation strategies, including bioaugmentation and biostimulation, effectively mitigate pollution, with cost reductions of up to 85% compared to traditional methods. Nanobiotechnology is also signified to enhance bioprocess efficiency, improving biocatalyst stability, activity, and scalability. The role of petroleum-, environmental-, and nano-biotechnology in developing innovative solutions that utilize agro-industrial wastes in the petroleum industry is also discussed to align with the principles of the circular economy by enhancing resource efficiency and reducing waste. Future efforts should prioritize integrating biotechnological solutions, advancing genetic engineering, and scaling pilot studies to commercial applications, underscoring biological innovations' potential to reshape the petroleum industry toward a more sustainable and environmentally responsible future.

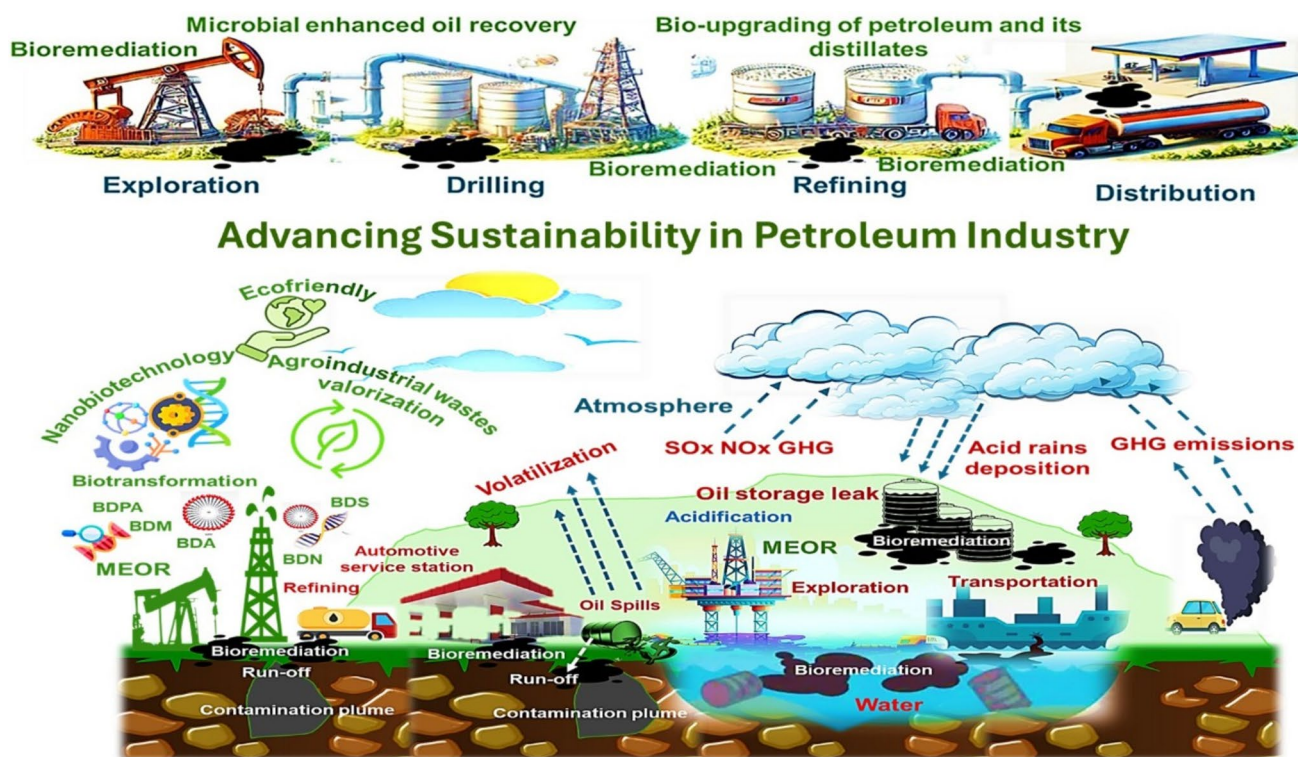
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Graphical Abstract



Keywords Microbial enhanced oil recovery · Bio-upgrading of petroleum and its distillates · Bioremediation · Nanobiotechnology · Agro-industrial wastes

Introduction

Energy use is steadily increasing over the world, which is projected to rise by 48% between 2012 and 2040 with the continuous increase in population and industrial activities (Finecomess and Gebresenbet 2024). Industrialization is widely recognized as a key driver of development and economic growth; however, it poses significant environmental challenges, primarily due to its heavy reliance on polluting, non-renewable energy sources (Usman and Balsalobre-Lorente 2022). These energy sources currently account for approximately 87% of the global energy demand (Aquilas et al. 2024). Furthermore, with over half of the global population now living in urban areas—and this proportion expected to rise to nearly 70% by 2050—energy consumption is projected to increase substantially, further intensifying environmental pressures (Finecomess and Gebresenbet 2024).

Global liquid fuel consumption worldwide is also expected to rise, from 95 mb/d in 2015 to 113 mb/d by 2040, with a great portion related to crude oil (Nassar et al. 2021a). The transport sector uses over 25% of the world's energy

and is expected to grow by 1.4%/year from 2012 to 2040 (El-Gendy et al. 2022). This comes with the global decline in high-grade oil reserves, i.e. the light crude oils (LCO) of low viscosity and low asphaltene, heavy metals, nitrogen, and sulfur contents and the elevation of the heavy crude oil (HCO) reserves, with its contradicted properties. The elevated viscosity of HCO is reported to potentially hinder its pumping, transportation, handling, processing, and refining (Pham et al. 2024). HCO reserves are distinguished by its high concentrations of mutagenic, genotoxic, and carcinogenic high molecular weight polyaromatic—hydrocarbons (HMWt-PAHs), sulfur heterocyclic (HMWt-PASHs), and nitrogen heterocyclic (HMWt-PANHs) compounds. Moreover, the increased viscosity, along with the asphaltenes' flocculation and deposition, complicates production and refining processes (El-Gendy and Speight 2022). This issue is exacerbated by strict global environmental rules that require a substantial decrease in the emissions of greenhouse gas (GHG) emissions and harmful sulfur dioxide and nitrogen oxides (Song et al. 2024). The notable expansion in oil consumption, especially the heavy one, has exacerbated operations related to oil extraction and utilization, leading to



environmental pollution stemming from oil spills occurring during exploration, transportation, and refining activities (Bi et al. 2025). This has a marked negative impact on soil, water, and air quality, raising concerns about their long-term sustainability and ecological consequences (Li and Alharthi 2024).

To attain the seventeen sustainability goals, which encompass the three pillars of economy, society, and environment, sustainable growth should fulfill current needs without jeopardizing the capacity of future generations. Thus, nowadays, the implementation of low carbon footprint processes in oil and gas industry is a vital point of study, as this sector is the foremost contributor to climate change (Dibia et al. 2025). This can be achieved through the integration of biotechnology into the upstream part including production, in addition to the downstream part, which includes the refining and processing of petroleum and its distillates. Petroleum biotechnology, as will be discussed in this review, pertains to the application of bioprocesses within the oil sector, starting from the exploration, production from reservoirs, and refining. That would be through the application of the microbial enhanced oil recovery (MEOR) and bio-upgrading of HCO and its distillates. Yet, the environmental biotechnology, as will be also discussed in this review, employs the biochemical capabilities of microbes and enzymes for environmental conservation, remediation, and reclamation. The promising integration of nanobiotechnology and agro-industrial wastes within the bioprocesses applied in the petroleum industry will be also conferred. This integration supports the principles of a green economy by decreasing the dependence on non-renewable resources and reducing the environmental impact. Furthermore, it aligns with circular economy practices by repurposing waste materials, enhancing resource efficiency, and reducing overall waste generation. Moreover, it supports circular economy principles by the reusing of waste materials, improving resource efficiency, and minimizing overall waste production. By adopting these innovative approaches, the petroleum industry can effectively lower its carbon footprint and water footprint, contributing to climate change mitigation and promoting a more sustainable future.

MEOR: scientific perspectives and technological advances

It has been reported that, even with typical oil recovery methods, a large amount of residual oil (around 70%) stays in the complex capillary networks in the pore spaces in oil reservoirs (Das et al. 2025a). Enhanced oil recovery (EOR), or tertiary oil recovery, refers to techniques that extract additional oil from reservoirs beyond the 25% to 35% recovery attained by the primary and secondary methods (Speight and El-Gendy 2018). EOR techniques include injecting

chemicals (such as surfactants or polymers), gases (like nitrogen, hydrocarbons, or carbon dioxide), steam to mobilize trapped oil, and MEOR (Speight and El-Gendy 2018). MEOR employs microorganisms to access and extract the oil, which remains stuck in the small channels of reservoir rocks. This technique is generally applied after the initial attempts with thermal and chemical recovery methods, when there is still a substantial amount of oil left in the reservoir (Wu et al. 2022). The conventional EOR techniques are expensive, energy intensive, and not environmentally friendly because of the use of hazardous chemicals and injection of CO₂. The cost of EOR reported to range between \$10 to over \$53 per barrel (Liu and Wei 2017). Alkaline–surfactant–polymer (ASP) flooding can irreversibly damage production infrastructure, such as widespread scaling on drill-pipes and pose contamination risks to formation waters and the surrounding environment (Gang et al. 2007). MEOR not only offers lower operational costs that priced between \$3 and \$9 per barrel and reduced energy consumption compared to thermal methods but also exhibits less dependence on fluctuating oil prices unlike many chemical approaches (Lin et al. 2022). Furthermore, MEOR is also offering advantages such as high efficacy, ease of process, robust adaptableness, and environmental safety. Additionally, microorganisms can be grown and maintained on low-cost sustainable raw materials, and biosurfactants can exhibit outstanding emulsification potentials (Xiao et al. 2023).

A petroleum reservoir is a complicated ecosystem comprised of living microorganisms and non-living minerals that interact within a dynamic network of nutrients and energy flows (Speight and El-Gendy 2018). (Wu et al. 2022) classified the MEOR into four classes; microbial selective plugging recovery (MSPR), microbial wax removal (MWR), microbialflooding recovery (MFR), and cyclic microbial recovery (CMR). Nevertheless, according to Su et al. (2018), MEOR can be performed via (1) augmentation with a selected microbial culture, (2) nutrient inoculation to stimulate indigenous microorganisms present in oil reservoirs, and (3) inoculation of exogenous microbial products. However, the primary mechanisms of MEOR depend on the degradation of the high-molecular-weight (HMWt) hydrocarbons and the production of biosurfactants (Nikolova and Gutierrez 2020). The principal methods of MEOR involve changes in porosity and permeability distribution within the reservoir, modifications to rock wettability, oil solubilization, and reductions in interfacial tension (IFT), emulsification, and improvements in the mobility ratio, as highlighted in several studies (Sharma et al. 2019). These processes utilize microorganisms, particularly bacteria, which interact with the crude oil to produce various gases (H₂, N₂, CH₄, CO₂) and solvents (biosurfactants and biopolymers, along with various acids) (Verma et al. 2020). These gases can either lower the viscosity of the oil or cause it to expand,

aiding in the displacement of the oil (Speight and El-Gendy 2018). The synthesis of low molecular weight fatty acids can also dissolve carbonate deposits and break down long-chain hydrocarbons like asphaltenes, resins, and waxes in the pore throats. This makes the reservoir more permeable and porous, and it also speeds up the flow of crude oil (Lazar et al. 2007). Biosurfactants are a viable option for MEOR because they are biodegradable, of low toxicity, and effective over a wide range of pressure, temperature, salinity, and pH (Purwasena et al. 2024). Furthermore, microorganisms can HCO-components, improving overall oil quality (Daryasafar et al. 2016). Factors influencing MEOR performance include temperature, permeability, pH, salinity, and oxygen content (Speight and El-Gendy 2018).

The indigenous bacterial populations could influence the viscosity and pH of formation water, oil/water interfacial tension, as well as the petroleum viscosity and its saturates, aromatics, resins, and asphaltenes (SARA) fractions (Bao et al. 2009). *Bacillus* strains have shown significant promise in oil recovery. McInerney et al. (2004) explored various *Bacillus* strains, including *Bacillus licheniformis*, *B. mojavensis*, *B. sonorensis*, and *B. subtilis*, for their biosurfactant production capabilities. Other studies have utilized petroleum degrader- and biosurfactant-producer *Bacillus* strains for enhancing the production in specific petroleum reservoirs, such as the Daqing oil field (She et al. 2011). For example, *B. subtilis* W19 generated biosurfactants that lowered interfacial tension from 20.9 to 1.85 mN/m. That caused a 28% oil recovery following MEOR in core flooding tests that used a mix of biosurfactant and chemical surfactant (Souayeh et al. 2014). Additionally, the biosurfactants from *B. subtilis* BS-37 demonstrated stability under extreme conditions, achieving a crude oil displacement efficiency of about 13.48% (Liu et al. 2015).

Some of the microorganisms that are often employed in MEOR practices are *Desulfovibrio desulfuricans*, *Pseudomonas aeruginosa*, *Xanthomonas campestris*, *B. licheniformis* (Kang et al. 2024a, 2024b). The identification of biosurfactants and their mechanisms has highlighted their role in reducing surface tension and enhancing oil displacement (Yernazarova et al. 2024).

Advancements in biotechnology have significantly improved MEOR techniques. Key developments include (Ke et al. 2024):

Extremophiles-microorganisms: The primary challenge in MEOR is isolating a bacterium capable of thriving and producing sufficient metabolic products under diverse environmental conditions. Table 1 gives some examples for the applicability of extremophiles in MEOR.

Genomic and metagenomic tools: These methods help in describing the microbial populations in reservoirs, which makes it easier to choose the best strains for oil recovery. This has also led to the creation of genetically modified

microbial enhanced oil recovery (GMEOR) and enzyme-enhanced oil recovery (EEOR), which are both considered as advanced MEOR technologies (Zhang et al. 2020a).

Bioprocess optimization: Enhanced nutrient injection strategies and fermentation processes have been developed to maximize microbial growth and metabolite production, improving recovery efficiency.

Biosurfactant production: New methods for cultivating and harvesting biosurfactants have emerged, enabling cost-effective production on an industrial scale. Figure 1 shows the screening criteria for identifying biosurfactant-producing extremophiles from various extreme environments. Biosurfactants significantly enhance oil recovery from oil reservoirs through various mechanisms, as depicted in Fig. 2. Some of these mechanisms are lowering the surface and interfacial tensions, changing wettability, and making oil/water or water/oil emulsions (Sen 2008).

Monitoring and control Systems: Advanced sensors and data analytics are being integrated into MEOR operations to monitor microbial activity and fluid dynamics in real-time, allowing for better management of the recovery process (Sun et al. 2025).

Global MEOR field cases

In the Chunfeng oilfield, the recovery of ultra-HCO was improved via the in situ MEOR. A combination of indigenous bacteria (*Dietz coli* Z4M8-2, *Pseudomonas* sp. XJZ3-1, and *B. subtilis* XJZ2-1) along with exogenous *B. subtilis* SLG5B10-17, and nutrients, in addition to an activator solution were injected into a production well over the course of 7 days, tailed by a shutdown period of 166 days. After 13 months, oil production elevated from 0.4 to 4.7 tons/day, recording a peak of 17 tons/day and water cut of 34%. Additionally, the study indicated a lengthier effective oil recovery period in the wells treated with MEOR relative to nearby wells that were subjected to steam treatment (Xuezhong et al. 2016). A large-scale pilot test was carried out in the Baolige oilfield, involving 78 injection wells and 169 production wells. Indigenous strains *Rhodococcus* sp. JH and *B. licheniformis* LC, identified through laboratory assays, were selected for the field trial due to the high surface activity of their biosurfactants. Over four injection cycles spanning 43 months, the cumulative additional oil production totaled 2.1×10^5 tons. However, in 15% of the production wells, no significant increase in oil output was observed. This was attributed to the location of these wells in formations with poor homogeneity or insufficient connectivity within the underground network, which hindered the flow of bacteria through the wells (Ke et al. 2018). In the same oilfield, a smaller trial was conducted shortly thereafter, involving two injection wells and eight production wells. The biosurfactant-producing bacterium *Luteimonas huabeiensis* HB-2,

Table 1 Extremophiles and their potential in enhanced oil recovery

Microbe parameter	Microorganisms	Contribution to MEOR processes	References
Aerobic	<i>Pseudomonas</i> , <i>Bacillus</i> , <i>Corynebacterium</i> , <i>Sireptococcus</i> , and <i>Xanthomonas</i>	Produce glycolipid, lipopeptide and glyco-lipopeptide biosurfactants	Saravanan et al. (2020)
Anaerobic	<i>Clostridium</i> , <i>Desulfovibrio</i> , <i>Leuconostoc mesenteroides</i> , and <i>B. licheniformis</i>	Produce glycoprotein at 96 °C and perform 37% oil recovery	Nikolova and Gutierrez (2020), Saravanan et al. (2020)
Facultative anaerobic	<i>Arthobacter protophormiae</i> , <i>Enterobacter cloacae</i> , <i>Enterobacter hormaechei</i>	Efficient in IFT reduction and wettability alteration by biosurfactant production	Hosseini and Tahmasebi (2020)
Piezotolerant (1–40 MPa)	<i>Rhodococcus ruber</i> Z25, <i>Rhodococcus erythropolis</i> , <i>Arthrobacter</i> sp. consortia, and <i>Pseudomonas</i> sp.	Degrade long-chain hydrocarbons, which reduces the crude oil viscosity	Ke et al. (2018)
Barophilic (40–50 MPa)	<i>Thermococcus barophilus</i>	Hydrocarbon biodegradation	Sakthipriya et al. (2017)
Piezophiles (50–65 MPa)	<i>Thermococcus piezophilus</i> , and <i>Shewanella benthica</i>	Biogas solubility, oil viscosity and IFT is influenced by increased pressure	Mamo and Mattiasson (2020)
Extreme piezophiles (65–100 MPa)	<i>Shewanella</i> and <i>Moritella</i>	High pressure affects the fluidity, permeability and carbonate dissolution	Sakthipriya et al. (2017)
Extreme psychrophile (–20 to 10 MPa)	<i>Rhodotorula</i> sp., <i>Rhodotorula psychrophila</i>	Produce sophorolipids	Perfumo et al. (2018)
Psychrophile < 20 °C)	<i>Pseudoalteromonas</i> , <i>Rhodococcus</i> sp., <i>Burkholderia</i> , <i>Flavobacterium</i> , and <i>Halomonas Neptunia</i> ANT-3b	Produce emulsifying glycolipid using n-hexadecane	Perfumo et al. (2018)
Mesophile (20–50 °C)	<i>Pseudomonas</i> , <i>Halomonas</i> , <i>Lysinibacillus</i> , <i>Achromobacter</i> ; <i>Acinetobacter</i> , and <i>Sphingomonas</i>	Degrade alkane fraction of crude oil	Nikolova and Gutierrez (2020)
Thermophiles (50–70 °C)	<i>Bacillus licheniformis</i> , <i>Brevibacillus thermoruber</i> , <i>Clostridium</i> , <i>Thiobacillus</i> , and <i>Pseudoxanthomonas</i> sp. G3	Reaction rate and bioavailability are improved via production of rhamnolipid	Astuti et al. (2019)
Extreme thermophile (70–100 °C)	<i>Thermotogales</i> , <i>Thermococcus</i> ; <i>Thermoanaerobacter</i> , <i>Methanobacterium thermagregans</i>	Degrade aliphatic hydrocarbon and produce endospore	Nikolova and Gutierrez (2020)
Extreme acidophiles (pH 0–2)	<i>Sulfolobus acidocaldarius</i> , <i>Acidianus brierleyi</i> , and <i>Ferroplasma acidarmanus</i>	Produce extremozymes such as oxidases, proteases, cellulases, amylases	Mamo and Mattiasson (2020)
Acidophiles (pH 2–5)	<i>Desulfovibrio desulfuricans</i> , <i>Acidophilium cryptum</i> , <i>Bacillus acidocaldarius</i> , and <i>Acidothermus</i>	Produce thermostable enzymes for surviving in the extreme reservoir surroundings	Debnath et al. (2019)
Neutrophiles (pH 5–8)	<i>Thermococcus celer</i> , <i>Pyrobaculum</i> ; <i>Rhodococcus</i> ; <i>Smithella</i>	Biosurfactant (trehalolipid) production	Eswari et al. (2019)
Alkalophiles (pH 8.5–11.5)	<i>Marinobacter</i> , <i>Micrococcus</i> , <i>Oceanobacillus</i> , and <i>Alkaliphilus transvalentis</i>	Synthesize alkaline active enzymes, for example, pectinases, xylanases, proteases	Mamo and Mattiasson (2020)
Extreme alkalophiles (pH 11.5–13.5)	<i>Ochrobactrum intermedium</i>	Potential candidates for ASP flooding	Mamo and Mattiasson (2020)
Halophiles (NaCl 2–5M)	<i>Halobacterium salinarum</i> , <i>Haloflex volcanii</i> , <i>Halobacterium distributum</i> , and <i>B. velezensis</i> BSA1	Salinity affects the microbial growth and metabolism along with the biosurfactant yield	Yin et al. (2023)



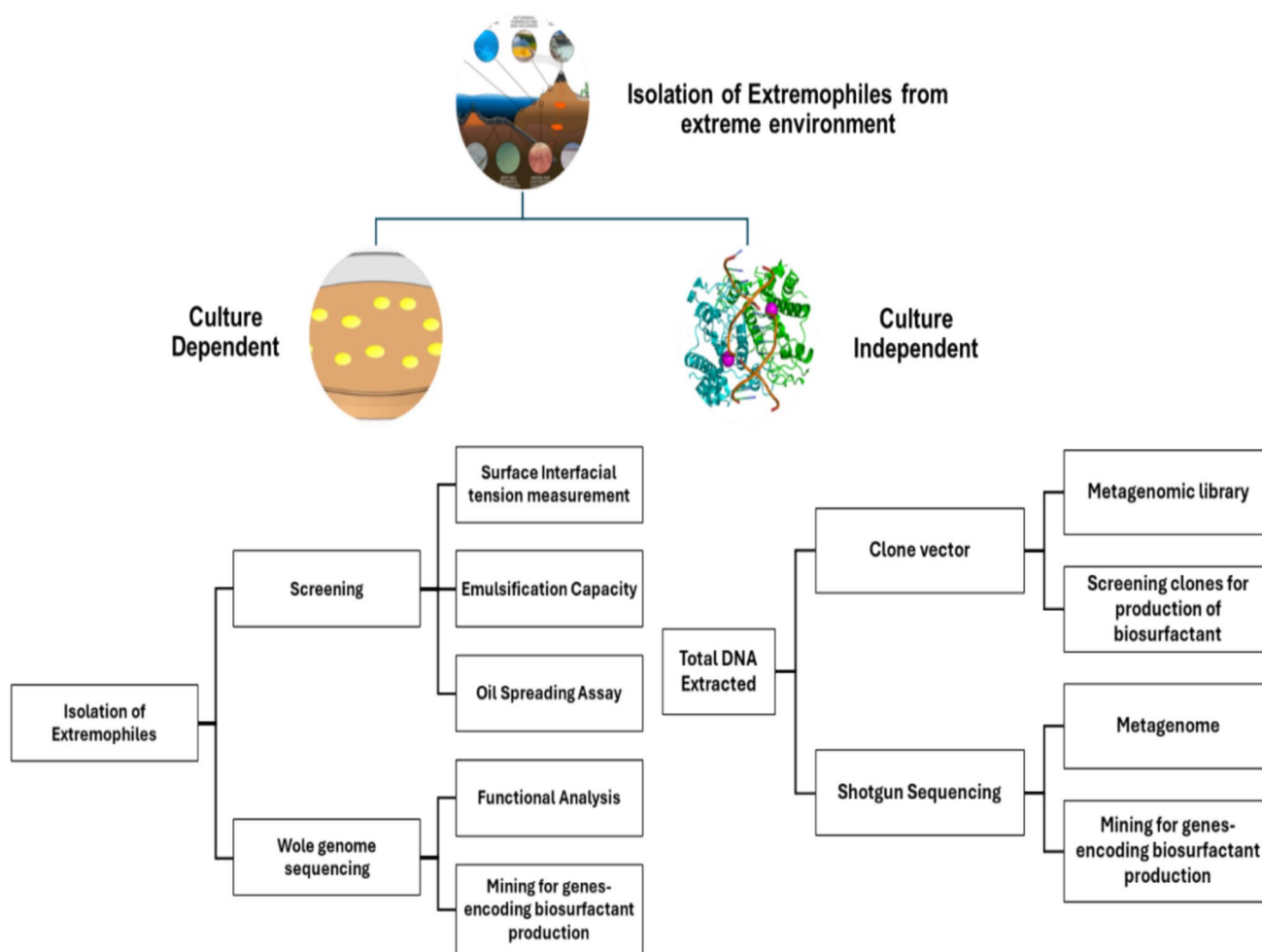


Fig. 1 Screening approaches for biosurfactant-producing extremophiles using culture-dependent and culture-independent techniques

which had demonstrated the ability to reduce oil–water interfacial tension and decrease oil viscosity in laboratory tests, was used in this trial. The field test comprised two bacterial injection cycles, each followed by a water flooding phase. By the end of the two cycles, an additional 2300 tons of oil were recovered, with the improvement primarily attributed to the reduction in oil viscosity (Zhang et al. 2020a). This underscores the potential of in situ biosurfactant production to improve oil recovery. However, beyond biosurfactants, both exogenous and indigenous microorganisms also produce other metabolites—such as gases, acids, and solvents—as well as biomass, all of which can further contribute to the observed enhancements in oil production (Speight and El-Gendy 2018). Table 2 summarizes other worldwide successful MEOR real field applications.

Research gaps and future opportunities in MEOR

Briefly, MEOR represents a convergence of microbiology, biochemistry, and petroleum engineering. Recent scientific

studies have deepened the understanding of microbial behavior in oil reservoirs, specifically how different strains can influence oil viscosity, interfacial tension, and fluid dynamics. Research has demonstrated that indigenous microorganisms can be leveraged for their natural oil-mobilizing capabilities, while engineered strains can be designed for specific recovery goals. Despite its potential, MEOR faces several challenges. The variability in reservoir conditions such as temperature, pH, and salinity can affect microbial growth and activity, making it difficult to predict and control the outcomes consistently across different sites. Moreover, the potential for microbial contamination and the ecological impacts of introducing non-native species into reservoir ecosystems require careful management and regulatory compliance. Likewise, the uncertainties in establishing suitable conditions for microbial groups involved in the metabolic pathways are another challenge to be solved. Furthermore, following microbial application, it is essential to eliminate the byproducts of microbial activity, along with the bacteria themselves, to preserve the composition of crude oil, as the

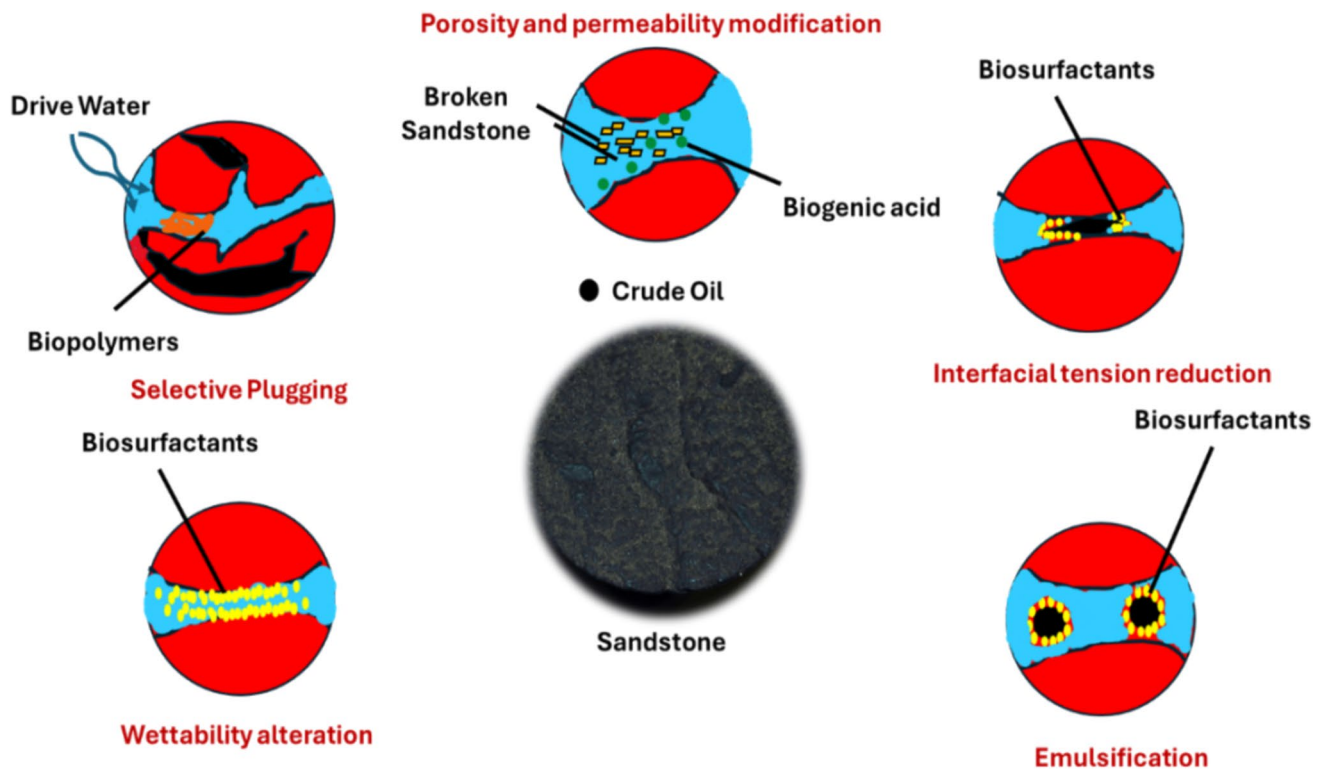


Fig. 2 Mechanisms of biosurfactant-enhanced oil recovery

Table 2 Successful field trials of MEOR

Country	Applied MEOR strategy	References
Argentina	Nutrients, biocatalyzers, and microorganisms Hydrocarbon degrading bacteria and anaerobic fermentation bacteria Facultative anaerobic bacteria	Strappa et al. (2004)
Azerbaijan	Microbial flooding recovery Microbial selective plugging recovery	Niu et al. (2020)
Bulgaria	Indigenous oil-oxidizing bacteria sourced from water injection and formation water	Shibulal et al. (2014)
Canada	Microbial flooding recovery	Town et al. (2010)
China	Different nutrient solutions were injected to stimulate the growth of indigenous bacteria Following treatment; <i>Pseudomonas</i> , <i>Bacillus</i> , and <i>Acinetobacter</i> were identified as the dominant genera	Su et al. (2018), Du et al. (2022)
Indonesia	Cyclic microbial recovery	Ariadji et al. (2019)
Romania	Microbial flooding recovery Cyclic microbial recovery	Safdel et al. (2017)
Russia	Microbial flooding recovery Microbial selective plugging recovery Stimulation of fermentative bacteria and suppression of sulfate reducers Hydrocarbon-oxidizingbacteria	Niu et al. (2020) Nazina et al. (2013) Nazina et al. (2020)
USA	Pure or consortia of <i>Bacillus</i> , <i>Clostridium</i> , and <i>Pseudomonas</i>	Nikolova and Gutierrez (2020), Ke et al. (2024)

biotransformation-induced loss of light fractions is undesirable. For instance, in situ bacterial cultivation can sometimes result in reduced oil production. Another potential drawback of MEOR is the process's unpredictability; however, this can largely be addressed by understanding the composition of crude oil, assessing the impacts of microbes on these components, and selecting suitable microbial strains for the application. Other manageable challenges associated with MEOR include: (i) plugging of reservoir rock by bacterial biomass in undesirable locations, (ii) in situ biotransformation of applied chemical compounds, (iii) acidification of crude oil caused by hydrogen sulfide production, (iv) the limited effectiveness of most MEOR-suitable bacteria in high-temperature or high-salinity reservoir environments, (v) the toxicity of heavy metal components in crude oil to microorganisms, and (vi) competitive inhibition and antagonistic interactions between injected microorganisms and indigenous microflora (Niu et al. 2020). To prevent microbial plugging of the wellbore and to ensure the effective transport of all necessary components to the target zone, some important actions should be implemented such as filtration prior to injection, minimizing biopolymer fabrication, and utilizing dormant cell forms, spores, or ultra-micro-bacteria to reduce microbial adsorption to rock surfaces (Sharma et al. 2023). Achieving desired in-situ metabolic activity requires careful control of some parameters, for example, pH, salinity, temperature, and pressure, along with isolating microbial strains that can adapt to extreme reservoir conditions. The issue of low metabolites concentration can be addressed using genetic engineering techniques, since advances in genetic engineering and biotechnology could lead to the development of more robust microbial strains tailored for specific reservoir conditions (Xiao et al. 2023). Recently, MEOR and low salinity water flooding (LSWF) reported as an efficient ecofriendly approach to alter wettability and enhance oil recovery by more than 44% (Bemani et al. 2025). However, the performance mechanism still needs more investigation and optimization.

Application of nanobiotechnology is also new trend to enhance EOR (El-Gendy et al. 2022). Nano-cellulose fluids have been identified as sustainable agents for improving oil recovery (Wang et al. 2021a). Nano-cellulose can be applied as emulsion stabilizing, foam stabilizing, and oil displacing agent in EOR (Rana et al. 2024). Additionally, nanofluids have shown potential in enhancing oil recovery processes that involve microbial interactions (Rezk and Allam 2019). Research by Khademolhosseini et al. (2015) studied the combined impacts of silica nanofluids and biosurfactants produced by *Acinetobacter calcoaceticus* (PTCC: 1318) in laboratory-scale flooding experiments using a glass micro-model. The study found that the combination of nano-materials and biomaterials led to significantly higher recovery of heavy oil compared to the individual effects of water,

nano-fluids, or biosurfactant flows. During the MEOR process, the gyrotactic microorganisms reported to enhance the thermal efficacy via porous media and enhancement of nanofluids stability (Ahmad et al. 2020). Lipid-based nanoparticles have been reported as surfactant nano-carriers within the porous media of the reservoir, enhancing the surfactant release and consequently the EOR (Rosestolato et al. 2019). In another study, biosurfactant used for the green production of silver nanoparticles (AgNPs), which expressed approximately the same oil recovery percentage as the chemically synthesized AgNPs, recording 14.28 and 14.94, respectively (Elakkiya et al. 2020). Recently, cellulose-based-alkali-resistant gel microspheres have been recently reported for EOR (Yin et al. 2024). Furthermore, enhanced monitoring techniques and bioreactor technologies could improve the scalability and control of microbial processes in MEOR applications (Speight and El-Gendy 2018). A major challenge in the use of biosurfactants is their high fabrication cost relative to their chemical alternatives. However, recent advancements have focused on utilizing low-cost substrates, such as agro-industrial wastes, for biosurfactant biosynthesis.

Agro-industrial wastes, such as agricultural residues, food processing by-products, and other organic materials, present a promising resource for MEOR. Utilizing these wastes helps in both waste management and contributes to sustainable oil recovery practices. Agro-industrial wastes are rich in organic compounds, including sugars, starches, and proteins, which can serve as excellent nutrient sources for microbial growth (Sundaram et al. 2024). By inoculating oil reservoirs with specific microbes and supplementing them with these waste materials, the microbial populations can be stimulated to flourish, enhancing their oil-mobilizing capabilities. Corn steep powder (Le et al. 2014), beet molasses (Shibulal et al. 2014), molasses (Wang et al. 2016; Sun et al. 2017), have been applied in MEOR and expressed remarkable enhancement in oil recovery. Nevertheless, further research is required to optimize production and recovery processes to improve their market competitiveness. From this standpoint, in situ biosurfactant production via MEOR emerges as a promising strategy. A key limitation, however, is the limited availability of microorganisms capable of surviving and producing biosurfactants under the specific conditions found in oil reservoirs. Although some promising examples have been identified in recent years, their efficiency has yet to be confirmed through field trials. In this regard, genetic engineering presents a potential solution by facilitating the development of biosurfactant-producing microorganisms with tailored properties for effective MEOR applications. Additionally, the production of biosurfactants through microbial processes utilizing agro-industrial waste is an active area of research, with efforts focused on optimizing conditions to maximize yield (Qamar and Pacifico 2023). Molasses and

wey (Joshi et al. 2008) and date molasses (Al-Bahry et al. 2013) have been testified for biosurfactant production with prospective application in MEOR. The integration of agro-industrial wastes into MEOR not only aids in oil recovery but also promotes the concept of waste valorization and circular economy, mitigate waste disposal issues and emissions of methane that results from natural degradation of biowastes, and reduces the reliance on chemical additives. Thus, this approach may become a cornerstone of sustainable oil extraction practices, promoting both economic and environmental advantages in the petroleum industry, in addition to the achievement of sustainable development goals, with its three pillars environment, society, and economy.

Bio-upgrading of petroleum and its distillates

Heavy oil reserves yield petroleum distillates containing high concentrations of recalcitrant thiophenic compounds, such as dibenzothiophene (DBT) and its derivatives, which can constitute up to 40% and 70% of the total sulfur content in Middle East and West Texas oils, respectively. (Nassar et al. 2021b; Akimbek et al. 2025). Non-basic nitrogen compounds (e.g., carbazole, CAR) constitute around 70–75% of the total nitrogen content in crude oil, while basic nitrogen molecules (e.g., quinoline, Qn) comprise about 25–30% (Speight and El-Gendy 2018). Nitrogen compounds are known to inhibit the hydrodesulfurization (HDS) process. Consequently, the removal of carbazole (CAR) and other nitrogen compounds could substantially improve catalytic cracking efficiency, leading to a higher gasoline yield (Zakaria et al. 2016). It has been reported that one of the major contaminants in crude oil is the high concentration of sulfur, which negatively affecting its quality and price, where, every extra percent of sulfur lowers the price by 0.056 USD/Brent dollar (Tamjidi and Esmaeili 2025). The presence of metals, nitrogen, and sulfur contributes to coke formation and deactivates upgrading and refining catalysts (Ancheyta 2016). This issue is exacerbated by strict global environmental rules that require a substantial decrease in the emissions of harmful sulfur dioxide and nitrogen oxides (Song et al. 2024). Hydrogen sulfide and ammonia poses acute toxicity risks, which can lead to fatalities in both natural environments and workplaces (Pham et al. 2024). The presence of sulfur in motor fuels adversely affects catalytic converters in engines, resulting in incomplete fossil fuel combustion and increased greenhouse gas (GHG) emissions. This exacerbates global climate change and poses risks to human health by contributing to the formation of pollutants such as nitrogen oxides (NO_x), sulfur oxides (SO_x), carbon monoxide (CO), carbon dioxide (CO₂), and

particulate matter (PM) (Ni 2022). NO_x and SO_x irritate the respiratory system, leading to symptoms such as shortness of breath, irritation of the mucous membranes, and sharp chest pains (Syrek-Gerstenkorn et al. 2024). Moreover, SO₂ has been reported to adversely affect photosynthesis, reduce carotenoid and chlorophyll content, and increase water use efficiency in plants. Thus, increasing the water footprint and negatively impacting the food supply chain (Lee et al. 2017). NO_x and SO_x are the core precursors of acid fog, snow, and rain (Syrek-Gerstenkorn et al. 2024). Those have negative impacts on ecosystems and environment; contribute to soil pollution, destroy green spaces, harms forest, damages crops, leather, vehicles, buildings, and monuments (Syrek-Gerstenkorn et al. 2024). Additionally, it contaminates open seas, oceans, lakes and rivers, pose health risks to human, and have detrimental effects on aquatic fauna and flora, including declines in fish populations, damaging coral reefs, and negatively impacting micro- and macro- algae (El-Gendy et al. 2024). Thus, to address the issue of climate change, global environmental regulations have imposed limits on sulfur and nitrogen levels in transportation fuels to reduce both tailpipe and evaporative emissions, including non-methane organic gases, NO_x, and SO_x. (Al-Khazaali et al. 2023). The U.S. Environmental Protection Agency (EPA) has amended the sulfur regulations to a minimum of 15 ppm for diesel fuel and 10 ppm for gasoline, concurrently, Euro 5 and Euro 6 regulations are striving for almost "sulfur-free" gasoline and diesel, with sulfur limits of 10 ppm or less (Al-Khazaali et al. 2023). Additionally, numerous countries, including Argentina, Australia, Brazil, Chile, China, Iran, Mexico, Russia, Saudi Arabia, and Taiwan have adopted ultra-low-sulfur diesel (ULSD) standards and in most developed countries, the permissible sulfur content in gasoline has been restricted to below 10 ppm (Shang et al. 2013). ULSD is estimated to reduce exhaust emissions by up to 90% compared to the period when sulfur levels were at 500 ppm (Sorate and Bhale 2015). To regulate shipping emissions, the International Maritime Organization (IMO) has recognized several Sulfur Emission Control Areas (SECAs) worldwide and implemented a global sulfur cap of 0.5%, which took effect in 2020 (Zhao et al. 2021). Several European Union countries, as well as the United States and Canada, have implemented stricter SECAs regulations, requiring marine operators to use fuels with a maximum sulfur content of 0.10 wt%, referred to as Ultra-Low-Sulfur Fuel Oil (ULSFO). (Nelson et al. 2022). Japan and the United States have imposed a tax on transportation fuels containing over 10 ppm sulfur (Zhao et al. 2021). Refineries are likely to face additional regulatory pressures to meet specifications for high cetane numbers and low aromatic content in diesel oil, all while simultaneously producing higher volumes of fuel

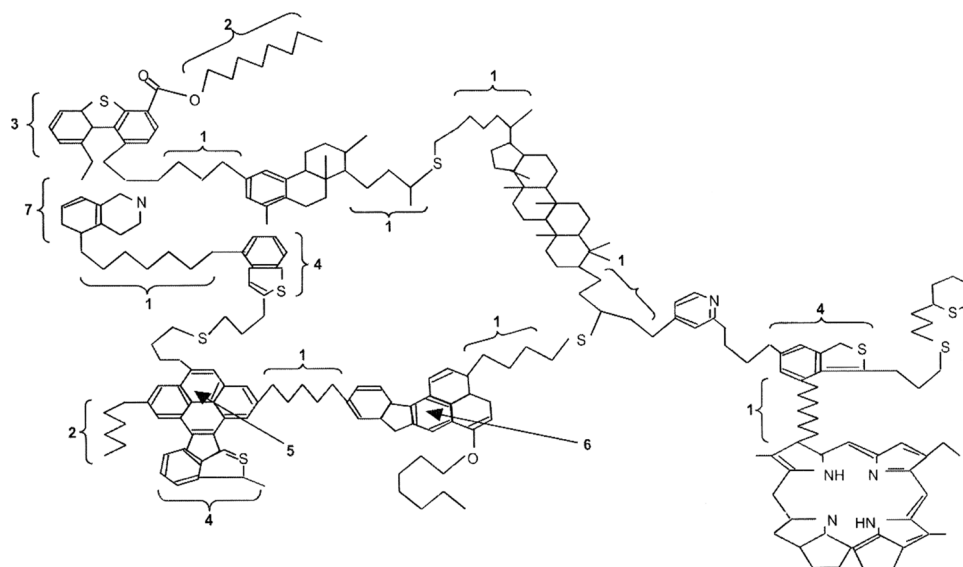
molecular structures are very complicated and vary a lot (Ali et al. 2012). It has oxygen (0.3–4.8%), nitrogen (0.6–3.3%), sulfur (0.3–10.3%), and a small amounts of metals like Fe, Ni, and V. The average molecular weight is between 600 and 2,000,000 (Tavassoli et al. 2012). Asphaltenes adversely impact petroleum extraction, transportation, and processing, as well as exhibit resistance to biodegradation after spills (El-Gendy and Speight 2022). Asphaltene constituents are characterized by relatively high molecular weights and large, highly hydrophobic structures, which can result in mass transfer limitations during aqueous reactions. Consequently, the rates of biotransformation are restricted by the mass transfer of objective molecules to the biocatalyst and, and, for complete microbial cells, through the cell membrane. (Leon and Kumar 2005). Despite these limitations, studies have demonstrated the bacterial transformation of such complex, HMWt-substrates. This transformation can significantly reduce the viscosity of crude oil by breaking down asphaltenes into smaller molecules and cleaving internal aliphatic linkages, such as sulfides, esters, and ethers, within the asphaltene structure (Gupta and Gera 2015). This is possible because these compounds provide essential elements for microbial survival, including carbon, hydrogen, sulfur, nitrogen, and oxygen. Figure 4 depicts the potential biotransformation pathways within the intricate structure of asphaltene constituents (Speight and El-Gendy 2018).

Bioprocesses have recently been recognized as a cost-effective and environmentally friendly alternative for breaking down asphaltenic structures, facilitating the conversion of low-value heavy oils into high-value light oils through bio-cracking (Hernández-López et al. 2015).

Finnerty and Singer (1983) reported that acidogenic microorganisms can reduce crude oil viscosity from 25,000 to 275 cP. Enzymatic treatment of asphaltene constituents

offers a promising substitute for heavy metal removal, aiming to mitigate catalyst poisoning during hydrotreatment and cracking processes. In a study on the asphaltene fraction of Castilla crude oil, which is characterized by high heavy metal content, three hemoproteins—chloroperoxidase, cytochrome C peroxidase, and lignin peroxidase—were tested in both aqueous buffers and organic solvents. Among these, only chloroperoxidase (CPO) effectively removed the Soret peak, eliminating approximately 27% of vanadium (V) and 53% of nickel (Ni) from petroporphyrin-rich fractions and asphaltene constituents (Mogollón et al. 1998). García-Arellano et al. (2004) reported the biotransformation of porphyrins and asphaltene constituents by a chemically modified cytochrome c. Ayala et al. (2007) demonstrated that peroxidase-catalyzed reactions show promise for the biotransformation of asphaltene constituents. Furthermore, Hernández-López et al. (2016) revealed that cytochrome P450 (CYP) monooxygenases play a role in the fungal biotransformation of asphaltene constituents and high molecular weight polycyclic aromatic hydrocarbons. Premuzic et al. (1999) documented a complex series of biochemical reactions involving selected microorganisms and HCO under controlled conditions, resulting in a significant reduction of N, S, O, and trace metal contents by approximately 24–40%. This process also led to a redistribution of hydrocarbons (HC), along with the biotransformation of asphaltene and associated polar fractions. Premuzic and Lin (1999) adapted and modified extremophilic microorganisms, including thermophilic, thermo-adapted, barophilic, and species adapted to extreme pH, high salinity, and toxic metals, such as *Achromobacter* sp., *Acinetobacter calcoaceticus*, *Arthrobacter* sp., *Leptospirillum ferrooxidans*, *Pseudomonas* sp., *Sulfolobus solfataricus*, *Thiobacillus ferrooxidans*, and *Th., thiooxidans* for the biotransformation of HCO feedstock.

Fig. 4 Regions susceptible to fragmentation and biodegradation in an asphaltene molecule. 1: photooxidation; 2: β -oxidation; 3: dibenzothiophene pathway; 4: similar to dibenzothiophene pathway; 5: pyrene pathway; 6: similar to that of benzo(a)pyrene pathway; 7: similar to CAR pathway (Speight and El-Gendy 2018)



Additionally, a fluorinated organosulfur compound, bis-(3-pentafluorophenylpropyl)-sulfide, was used as a substrate to isolate a bacterium capable of cleaving sub-terminal C–S bonds within alkyl chains (Van Hamme et al. 2004). This process could facilitate not only the desulfurization of HCO but also viscosity reduction, as this bacterium can cleave alkyl C–S bonds in asphaltene molecules without affecting the calorific value of the oil feedstock. Hao et al. (2004) reported that a Gram-negative thermophile strain, TH-2, isolated from the Shengli oil field in China and capable of withstanding temperatures up to 85°C, biodegrades asphaltene constituents, resins, and HMWt-PAHs. This strain also reduces the sulfur and nitrogen contents of HCO. The bioconversions within the crude oil led to an increase in lighter hydrocarbons and a redistribution of saturates, alongside a decrease in viscosity and paraffin content by 10.1–55.4% and 16.2–37.7%, respectively. Lavania et al. (2012) documented a viscosity reduction in HCO from 3520 to 2029 cP due to the anaerobic biodegradation of asphaltene by *Garciaella petrolearia* TERIG0. Similarly, Zargar et al. (2021) reported a microbial consortium capable of achieving a 91% reduction in the viscosity of Maya crude oil through the biotransformation of its asphaltene constituents. This process also resulted in an 80% reduction in sulfur and nitrogen content and the incorporation of oxygen into the asphaltene structure.

Biodearomatization

Diesel fuels have a higher cetane number, lower aromatic content, and negligible sulfur content, making them ideal for ultra-clean diesel engine combustion and positioning them as a fuel of the future. Hydrogenation processes, involving selective fragmentation and aromatic ring opening, can reduce density and improve the cetane number of diesel (Jia et al. 2024). There is an inverse correlation between the cetane number and emissions of particulate matter (PM) and nitrogen oxides (NO_x), making the cetane number—an indicator of diesel fuel's ignition quality—a critical parameter to regulate (Fayad et al. 2022). Catalytic hydrotreatment is the primary method for dearomatization; however, achieving an optimal balance between hydrogenolysis and hydrogenation reactions can limit process efficiency and produce low molecular weight volatile products, which may be lost from the diesel fraction. Additionally, higher hydrogen input is required for the dearomatization of heavy oils due to their elevated aromatic content. (Henpraserttae et al. 2023). Biodearomatization provides the advantages of high chemo-, regio-, and stereo-selectivity compared to traditional chemical methods (Turner and Gerlach 2024).

The naphthalene degradation route underpins the development of a biodearomatization process (Fig. 5). The biotransformation of aromatic rings in crude oil is reported

to initially involve ring hydroxylation (Fig. 5). This initial activation is followed by conventional hydrogenation and/or hydrogenolysis, leading to the production of cracked and ring-opened compounds (Speight and El-Gendy 2018). The naphthalene degradation pathway has been extensively characterized in species such as *Burkholderia*, *Comamonas*, *Mycobacterium*, *Nocardioides*, *Polaromonas*, *Pseudomonas*, *Rahnella*, *Ralstonia*, *Rhodococcus*, and *Sphingomonas* (Mohapatra and Phale 2021).

It has been proposed that interrupting step 5 (Fig. 5) could result in the formation of a more linear oxidized hydrocarbon with the same carbon content as naphthalene (Foght 2004). This modification would reduce the aromaticity of the compound and produce hydrocarbons that could be hydrogenated under milder conditions. Mutants of *Sphingomonas yanoikuyae* N2 and *P. fluorescens* LP6a have been shown to be active in water–oil biphasic reactors, capable of opening the aromatic rings of naphthalene, methyl naphthalene, DBT, 4-MDBT, phenanthrene, and CAR. These processes retained the carbon content while reducing the aromatic content in light gas oil (Kotlar et al. 2004). Additionally, Leon and Kumar (2005) reported the isolation of specific bacteria from the Guanaco asphalt lake in Venezuela that degrade DBT via the Kodama pathway (Fig. 6), leading to the production of 3-hydroxyformyl benzothiophene. These bacteria are recognized for their capacity to proliferate on bitumen and to diminish the asphaltene components, resin, sulfur, and nitrogen levels in HCO, leading to a documented decrease in viscosity.

Biodemetalization

The primary demetalization process used in the petroleum industry is physical deasphalting. Porous membrane filtration effectively removes N, S, aluminum, chromium, copper, Ni, V, and asphaltene from lubricating oil, spent diesel, heavy oils, crude oil, and bitumen (Kutowy et al. 1989). Separating lighter oils from heavier asphaltene components by mixing heavy oil or residue with low boiling solvents like butane, isobutene, or propane (Magomedov et al. 2017) or deep eutectic solvents (Mohammed et al. 2023). Adsorptive demetalization (Majeed et al. 2023), oxidative demetalization of petroleum asphaltene components and residues (Gould 1980), as well as hydro-demetalization (Yang et al. 2004), are other common methods for demetalizing crude oils. However, these processes can be costly and often result in secondary environmental pollution.

Biocatalytic techniques for the extraction of Ni and V from petroporphyrins have been documented, particularly employing CPO (Mogollón et al. 1998). The degradation of porphyrin ring structures via a chlorination reaction employing the *Caldariomyces fumago*-CPO enzyme facilitates the biocatalytic demetalization of petroporphyrins and

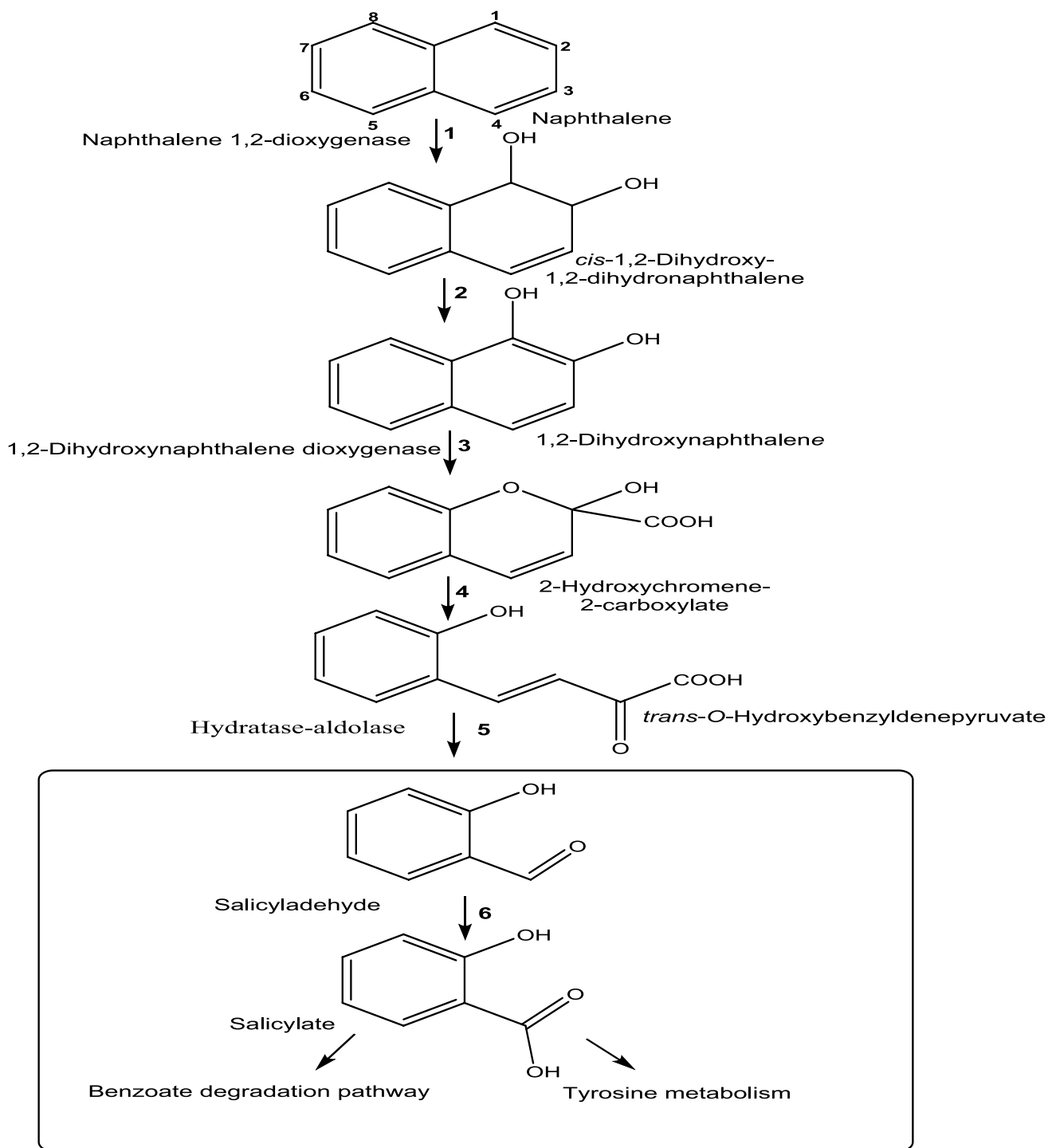


Fig. 5 Naphthalene biodegradation pathway (Speight and El-Gendy 2018)

asphaltene components, achieving reductions of approximately 53% and 93% in vanadyl octaethyl porphyrin and nickel octaethyl porphyrin, respectively (Fedorak et al. 1993). However, chlorination generates chlorinated compounds that undesirably negatively impact the environment. Unlike peroxidases, oxygenases from various sources such

as animal and plant cells (like mung beans and *Arabidopsis thaliana*), in addition to *Escherichia coli*, and yeast (like *Candida tropicalis*), can degrade porphyrin molecules without using chlorine or peroxide (El-Gendy and Speight 2022). BDM of petroporphyrins and crude oil has been also accomplished via the oxidation of porphyrinic rings utilizing



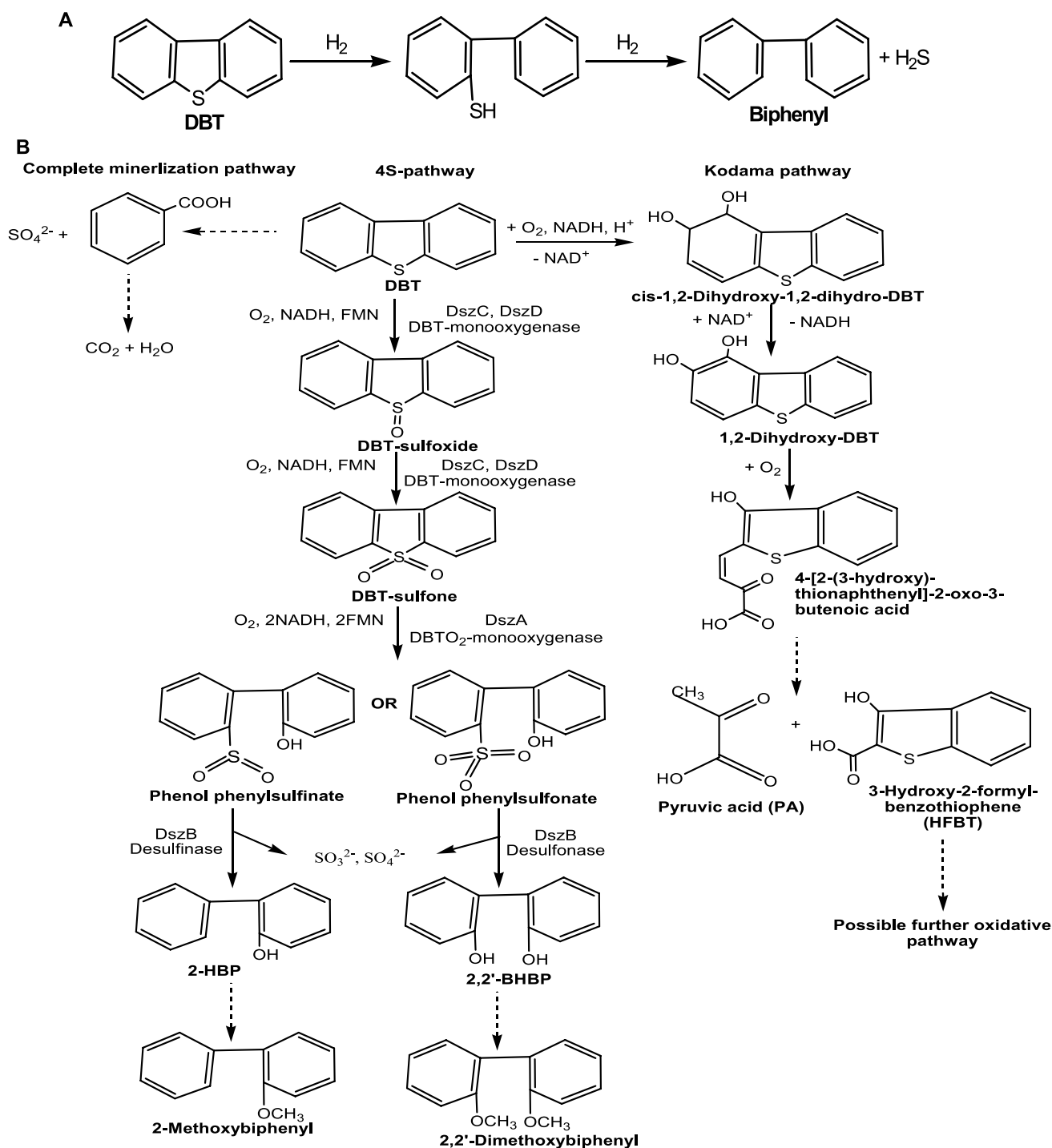


Fig. 6 Possible biodesulfurization pathways; (A) anaerobic and (B) aerobic (El-Gendy and Speight 2022)

hemoproteins (cytochrome C reductases) derived from *B. megaterium* and *Catharanthus roseus*, in conjunction with the cofactor nicotinamide adenine dinucleotide phosphate (NADPH) (Xu et al. 1998). Salehizadeh et al. (2007) reported the potential of crude oil BDM using *Aspergillus* sp. MS-100, a strain isolated from contaminated soil at the

Isfahan refinery. This microorganism is capable of metabolizing vanadium oxide octaethyl porphyrin (VOOEP) as its sole carbon source. Additionally, horse myoglobin and plant peroxidase were identified as effective proteins for synthesizing agents designed to remove porphyrins from uncracked fuels (Paul and Smith 2009). Furthermore, extracellular

enzymes produced by *Aspergillus terreus* HJ2 and *A. nidulans* HJ4 have been shown to facilitate the biotransformation of HCO. These enzymes led to reductions in asphaltenes, resins, Fe, V, and Ni by approximately 61.77%, 47.95%, 54%, 31.6%, and 44.1%, respectively, along with a 12.7% decrease in viscosity. Concurrently, there was an increase in saturates and aromatics (Zhang et al. 2024). A significant advantage of BDM is its operational flexibility. It can be conducted in batch, semi-continuous, or continuous modes, either independently or in combination with other biorefining processes, such as BDS. The process can be carried out in sealed or open vessels and under conditions with or without light (El-Gendy and Speight 2022). Kilbane (2005) highlighted that, since most metals in petroleum are associated with organonitrogen compounds, it is possible to simultaneously perform BDN and BDM. This approach reduces both nitrogen and metal content in a single operation, eliminating the need for two separate processes.

Biodesulfurization

To promote innovative breakthroughs and address the challenges posed by sulfur compounds in the industry, several techniques have been proposed, including adsorptive desulfurization (ADS), extractive desulfurization (EDS), HDS, oxidative desulfurization (ODS), and BDS (El-Gendy and Nassar, 2018). These technologies are directly linked to environmental safety and sustainability, significantly contributing to the reduction of sulfur levels in fuel oil (Qiu et al. 2024). However, each has advantages and some drawbacks as summarized in Table 3.

The concept of BDS is as old as 75 years ago (Zobell 1953). Similar to HDS, anaerobic-BDS (ABDS) desulfurizes fuels, resulting in the production of H₂S (Fig. 6A). ABDS of petroleum and its distillates has been documented using various microorganisms including *Desulfomicrobium* sp. (Yamada et al. 2001), *Desulfovibrio* sp. (Srivastava 2012), *Thiobacillus* sp. (Eckart et al. 1982), *Pyrococcus* sp. (Tilstra et al. 1992), and *Nocardioform* sp. (Finnerty 1993), *Desulfatiglans* sp. (Kareem et al. 2016), as well as *Anaerolineaceae*, *Clostridium*, and *Rummeliibacillus* sp. (Stylianou et al. 2023). ABDS offers two primary advantages: (1) the generated H₂S gas can be processed using current refinery desulfurization facilities (e.g., Claus process), and (2) there is a low generation of undesirable colored and gum-forming byproducts that would arise during the bio-oxidation of hydrocarbons (Javadli and de Klerk 2012). Despite its potential, anaerobic biodesulfurization (ABDS) remains underdeveloped due to low reaction rates, safety concerns, and economic challenges. Additionally, the enzymes responsible for ABDS have yet to be identified, and no efficient anaerobic microorganisms suitable for large-scale applied petroleum ABDS have been isolated (El-Gendy and Speight

Table 3 Advantages and disadvantages of desulfurization technologies

	HDS	EDS	ADS	ODS	BDS
The earliest and most mature industrial desulfurization process					
High temperature and pressure					
Expensive catalyst and hydrogen feed					
Recalcitrance of alkylated-DBTs					
Energy-intensive					
High operational and capital costs					
Emitting polluting H ₂ S					
	Low temperature and pressure No hydrogen requirement Expensive and non-ecofriendly solvents Selectivity of solvents is challenge-able Recovery and recycling of solvents are challengeable Disposed hazardous solvents containing concentrated carcinogenic S-compounds, creating a new waste-management problem	Low temperature (20–150°C) and atmospheric pressure Adsorbents can be prepared from agricultural wastes (e.g. biochar, activated carbon, silica-based adsorbents, etc.) Adsorbents' capacity, stability, Selectivity, and regeneration are challengeable Disposed adsorbents carrying elevated amounts of carcinogenic S-compounds, creating a new waste-management problem	Refractory S-compounds can be removed Low operating temperature and pressure Lower operating and capital costs relative to HDS Corrosive oxidizers; e.g. H ₂ O ₂ , acetic acid Separation of produced sulfoxide- and sulfone- derivatives from oxidized fuels is challengeable Creating sulfonic wastes to be treated	Cost-effective Ecofriendly process Low temperature and pressure High selectivity No adverse effect on fuel calorific value or gasoline octane number Low desulfurization rate Short life-time of applied enzymes or microorganisms Tolerance of applied biocatalyst to oil concentration and inhibitory byproducts are challengeable Mass transfer limitation from oil/water interface to the microbe Biocatalyst separation	



2022). Consequently, aerobic-BDS has been the focus of most of the research in BDS (Tamjidi and Esmaili 2025).

There are three key pathways for aerobic-BDS (Fig. 6B). The partial degradation Kodama pathway where DBT is used as a carbon source only, the complete degradation pathway where DBT serves as both a sulfur and carbon source, and the sulfur-specific 4S pathway. The 4S pathway is commercially useful for fuel desulfurization as it retains the hydrocarbon structure, preserving the fuel's calorific value (Jatoi 2025). Integration of ADS and BDS has been reported, where, *Pseudomonas*-desulfurizing strain, is used to regenerate the applied the DBT compounds-selective adsorbents (Li et al. 2009). A three-step process; BDS/ODS/reactive-ADS has been reported by Agarwal and Sharma (2010). Aerobic and anaerobic BDS of LCO and HCO with sulfur content of 0.378% and 1.88%, respectively has been performed using *Pantoea agglomerans* D23W3. That recorded a BDS efficiency of approximately 63.29% and 61.4%, respectively, and the anaerobic-BDS expressed marginally enhanced performance than aerobic-BDS. Then, the sequential ODS and reactive-ADS recorded S-removal of approximately 94% and 95%. Moreover, the application of thermophilic *Klebsiella* sp. 13 T achieved 62% and 68% sulfur removal from the LCO and HCO in the initial ABDS step, respectively.

Biodenitrogenation

The established CAR-BDN pathway (Fig. 7A) closely matches the DBT-Kodama pathway (Fig. 6B). It is evident that it is economically unviable due to the concomitant loss in the hydrocarbon structure, which subsequently results in a decrease in fuel value. Nevertheless, most CAR-degrading microorganisms initiate the CAR-BDN pathway by producing 2'-aminobiphenyl-2,3-diol as the first intermediate (Fig. 7B). Therefore, recovering CAR-derived nitrogen in the form of anthranilic acid (ANA) and/or 2'-aminobiphenyl-2,3-diol, which are less inhibitory to refining catalysts, is recommended to mitigate part of this issue. This recovery can be achieved using recombinant or mutant strains. Kilbane et al. (2001) reported that *P. ayucida* strain IGTN9m (ATCC No. PTA-806) selectively removes nitrogen from quinolone (Qn) via BDN without altering its carbon skeleton. Another mechanism involves the release of nitrogen from CAR as ammonia (Rhee et al. 1997). While most of BDN-mechanisms occur aerobically, anaerobic-BDN (ABDN) has also been documented (Fallon et al. 2010).

Global field cases for bio-upgrading of petroleum and its distillates

Although many petroleum biotechnologies have primarily undergone laboratory testing, pilot plants for BDS were established in the mid-1990s by US-based ENCHIRA

Biotechnology Corporation (ENBC). The first ABDS-process was patented by ESSO Research and Engineering Company on 1961. The leading BDS patent holders are Exxon Research & Engineering Company (2), the Korean Advanced Institute of Science and Technology (3), the US-based Institute of Gas Technology (4), the Japanese Petroleum Energy Centre (4), and ENBC (21) (El-Gendy and Speight 2022). The US Department of Energy (DOE) allocated ENBC \$900,000 in 2000 to study the use of new gene-shuffling technologies to make a biocatalyst for the selective-BDS of both petrol and diesel fuel (Grisham 2000). The current microorganisms' BDS rate is still not good enough; hence Monticello (2000) suggested a prototype plant with three bioreactors to get low sulfur levels (Fig. 8).

Petro Star and Diversa funded genetic engineering studies for the BDS of sulfones produced from Petro Star Conversion Extraction Desulfurization (CED) route (Bonde and Nunn 2003). An investigation funded by the Japan Cooperation Center, Petroleum (JCCP) utilized resting biomass of thermophilic *Mycobacterium phlei* WU-F1 that achieved full BDS of 149 ppm DBT within 90 min at 50°C. WU-F1 also demonstrated desulfurization capabilities towards naphthoathiophene found in hydrodesulfurized-light gas oil and untreated-gas oil at 45 °C and 50 °C, respectively (Furuya et al. 2002). Further, WU-F1 reported to biodesulfurize a hydrodesulfurized-gas oil, recording from 60 to 70% (Furuya et al. 2003).

In 1999, ENBC estimated a great decrease in energy consumption and CO₂ emissions upon applying successive HDS and BDS processes (Pacheco et al. 1999). Later in 2006, PetroStar Inc. performed a cost analysis for desulfurizing 5000 ppm S-diesel oil into ULSD of 10 ppm S using HDS applied in PetroStar's Valdez Refinery and BDS using *Rhodococcus* sp. (Bonde and Nunn 2003). Both studies prove the feasibility of applying BDS as complementary to HDS (Table 4).

However, in a recent publication, BDS is estimated to emit 80% fewer GHG emissions than the HDS process, and the operational and capital costs for establishing a BDS process are predicted to be 10–20% and 50% cheaper than those of HDS, respectively (Ahmad et al. 2023a).

Sugaya et al. (2001) developed a petroleum-BDN bioprocess, where, a QN-BDN *Comamonas* sp. TKV3-2-1 has been applied for BDN of petroleum under storage (Fig. 9). That recorded BDN-rate of approximately 1.6 mmol/g cell/h at 83% v/v (O/W) and 28.5 g biomass/L.

Gordonia sp. strain F.5.25.8 discovered by the Brazilian oil PETROBRAS Company can metabolize both DBT and CAR via the 4S-pathway and as its exclusive N-source, respectively without altering the fuel-calorific value. Another advantage of F.5.25.8 is its ability to withstand temperatures as far as 42 °C, promoting its real-field application in BDS/BDN processes of hydrotreated oil feeds (Santos



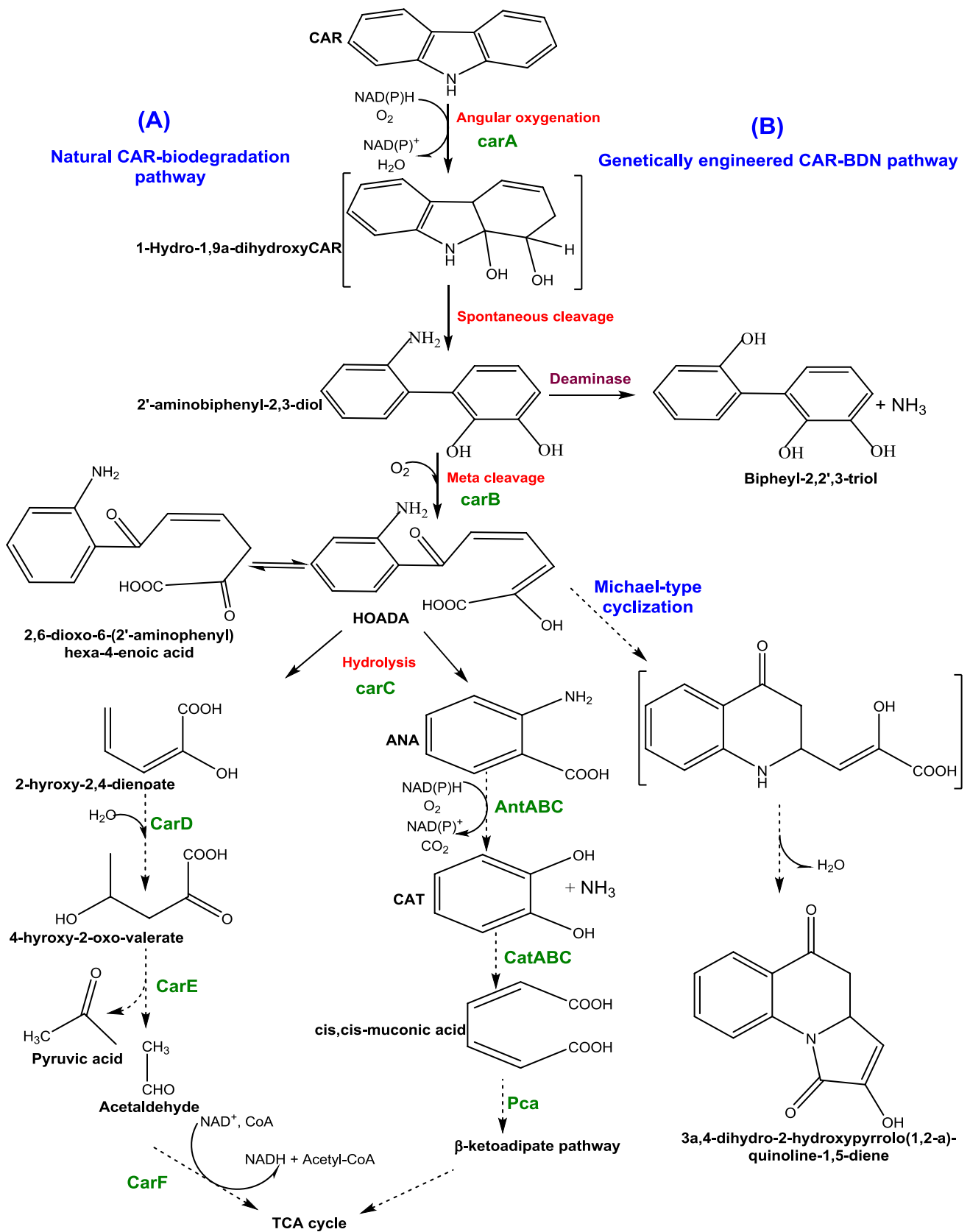


Fig. 7 Possible biodenitrogenation pathways (El-Gendy and Speight 2022)

Table 4 Summary of the estimated energy requirements, CO₂ emissions, and operational cost of diesel oil desulfurization

An assumption performed by EBC in 1999					
Desulfurization technology	Desulfurization of diesel oil from 0.2 to 0.005%		Desulfurization of diesel oil from 0.05 to 0.005%		CO ₂ emission lb/bbl
	Energy consumption MBTU/bbl	CO ₂ emission lb/bbl	Energy consumption MBTU/bbl	CO ₂ emission lb/bbl	
HDS	260	29.9	138	15.8	
BDS	56	6.1	37	4.3	
HDS/BDS	Decrease from that of HDS alone 21.5%	20.4%	26.9%	26.9%	
An assumption performed by EBC in 1999					
5000 ppm S-diesel oil into ULSD of 10 ppm S					
Desulfurization process	Annual operating cost		Cent/gallon ULSD		
	\$				
BDS alone	9,232,900				10.7
HDS alone	5,800,000				6.8
BDS followed by HDS	9,400,000				11.1
HDS followed by BDS	8,000,000				9.4



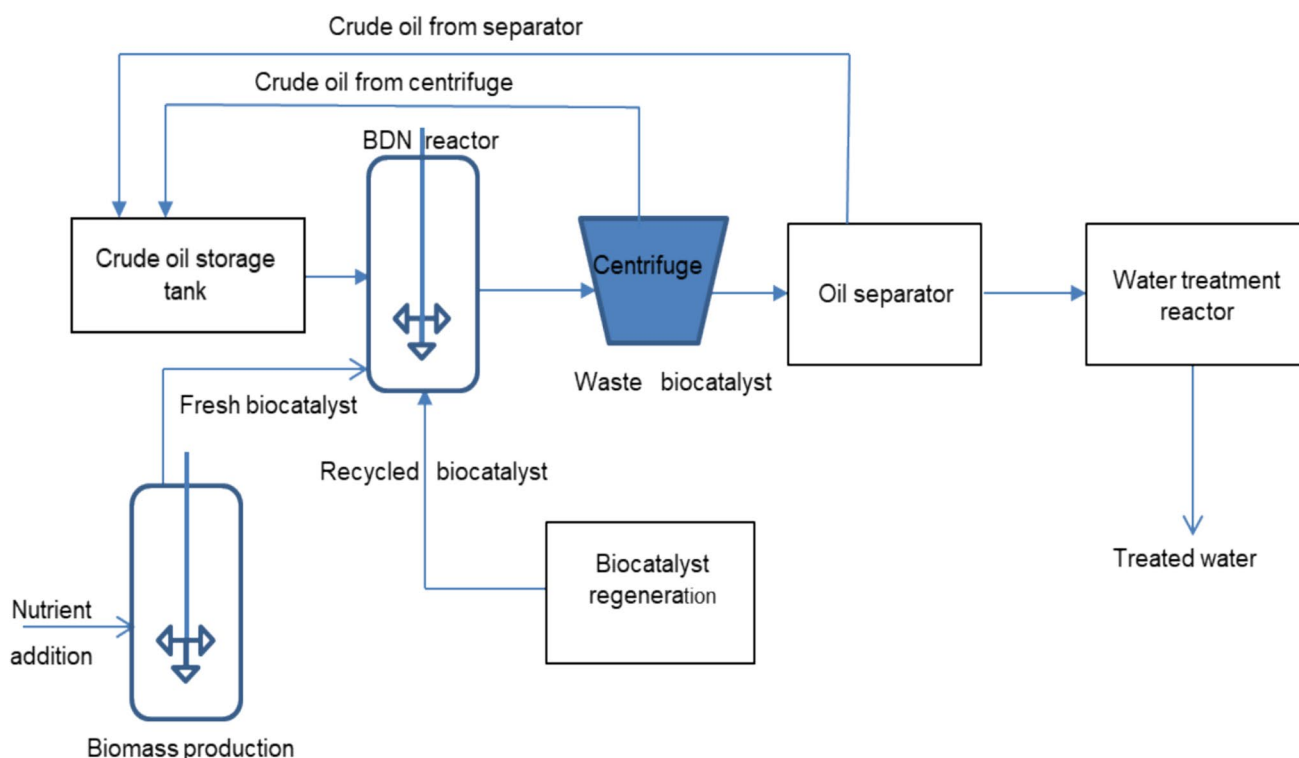


Fig. 9 A suggested petroleum BDN process (El-Gendy and Speight 2022)

During Fuel-BDS or BDN, a three-phase bioprocess made up of oil, water, and biocatalyst is involved. The transfer of PASHs or PANHs compounds from oil to water and subsequently into cells, where oxidation reactions take place in the cytoplasm, poses a limitation on the metabolism of such compounds (i.e. it is a rate limiting pace) (Setti et al. 1999). The production of biosurfactants may enhance the dispersion of organic compounds within the aqueous phase, thereby improving the mass transfer of PASHs and PANHs within the organic/aqueous phases. Thus, it is essential to obtain microbial isolates tolerating high concentrations of oil-feed, capable of producing biosurfactants, and characterized by wide enzymatic versatility to biodesulfurize and denitrogenize diverse S- and N-compounds, to biotreat higher amount of oil feed with lower bioreactor size and operating cost. Future advancements in this field are likely to depend on either the genetic modification of existing microbial strains (Bagchi and Srivastava 2024) or the identification of novel (Ahmad et al. 2023b) BDS or BDN agents. Different bacterial strains have been reported for efficient BDS capabilities over a wide spectrum of thiophenic compounds; *B. subtilis* WU-S2B (Kirimura et al. 2001), *Mycobacterium* sp. X7B (Li et al. 2003), *Gordonia desulfuricans* strain 8N (Akhtar et al. 2016), *Rhodococcus jialingiae* HN3 (Nassar et al. 2021b), *Paenibacillus glucanolyticus* HN4 (Nassar et al. 2021c), and *Gordonia rubripertincta* W3S5 (Parveen

et al. 2024). *R. erythropolis* HN2 (Nassar et al. 2021a), *Paenibacillus glucanolyticus* (Nassar et al. 2021c), and other strains; *P. putida*, *R. globerulus*, *Gordonia alkanivorans*, and *Bacillus subtilis*, reported to produce biosurfactants, which enhance the BDS-process (El-Gendy and Speight 2022). Remarkably, the DBT-BDS efficiency of a recombinant strain M29 significantly exceeded that of the wild *R. erythropolis* DS-3, and displayed no product feedback inhibition (Ma et al. 2006). A recombinant solvent-tolerant biodesulfurizing *Pseudomonas putida* A4 obtained from integration of *dsz*-genes from *R. erythropolis* XP into the solvent tolerant *P. putida* Idaho, expressed an efficient BDS-rate of approximately 1.29 mM DBT/g DCW/h (Tao et al. 2006). Recently, a genetically engineered *P. putida* DS006 reported for efficient DBT-BDS and elevated solvent tolerance (Glekas et al. 2025). Various solvent tolerable *Pseudomonas* sp. have been reported for BDN; *P. cepacia* strain F297 (Grifoll et al. 1995), *Pseudomonas* strain LD2 (Gieg et al. 1996), *P. rhodesiae* strain KK1 (Yoon et al. 2002), and *P. stutzeri* (ATCC 31258) (Larentis et al. 2011). A solvent tolerant Gram-positive *Bacillus Clausii* BS1 isolated by Zakaria et al. (2016) for CAR-BDN, reported for being tolerable to the elevated concentrations of the inhibitory by-products anthranilic acid (ANA) and catechol (CAT). Genetic studies to halt DBT-BDS at the step of 2-hydroxybiphenyl sulfinate (2-HPBSi) production is a very important point of future



research as it can be applied in MEOR and detergent industries and open a new era for biopetrochemicals (Martzoukou et al. 2025). Additional efforts are required to optimize and clarify the mechanisms of anaerobic BDS.

The application of thermophilic strains may offer significant advantages for the treatment of crude oil, particularly at elevated temperatures, as this reduces viscosity and obviates the need for cooling fractions to ambient temperatures. Such conditions would facilitate the integration of biodesulfurization (BDS) processes into refining operations subsequent to FCC and/or HDS and could potentially yield cost savings by eliminating the need to significantly cool refinery streams (Stanislaus et al. 2010). Table 5 summarizes the BDS efficiencies of the published thermophilic biodesulfurizing microorganisms. *Anoxybacillus rупiensis*, a thermophilic CAR-BDN bacterium, which withstands temperatures up to 80 °C, has also been reported with maximum BDN activity within 55–65 °C (Fadhil et al. 2014).

From a practical perspective, a dual selective BDS and BDN bioprocess would facilitate the large-scale viability of biorefining and bio-upgrading processes of petroleum and its fractions (Kilbane 2006). Nojiri et al. (1999) reported the production of carbazole-1,9a-dioxygenase (CARDO)—a multicomponent enzyme—by *P. resinovorans* CA10. This CARDO facilitates the angular dioxygenation of CAR, DBT, dibenzofuran (DBF), and other PASHs, PANHs and PAOHs compounds. The recombinant strain *R. erythropolis* SN8 reported to express an efficient simultaneous selective crude oil BDS and BDN from DBT and CAR, along with their derivatives, respectively (Yu et al. 2006). *R. erythropolis* ATCC 4277 has been reported for an efficient simultaneous BDN and BDS batch bioprocess with an elevated concentration of heavy gas oil (HGO; 40% v:v O/W). That recorded approximately a BDN rate of 162 mg N/kg HGO/h and BDS

rate of 148 mg S/kg HGO/h (Maass et al. 2015). However, the optimization of medium components is reported to be critical, as the elevated N-concentration in biomass enrichment media would suppress the development of the BDS oxidoreductase and desulfinase enzymes (Porto et al. 2017). Thus, consequently enhance BDN on the expense of BDS (Todescato et al. 2017).

Nanobiotechnology in bio-upgrading of petroleum and its distillates

Immobilized biocatalysts has several advantages over free biocatalysts, including; enhanced storage and operational stability, ease of separation, reduced contamination risk, extended lifespan, enhanced activity, overcome the mass transfer limitations, protect the biomass from the byproducts' inhibitory effect, enhance the bioelarnace against high concentration of solvents and oil feed, offer biocatalyst reusability, and can be applied in both batch and continuous bioprocesses (Hou et al. 2005). However, immobilized biocatalysts via entrapment in hydrogels, for example, alginate or polyvinyl alcohol (PVA) suffer from some limitations. Loss of activity with successive cycles, due to the decline in co-factors concentrations (e.g. FMNH₂ and NADH₂) and mass transfer limitations, especially with alkylated high molecular weight compounds (Yan et al. 2008). Iron oxide nanoparticles (ION) have recently been utilized throughout the petroleum industry, including exploration, production, petroleum processing, refining, and petrochemical industries, as well as in the treatment of produced water from petroleum production (PPPW) for reinjection and wastewater recycling, etc. (El-Gendy et al. 2022). The green-synthesized IONs have better biocompatibility, lower toxicity, a larger specific surface area, and better magnetic properties than the

Table 5 BDS of DBT using thermotolerant biodesulfurizing microorganisms

Bacteria	Temperature/substrate specificity	BDS efficiency	References
<i>Paenibacillus</i> sp. A11–2	50 °C DBT, 4-MDBT, 4,6-DMDBT	Complete desulfurization of 0.065 mM DBT	Konishi et al. (1997)
<i>Bacillus subtilis</i> WU-S2B	50 °C DBT, 4-MDBT, 4,6-DMDBT	Complete desulfurization of 0.54 mM DBT	Kirimura et al. (2001)
<i>Mycobacterium phlei</i> WU-F1	50 °C DBT, 2,8-DMDBT, 4,6-DMDBT	Complete desulfurization of 0.8 mM DBT	Furuya et al. (2001)
<i>Mycobacterium phlei</i> GT1S10	50 °C Th, BT, DBT, 2-MDBT	1.09 μmol 2-HBP/g DCW/min	Kayser et al. (2002)
<i>Mycobacterium</i> sp. X7B	45 °C DBT	82.5% of 0.4 mM DBT	Li et al. (2003)
<i>Mycobacterium phlei</i> SM120–1	45 °C DBT, light gas oil	0.17 μmol 2-HBP/g DCW/min	Srinivasaraghavan et al. (2006)
<i>Mycobacterium goodii</i> X7B	40 °C DBT	90% of 0.5 mM DBT	Li et al. (2007)
<i>Sulfolobus solfataricus</i> P2	70 °C DBT	1.23 μM 2-HBP/g DCW/h	Gün et al. (2015)



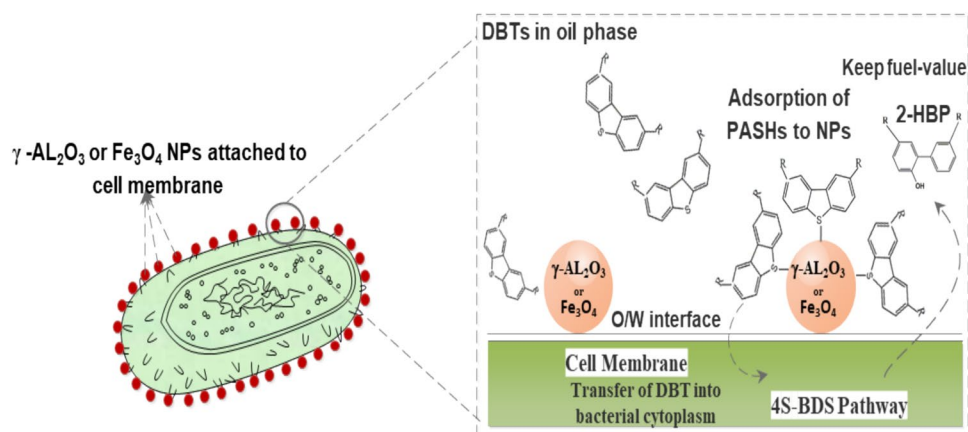
chemically generated and functionalized ones. This makes them more useful in bioprocesses and environmental remediations. (Fahmy 2020). The one-pot green synthesis of NPs is a better choice than traditional chemical methods since it is cheaper, better for the environment, consumes less time and energy, has a smaller water and carbon footprint, and because of its ease of manipulation (El-Gendy and Omran 2019). It can be considered as sustainable process, upon the application of different agro-industrial wastes extracts in its preparation (Nassar et al. 2021a). Application of magnetic nanoparticles (MNPs) in bio-upgrading processes is found to be very promising due to their favorable attributes like high surface-to-volume ratios, superparamagnetic characteristics, negligible toxicity, and it achieve all advantageous of immobilization (Li et al. 2013).

The initial application of NPs in fuel bio-upgrading is in BDS (Shan et al. 2003). The increase in bio-upgrading rate is attributed to the selective adsorption capacity toward the heterocyclic compounds, which is electrostatic and reversible (Zhang et al. 2008). Thus, overcome the mass transfer limitation, collecting PASHs, and PANHs compounds from the non-aqueous oil phase to the cell, and then returning back the desulfurized and denitrogenized compounds, enhancing the transfer and bioprocess rate, overcoming the byproducts inhibitory effects, and keeping the fuel hydrocarbon skeleton and calorific value (Fig. 10). BDS can be integrated with ADS via the application of nanobiotechnology, through the bioregeneration of the adsorbents to be reused. A critical factor for successful bioregeneration is the adsorbent's porous structure, which facilitates contact between the oil containing PASHs compounds, water, and biocatalyst through agitation, creating emulsified microdroplets at the O/W interface, creating emulsified microdroplets at the O/W interface. Li et al. (2006) put out a microbial process for regenerating desulfurization adsorbents, utilizing *P. delafieldii* R-8 for the regeneration of Cu-modified zeolite sorbent used in ADS. The adsorption capability of the bioregenerated sorbent was found to be 85% of that of a fresh one, allowing its reusability. However, a challenge arose due to the resemblance in

the adsorbent size and the biodesulfurizing cells size, which complicates their separation. Later, Li et al. (2008) solved this by applying *P. delafieldii* R-8 decorated by MNPs, for the bioregeneration of AgY zeolite desulfurization adsorbents. The biocatalyst separation has been accomplished using an external magnetic field and the adsorption capability of the bioregenerated sorbent was found to be 93% of that of a fresh one, allowing its reusability.

The modification of the nano-adsorbents $\gamma\text{-Al}_2\text{O}_3$ with gum Arabic (GA) found very promising in the integration of ADS with BDS (Zhang et al. 2007). The ADS was enhanced by 1.12 fold. Moreover, *P. delafieldii* R-8 cells decorated with modified alumina improved the BDS efficiency recording 25.7 mM S/kg/h relative to those decorated by unmodified alumina, which recorded 17.8 mmol S/kg/h. That was attributed to the role of GA, which improved the dispersion, biocompatibility, and affinity of the NPs for the bacterial cells while reducing toxicity. In a further work (Zhang et al. 2008), various NPs— γ -alumina, molecular sieves, and activated carbon (AC)—were compared for their effectiveness in the in situ simultaneous application of ADS and BDS utilizing *P. delafieldii* R-8. The results indicated that Na-Y molecular sieves inhibited R-8 cell activity, and AC was ineffective in desorbing the adsorbed DBT, making them unsuitable for this application. In contrast, $\gamma\text{-Al}_2\text{O}_3$ NPs quickly took up DBT from the oil phase, released it, and moved it to R-8 cells for BDS, which sped up the pace of desulfurization. The size of NPs used for cell decoration found to be a critical parameter. The MNPs < 5 nm, are reported to be more effective in BDS enhancement (Bardania et al. 2013). However, 13 nm MNPs-coated *R. erythropolis* IGTS8 proved an enhanced BDS efficacy (Rahpeyma et al. 2018). Application of biosurfactants, oleate or glycine found to decline the NPs agglomeration and perform better distribution and arrangement of NPs on cells, without negatively affecting their BDS efficiency (Etemadifar et al. 2014). Glycine-modified-MNPs proved to enhance the BDS efficacy of *R. erythropolis* IGTS8 and *R. erythropolis* FMF, where decorated cells kept

Fig. 10 Enhancement of bio-upgrading process by using NPs-coated cells



their activity for five- consecutive cycles (Bardania et al. 2019). The non-toxic carbon nanotubes (CNTs) can selectively adsorbing PASHs, would enhance its availability to microbial cells and increase its membrane-permeability, enhancing their BDS-efficiency and byproducts tolerance (Sadare and Daramola 2019). Decoration of *R. erythropolis* IGTS8 and *Gordonia rubropertinctus* PTCC 1604 by polyethylene glycol (PEG)-modified CNTs enhanced the DBT-BDS rate by approximately 12% and 16%, respectively, compared to free cells (Karimi et al. 2018a). Further, adding MNPs synergistically extra-enhanced DBT-BDS effectiveness of *R. erythropolis* IGTS8 (Karimi et al. 2018b). ZnO NPs also reported to improve the BDS efficiency of *R. erythropolis* IGTS8 and *P. aeruginosa* PTSOX4 (Rahpeyma et al. 2017). Also, immobilization via silica NPs enhances the DBT-BDS efficiency of *R. erythropolis* IGTS8 by approximately 20% relative to free one (Canales et al. 2018). The 241 magnetite–silica nanocomposite-coated *R. erythropolis* IGTS8 expressed higher BDS efficacy than the MNPs-coated IGTS8 (Rahpeyma et al. 2018). The cell ornamentation of the thermophilic *B. thermoamylovorans* by 20 nm and 30–40 nm either starch-functionalized- MNPs or iron NPs has boosted the DBT-BDS efficacy by 10% and 22%, relative to free cells, respectively (Etemadi et al. 2018). Later starch-functionalized-CNT reported to enhance the BDS efficacy, as it enriched both the selective adsorption of PASHs and their oxidation (Sabaghian et al. 2019a, 2019b).

Nanobiotechnology can be also applied for anaerobic-BDS, where NPs enhanced the BDS performance of *Desulfobacterium indolicum* in kerosene feed, recording approximately 71.73%, within 3 d, at room temperature, under atmospheric pressure (Kareem 2014). Applying nano-materials, such as latex-NPs or alumina nano-rods, for enzyme immobilization, performed promising application in ODS via the nano-immobilized chloroperoxidase (CPO) and myoglobin enzyme (Vertegel 2010; Juarez-Moreno et al. 2015).

Wang et al. (2007) demonstrated that *Sphingomonas* sp. XLDN2-5 immobilized in a gellan gum (GG)-modified by MNPs enhanced the CAR-BDN, achieving a rate of 3479 μg CAR/g wet weight cell/h. This was significantly higher than the rates observed for GG-immobilized cells (1761 μg CAR/g wet weight cell/h) and free cells (3092 μg CAR/g wet weight cell/h). It is worth to mention that the immobilized XLDN2-5 immobilized into GG-modified-MNPs have been used for eight cycles with an obvious increase in CAR-BDN, which recorded 4638 μg CAR/g wet weight cell/h and overcome also the steric hindrance and mass transfer limitations occurred with GG-immobilized cells. That was due to the robust cell progression within the GG-modified-MNPs beads and the ability of MNPs to weaken the binding of the GG layers and creation of plentiful pores, facilitating better nutrient and substrate access towards the immobilized XLDN2-5 cells. Later, magnetically immobilized

Sphingomonas sp. XLDN2-5 found to exhibit an equivalent CAR-BDN efficiency to that of the free cells (Li et al. 2013). However, total elimination of 3500 μg CAR was achieved within 9 h during the 6th cycle and within 2 h during the 7th and 10th, indicating progressive enhanced BDN efficiency in successive reusability. Yet, the MNPs/cells ratio is an important factor to be studied. Sun et al. (2017) observed that the increment of MNPs concentrations than 1:1 w:w (MNPs/wet cell weight), decreased in the BDN activity MNPs coated-*Sphingomonas* sp. XLDN2-5. Nassar et al. (2021a) documented an enhancement in BDS efficiency of super-paramagnetic iron oxide NPs (SPION)-decorated *R. erythropolis* HN2 at a concentration of 0.9 g SPION/g cell, which recorded 1.33 fold BDS-enhancement than free cells. The SPION can overcome mass transfer limitations, enhance the selective adsorption of PASHs, and improve their permeability across the cell membrane. This process drives inhibitory end byproducts back into the non-aqueous oil phase, thereby preserving the oil fed heat value. The BDN rate of 1000 ppm CAR was doubled applying MNPs coated *B. clausii* BS1, retaining its activity up to 672 h operation (Zakaria et al. 2015). MNPs enhanced *B. clausii* BS1 tolerance towards the inhibitory ANA and CAT byproducts, it can be stored for 30 d at 4 °C without losing its activity. The enhancement of the BDS efficacy of MNPs-decorated *R. erythropolis* HN2 was also reported (Nassar et al. 2021a). It also overwhelmed the O/W phase ratio, steric hindrance and mass transfer limitations recording an enhancement in Th, BT, DBT, 4-MDBT, and 4,6-MDBT BDS rate by approximately 2.68, 1.91, 1.40, 1.28, and 1.56 relative to free cells, respectively in a 30% (O/W) biphasic batch bioreactor. Not only that, but it also has a long life-span, operational and storage stability. It can be operated for 480 h and stored for 28 d at 4 °C without losing its activity. Table 6 summarizes some published examples for the enhancement of BDS and BDN by applying nanobiotechnology.

Agro-industrial wastes and its applications in bio-upgrading of petroleum and its distillates

Recently readily available, sustainable, and cost-effective agro-industrial wastes are applied to improve the BDS-efficiency. Alves et al. (2008) reported that the enzyme hydrolyzate of recycled paper sludge can improve the DBT-BDS effectiveness of *Gordonia alkanivorans* strain 1B. That recorded 1.1 μmol 2-HBP/g DCW/h productivity with a concomitant removal of approximately 250 μM DBT, within 96 h. Later, the total reducing sugars in *Jerusalem artichoke* juice have replicated the BDS efficacy of the *G. alkanivorans* strain 1B (Silva et al. 2013). The improvement of DBT-BDS utilizing *G. alkanivorans* strain 1B has also been documented, employing sugar beet molasses as a carbon source (Alves and Paixão, 2014). That achieved a 1B



Table 6 Application of nano-materials in BDS and BDN processes

Bacteria	Nano-matrix	BDS/BDN efficiency	References
<i>P. delafieldii</i>	Magnetic-PVA beads	9 mM S/kg DCW/h Super-paramagnetic Ease of collection Reusability for 7-cycles	Shan et al. (2003)
<i>P. delafieldii</i>	Decorated by MNPs	Enhanced BDS-rate 16.4 mM S/kg DCW/h Complete DBT removal Reusability for 5-cycles Better operational stability	Shan et al. (2005)
<i>P. delafieldii</i> R-8	Decorated by nanosorbent γ -Al ₂ O ₃	Two-fold BDS-enhancement than free cells	Guobin et al. (2005)
<i>P. delafieldii</i> R-8	Magnetic-PVA beads	Magnetization 8.02 emu/g Enhanced BDS-rate 40.2 mM S/kg DCW/h	Guobin et al. (2006)
<i>R. erythropolis</i> LSSE8-1	Decorated by MNPs	Enhanced BDS rate 14.1 μ M DBT/g DCW/h Completely desulfurize 2.5 mM DBT in n-tetradecane within 10 h Super-paramagnetic Ease of collection Reusability for 7-cycles, keeping 80% of its activity Free cells kept 15% of its activity	Li et al. (2009)
<i>R. erythropolis</i> IGTS8	Decorated by MNPs	Enhanced BDS by 56%, relative to free cells	Ansari et al. (2009)
<i>R. erythropolis</i> LSSE8-1-vgb	Decorated by nanosorbent γ -Al ₂ O ₃ and MNPs (5:1 w:w)	20% higher BDS, relative to MNPs-coated cells (1:50 MNPs:cells) Complete removal of 2 mM DBT in n-octane (1/2 O/W) within 9 h Reusability for 3- cycles, keeping 90% of its activity	Zhang et al. (2011)
<i>R. erythropolis</i> FMF <i>R. erythropolis</i> IGTS8	Decorated by MNPs	70—73% BDS efficiency relative to 67 and 69% of free cells Better reusability and separation	Bardania et al. (2013)
<i>Brevibacterium lutescens</i> CCZU12-1	Magnetized alginate beads	Complete removal of 2 mM DBT in n-octane (1/9 O/W) No mass transfer limitation Reusability for 4-cycles Ease of separation	Dai et al. (2014)
<i>R. erythropolis</i> R1	γ -Al ₂ O ₃ /alginate beads with the addition of cofactors 10 mM nicotinamide and 40 mM riboflavin	Enhanced BDS efficiency by 30% after 4-cycles	Derikvand et al. (2014)
<i>R. erythropolis</i> IGTS8	Decorated by mesoporous silica nanosorbents	Enhance BDS efficiency 0.34 μ M DBT/g DCW/min 0.126 μ M 2-HBP/g DCW/min 19% and 16% better than free cells, respectively	Nasab et al. (2015)
<i>R. erythropolis</i> IGTS8	Decorated by MNPs	Enhanced BDS efficiency, 15.80% better than free cells	Karimi et al. (2017)
<i>Pseudomonas</i> sp. GBS.5	Decorated by MNPs	Enhanced CAR-BDN efficiency, 77.14% better than free cells Reusability for 5-cycles, keeping 77% of its BDN capacity	Mehndiratta et al. (2014)
<i>R. erythropolis</i> HN2	Decorated by SPION	Enhanced diesel oil BDS 30% v/v oil/water, removed 96% of 690 mg S/L, with 1.2-fold relative to free cells	Nassar et al. (2021a)



biomass progression rate of 0.0795 h^{-1} , with a concomitant production of 2-HBP of approximately $250 \mu\text{mol/L}$. Eight Carob pulp liquors, have also been identified as an economical carbon source for BDS (Pacheco et al. 2019). Cassava and trub agro-industrial wastes have been also stated to enhance the BDS efficiency of HGO, applying a selective biodesulfurizing of *R. erythropolis* ATCC 4277 (Porto et al. 2017). Nassar et al. (2021b) documented the utilization of corn-steep liquor (CSL), by-products of the starch industry, and bioglycerol, a byproduct from biodiesel production process, as nitrogen and carbon co-substrates in BDS process. That enhanced the biomass production and DBT-BDS-efficacy of a novel biodesulfurizing *R. jialingiae* strain HN3 by approximately two hundred- and six-fold, respectively. The integration of agro-industrial wastes into BDS process not only overcomes the cost of media constituents, which is required for biomass growth and enzymatic enrichment, but it also enhance the BDS rate.

Researches on the green synthesis of silica- and C-based catalysts applied in HDS processes should be also addressed to decrease the overall catalyst cost. Moreover, acetic acid as a C-co-substrate has been also reported to enhance the BDS efficacy of *P. putida* CECT5279 (Martinez et al. 2015). Thus, the integration of the fermentation process of lignocellulosic and other agro-industrial wastes producing biorefineries such as lactic, acetic, and citric acids, into BDS process is an interesting point of research to open new era for green and circular economy in petroleum industry. The hot aquatic extract of mandarin peels has been testified for the green synthesis of 20–63 nm $\alpha\text{-Fe}_2\text{O}_3$ (Ali et al. 2017) and Fe_3O_4 NPs (Nassar et al. 2021a) that have 2.4168 emu/g and 51.12 emu/g magnetization, respectively. Those expressed wide applications in enhancing the rate of wastewater treatment and oil feed BDS. Alginate can be produced from the proliferated brown macroalgal biomass, as a bioremediation tool for marine ecosystem (El-Gendy et al. 2024) can be applied for immobilizing the BDS and BDN microorganisms. Agro-industrial wastes can be also applied for the preparation of C- and silica- based adsorbents applied in ADS and ADN (Azeez and Ganiyu 2023; Qiu et al. 2024) and also for ODS and ODN (Roman et al. 2021). Many other agro-industrial wastes, for example palm tree cobs, carbonized slash pine bark, chromium tanned leather, and oil palm shell can be valorized into cost efficient AC for ADS and ADN (Anisuzzaman et al. 2014). Omran et al. (2021) published the preparation of AC from the spent waste mandarin peels disposed from the green synthesis of Ag-nanoparticles, as a tool for reaching to zero-waste green industry and to decreases the overall cost of AgNPs preparation. Other domestic waste biomass, for example; coconut shells, almond shells, olive stones, apricot pits, palm fruit bunches, and peach pits can be also utilized as sustainable sources for AC-synthesis for ADS and ADN (Faria et al. 2023). Olive

and rosemary leaves testified for the green production of Ag and ZnO NPs, then Ag-activated carbon (Ag/AC), ZnO-activated carbon (ZnO/AC), and Ag/ZnO/AC nano-composites have been prepared via precipitation. Upon their application for ADS, they expressed efficient S-removal according to the following order $\text{AC} < \text{Ag/AC} < \text{ZnO/AC} < \text{Ag/ZnO/AC}$ (Saleem et al. 2023).

Other important points of research to be covered can be summarized as follows; (1) isolation of halotolerant microbial isolates to lessen the fresh water consumption; (2) recycling of obtained wastewater to decrease the water-footprint of the applied bioprocess; (3) isolation of more solvent tolerable, hydrophobic microorganisms capable also of biosurfactant production to overwhelm the issue of transfer limitation between oil/water phases and biotreat more feed volume/cycle, (4) use genetic engineering to increase the number of copies of specific BDS and BDN genes and/or create recombinant microorganisms with better BDS and BDN efficacy and can tolerate high levels of hydrocarbons and inhibitors, (5) apply statistical and computational machine learning tools for optimization, modeling, and simulation of the applied bioprocess, as this will lower the cost of optimizing and scaling up of the process, (6) minimize the overall cost as much as possible, via the production of valuable byproducts during the applied bioprocess stream, and (7) design new bioreactors with efficient capabilities to control the process factors, including; temperature, pH, purge of required gases (O_2 or H_2), nutrient concentration, O/W ratio, etc.

Biological approaches to mitigate petroleum hydrocarbon pollution

There are different sources of pollutants disposed from oil and gas industry; the formation water that produced upon EOR, refinery and petrochemical industrial wastewater, sludge from tank cleaning, and oil spill that might occur during transportation or storage (El-Gendy and Speight 2015). The traditional physico-chemical techniques applied for remediation are primarily focus on contaminant separation rather than degradation, failing to eliminate environmental pollutants effectively. The chemical remediation techniques utilize expensive and non-ecofriendly cleaning agents including synthesized organo-clays through ion exchange, dispersants, chemical absorbents, synthetic surfactants, and additives (Ossai et al. 2022). However, these agents are associated with several disadvantages, such as high costs, non-biodegradability, non-recyclability, and potentially significant acute and chronic toxic effects on ecosystem (Matilda and Samuel 2024). In contrast, bioremediation technology, employing microbial populations, offers a more environmentally friendly and cost-effective solution (Elshafei and Mansour 2024). This method not only

degrades pollutants completely but also aligns with sustainable development goals, making it increasingly preferable (Tedesco et al. 2024). The costs associated with bioremediation are estimated to range from \$5 to \$300 per cubic meter, depending on the specific techniques employed. In contrast, physico-thermal treatments and non-ecofriendly incineration incur costs of approximately \$600 and \$2000 per cubic meter, respectively, making them significantly more expensive than bioremediation (Bianco et al. 2023). Despite its demonstrated successes, challenges remain in the implementation of bioremediation techniques. It is usually applied as complementary for physical and/or chemical treatment (El-Gendy and Speight 2015).

Global bioremediation field cases

Bioremediation proved a successful real field application in the two main historical petroleum spills; BP Deepwater Horizon and Exxon Valdez (Atlas and Hazen 2011). There are some other successful field trials for various bioremediation strategies (Table 7), yet, addressing issues such as the selection of appropriate microbial strains, the design of effective remediation processes, and enhancement of the process rate are essential for future advancements in this field.

Research gaps and future opportunities for the applicability of bioremediation

Bioremediation is categorized into natural attenuation that occurs spontaneously, relying on existing environmental conditions without human intervention and engineered bioremediation, including biostimulation of indigenous microflora and bioaugmentation via injection of nutrients and microorganisms (Wang et al. 2021b). Engineered bioremediation typically operates more rapidly than natural attenuation because it actively stimulates biological degradation

and allows for the precise regulation of nutrients, oxygen levels, moisture content, temperature, and pH (Bala et al. 2022). There are two main types of engineered bioremediation: in-situ and ex-situ bioprocesses.

In-situ denotes to the biotreatment of contaminated materials right away at the polluted site, allowing for the natural environmental conditions to facilitate the process. Conversely, ex-situ bioremediation means taking away the contaminated materials for treatment at a separate location, which may include methods such as water pumping or soil excavation (Perez-Vazquez et al. 2024). Factors affecting the bioremediation of petroleum hydrocarbons are summarized in Fig. 11.

The introduction of various stimulatory materials, including bulking agents (Helmy and Kardena 2024), nutrient amendments (Bertha et al. 2021), bio-surfactants (Sah et al. 2022), biopolymers (El-Gendy and Nassar 2015), and slow-release fertilizers (Sun et al. 2023) is essential to enhance and support the growth and enzymatic activities of microorganisms for achieving a successful bioremediation process. The sustainable, readily available and cost-effective organic wastes (Fig. 12) can be utilized to fulfill this approach (El-Gendy and Farah 2011; Ali et al. 2014; El Mahdi et al. 2016; Zhang et al. 2020b; Valdivia-Rivera et al. 2024). The bioaugmentation with enriched wild microorganisms (Gao et al. 2023) or genetically engineered ones GEMs (Rafeeq et al. 2023) that are capable of mineralizing the recalcitrant and carcinogenic PAHs, PASHs, PANHs, and phenolic compounds is very important. However, the complexity and cost of genetic manipulations, in addition to the global concern and restrict regulations for GEMs applications, besides their possible negative impact on indigenous microflora, withdraw their commercialization in bioremediation field (Rebello et al. 2021). Thus, the application of wild microbes with enriched and well adapted enzymatic system is still the most global commercialized bioremediation scenario (Maglione et al. 2024). *Staphylococcus gallinarum* NK1 reported to

Table 7 Successful field trials of various bioremediation strategies

Country	Bioremediation strategy	Pollutant	%BD	Reference
India	Biostimulation	Petroleum polluted soil	75	Gogoi et al. (2003)
Australia	Bioaugmentation	PAH-polluted soil	81	Juhasz et al. (2005)
Spain	Biostimulation	PAH-polluted soil	94.4	Pelaez et al. (2013)
Canada	Biopile	Petroleum polluted soil	47	Akbari (2014)
Hong kong	Bioaugmentation	PAH-polluted soil	99.4	de Almeida et al. (2021)
Romania	Ex-situ bioremediation	Petroleum polluted soil	83	Micle and Sur (2021)
Peru	Bioaugmentation and Biostimulation	Crude oil	95.23	Jimenez and Maldonado (2022)
Iran	Biostimulation	Petroleum sludge	75	Koolivand et al. (2022)
Australia	Bioaugmentation	PAH-polluted soil	96	Guerin (2022)
Spain	Bioaugmentation	Petroleum polluted soil	90.3	Curiel-Alegre et al. (2024)
Indonesia	Land farming bioremediation	Petroleum polluted soil	99.75	Helmy and Kardena (2024)



Fig. 11 Factors affecting the bioremediation of petroleum hydrocarbons pollutants

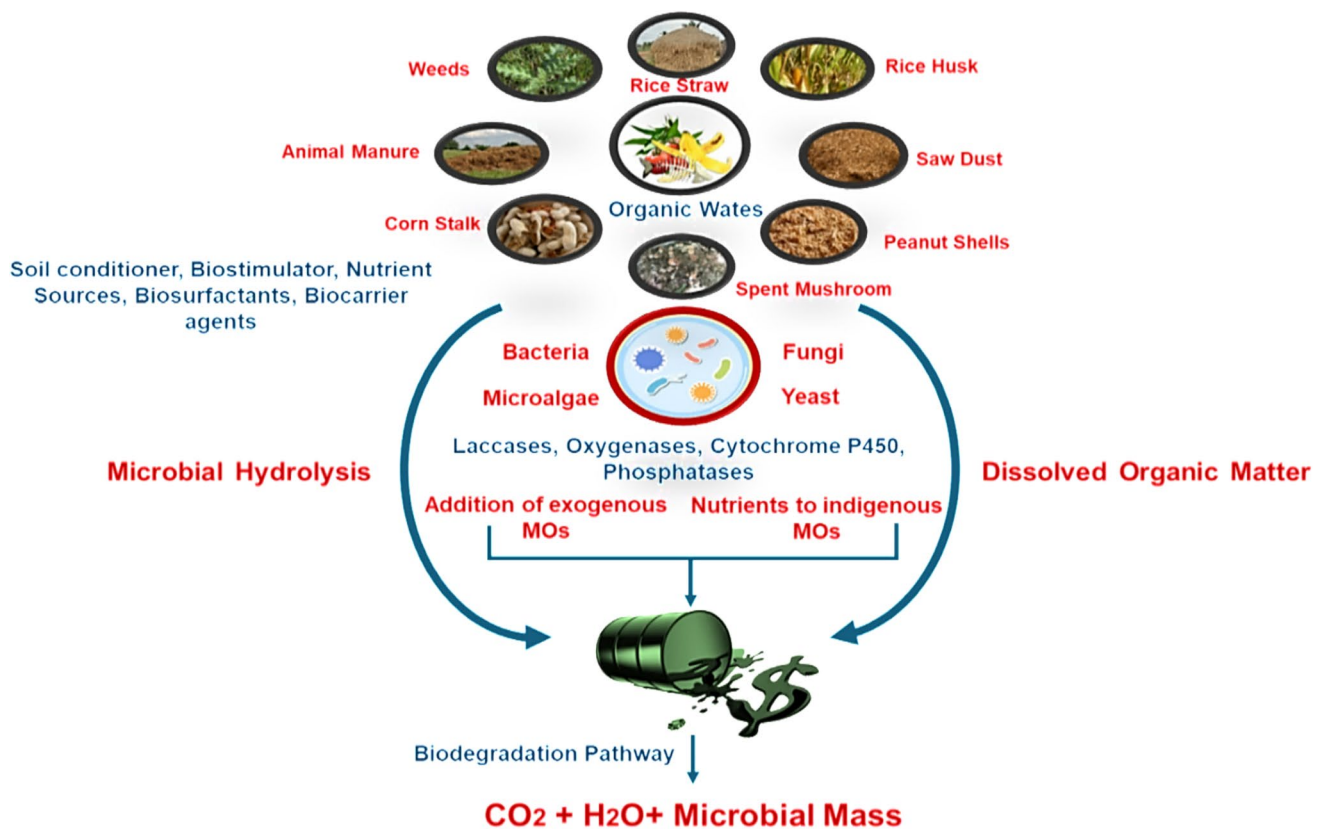


Fig. 12 Enrichment of hydrocarbon-degrading microbes (MOs) applying organic wastes

degrade both PAHs and PASHs (El-Gendy et al. 2009). *Bacillus sphaericus* HN1 is reported for degradation of the recalcitrant PASHs (Deriase et al. 2015). The halotolerant *Corynebacterium variabilis* Sh42 is reported for its capability for degrading DBT and its derivatives in addition to the recalcitrant phenolic compounds 2-hydrobiphenyl and

2,2'-bihydrobiphenyl (Deriase et al. 2013). The design of microbial consortia is reported to express a positive synergistic effect on the bioremediation process, as it would overcome the obstacles of; (1) wide variation in polluting compounds' degradation rates, and (2) the presence of inhibiting byproducts (Aruotu et al. 2023). Moreover,

thermophilic consortia are useful for bioremediation of petroleum hydrocarbons polluted environment, especially in high hot environment exceeding 45 °C, since they can multiply and metabolize rapidly, producing biosurfactants which enhance the bioavailability of pollutants, especially the complex hydrocarbons, consequently speeds up the biodegradation process (Peng et al. 2024). In a microcosm scale study, the bioaugmentation of petroleum hydrocarbons polluted soil with a thermophilic bacterial consortium of *Caldibacillus*, *Mycobacterium*, *Luteimonas*, *Chelatococcus*, *Pseudoxanthomonas*, *Alcaligenaceae*, *Kyrpidia*, *Aeribacillus*, *Conexibacter*, and *Geobacillus* incubated at 55 °C for 140 days decrease the petroleum hydrocarbons concentration from 13,890 to 3703 mg/kg, with a predominant efficacy towards medium- and long-chain hydrocarbons recording 87.1% and 67.2%, respectively (Wang et al. 2025).

Application of lignocellulosic wastes as bulking agent (Nwankwegu et al. 2017) and carrier for nutrients, enzymes, and microorganisms (Ubani and Atagana 2024) in bioremediation process is reported to be very promising. Moreover, the utilization of biosurfactant producer hydrocarbon degrading microorganisms (MOs) is very recommendable (Table 8), as it will minimize the cost of ex-situ production of biosurfactant and then purge it to the area under reclamation (Guo et al. 2022). *Agro-industrial wastes* can be also applied in bioremediation processes. Ali et al. (2014) implemented a novel process to bioremediate petroleum contaminated wastewater of API separator using only CSL as the sole N, P, and K source and produce rhamnolipid biosurfactant as a value added product that has wide industrial application. After 21 d of incubation, the gravimetric analysis of the biosurfactant showed a yield of 1.3 g/L with

a concomitant loss of 2.9 g/L of crude oil (i.e. 58% biodegradation). Bioaugmentation of an oily sludge-contaminated soil with enriched biosurfactant producer *Micrococcus lutes* RM1 and CSL was also reported to be better than applying biostimulation alone (Soliman et al. 2014). That was also confirmed by El-Gendy et al. (2014b) upon the bioaugmentation of petroleum polluted seawater by the halotolerant, asphaltene degrader, and rhamnolipid producer *Pseudomonas aeruginosa* Asp2 and CSL. Asp2 proved versatile capability in biodegrading the most recalcitrant petroleum hydrocarbons constituents, not only the asphaltene and resins fractions, but also the pristine, phytane, terpanes, steranes, diasteranes, and hopanes biomarkers.

Nowadays, recycling of wastewater to be reused in EOR, cooling towers, and downstream processes is recommendable to decrease the water footprint of this industry and minimize pollution (Patni and Ragunathan 2023; Shahbaz et al. 2023). One of the expensive fundamental ideas is to refrain from amalgamating various types of wastewater streams to alleviate the load on treatment facilities, as illustrated in Fig. 13.

The spent waste rice straw (SWRS) and bagasse (SWB) disposed of bioethanol production process act as good biosorbent for phenols Younis et al. (2014) and PAHs Younis et al. (2015) in refineries wastewater. Integrated processes for producing different types of biofuels and recycling of wastewater are very recommendable to achieve zero-waste process, decrease the carbon and water footprints, and implement the concept of circular economy. Bagasse (Abo-Staté et al. 2013) and rice straw has been used to produce 226 kg of ethanol/ton bagasse and 110 gallon bioethanol/ton rice straw (Nassar et al. 2022a) with calorific value (CV) of approximately 25 MJ/kg. The disposed SWB proved to act as good biosorbent for kerosene and diesel oil contaminated water and the obtained polluted sorbent was used as a solid biofuel of 33 MJ/kg CV (El-Gendy and Nassar 2016). The disposed SWRS proved as an efficient biosorbent to petroleum hydrocarbons, heavy metal, and the scale formation inducers and the obtained polluted sorbent was used as a solid biofuel of 33 MJ/kg CV of 38.56 MJ/kg CV. It is worth to mention that the treated water obtained from the suggested integrated process was relevant to the Egyptian law for safe discharge on open water streams and to the international standards for water reuse in EOR.

Application of nano-bioremediation is very promising. The phenol biodegradation rate was doubled by the usage of immobilized laccase onto magnetic mesoporous silica-NPs (Wang et al. 2012). It is worth to mention that the immobilized enzymes used for 10 successive cycles, with retaining 73% of its activity. The decoration of *Micrococcus lutes* RM1 and halotolerant biosurfactant fabricator *P. aeruginosa* NSH3 cells with MNPs enhanced the biodegradation rate of the recalcitrant HMWt pyrene in aqueous media

Table 8 Overview on biosurfactants producers for enhanced hydrocarbon degradation

Biosurfactants	Microbial strains	References
Surfactin	<i>Bacillus subtilis</i> ATCC 21332	Karlapudi et al. (2018)
	<i>B. subtilis</i>	
	<i>B. licheniformis</i>	
Rhamnolipid	<i>P. aeruginosa</i> MM1011	Amani et al. (2013)
	<i>P. aeruginosa</i> Asp2	Ali et al. (2014)
	<i>P. fluorescens</i>	Lavanya (2024)
Glycolipid	<i>Aeromonas</i> sp.	Ambust et al. (2023)
	<i>Bacillus</i> sp.	
	<i>P. aeruginosa</i>	
Sophorolipid	<i>Candida tropicalis</i>	Karlapudi et al. (2018)
Lipopeptide	<i>Acinetobacter baumannii</i> OCB1	Zahed et al. (2022)
	<i>Staphylococcus pasteurii</i> CO100	
di-rhamnolipid	<i>Pseudomonas</i> sp. S1WB	Phulpoto et al. (2022)

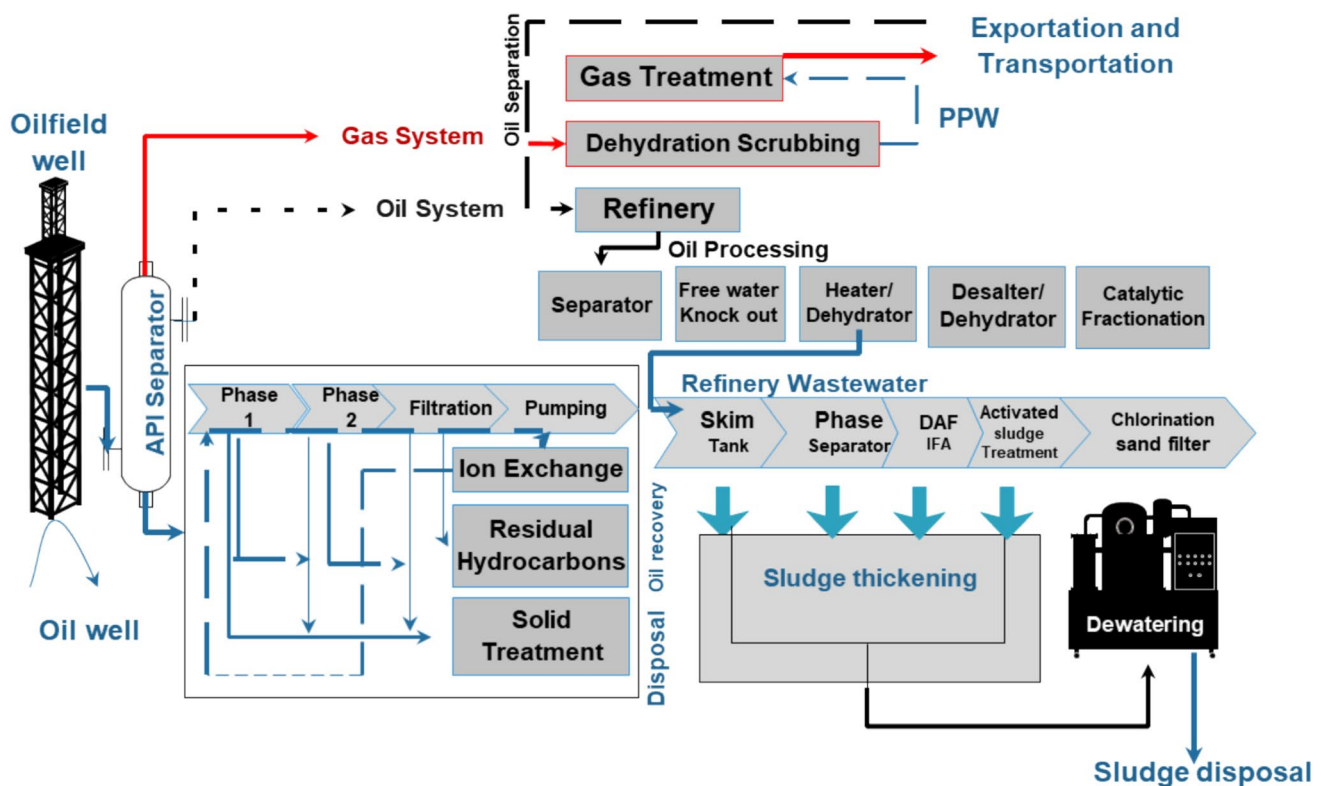


Fig. 13 Wastewater sources and treatment in petroleum industry (El-Gendy et al. 2022)

(Saed et al. 2014) and HMWt-PAHs and PASHs in polluted sediment (Nassar et al. 2022b), respectively. El-Sheshtawy et al. (2014) mentioned that the amendment of media with $\alpha\text{Fe}_2\text{O}_3$ and $\text{Zn}_5(\text{OH})_8\text{Cl}_2$ enhanced the biodegradation rate of *P. xanthomarina* KMM 1447 and *P. stutzeri* ATCC 17588 consortium. The MNPs-coated *Thiobacillus versutus* D301 has been applied for treating alkaline water polluted by polysulfide, sulfide, and thiosulfate (Xu et al. 2015). *Citrobacter* coated with magnetized silica NPs recorded a 450-fold increment in sulfide reduction of wastewater highly contaminated with sulfate (Zhou et al. 2015). *Comamonas* sp. JB immobilized into r- Fe_2O_3 NPs/gellan gum beads enhanced the bioremediation of petrochemical wastewater highly contaminated with BTEX and the immobilized-JB used for eight-successive cycles without losing its catalytic activity (Jiang et al. 2015). Immobilized horseradish peroxidase onto $\text{Fe}_3\text{O}_4/\text{Au}@CA$ proved high operational stability and activity in the bioremediation of wastewater highly contaminated with 4-chlorophenols (Sarno and Iuliano 2020).

Conclusion and recommendations

MEOR and bio-upgrading of petroleum and its distillates are cost-effective and eco-friendly processes and hold great potential as a viable alternative and/or

complementary to the traditional chemical and physical methods. A brief evaluation of the existing data, the estimated practicality, and the benefits of applying biotechnology in the petroleum industry is summarized in Table 9. However, uncertainties remain regarding the engineering design criteria necessary for the implementation of microbial processes in practical applications. Consequently, an enhanced comprehension of bioprocesses and mechanisms from an engineering standpoint is imperative, alongside systematic assessments of key factors affecting the process, including (i) the composition and quality of the crude, (ii) the heterogeneity, features, and geology of the reservoir, (iii) selection of appropriate microbial consortia, (iv) application of advanced technologies, for example genetic engineering, nanobiotechnology, and incorporation of cost-effective, readily available, and sustainable agro-industrial wastes. Thus, continued scientific research, coupled with technological innovations, will be paramount in overcoming the current limitations and realizing the full potential of MEOR and bioprocesses in the oil and gas sector. Not only that, but more large-scale pilot tests and field trials are needed to validate laboratory findings and address practical challenges in diverse environmental and reservoir conditions. These trials will provide critical insights into the adaptability and efficacy of biotechnological methods under real-world scenarios.

Table 9 A brief evaluation of the existing data, the estimated practicality, and the benefits of applying biotechnology in the petroleum industry

Biotechnological application	Existing data	Estimated practicality	Benefits
Microbial enhanced oil recovery (MEOR)	<p>Microorganisms: e.g., <i>Pseudomonas</i> and <i>Bacillus</i> produce biosurfactants to reduce interfacial tension (IFT) and mobilize trapped oil</p> <p>Some reported field trials in China, USA, and Argentina demonstrated significant oil recovery improvement</p> <p>In some field trials; oil production increased from 0.4 to 4.7 tons/day and in others; MEOR yielded 2.1×10^5 tons additional oil</p> <p>Cost: \$3–\$9 per barrel compared to \$10–\$53 for traditional methods</p>	<p>Effective in heterogeneous reservoirs where traditional methods fail</p> <p>Adaptable to extreme conditions (e.g., high salinity, temperature, and pressure)</p> <p>Low cost for implementation in reservoirs</p> <p>Cost-effective for heavy oil recovery</p> <p>High adaptability to local reservoir conditions</p>	<p>Microorganisms can be sustained on low-cost sustainable raw materials, and biosurfactants exhibit excellent emulsification properties</p> <p>MEOR products are all biodegradable and will not be accumulated in the environment, thus, it is environmentally compatible</p> <p>Reduces energy consumption, operational costs, and GHG emissions</p> <p>Utilizing agro-industrial wastes as nutrient sources, promotes circular economy principles</p> <p>Enhances oil recovery efficiency by modifying reservoir properties (e.g., wettability and viscosity)</p> <p>Supports domestic energy security</p> <p>Reduces reliance on energy-intensive methods</p> <p>Provides economic and regulatory advantages through cleaner production</p> <p>Cost-effective and eco-friendly</p>
Bio-upgrading of petroleum and distillates	<p>Bioprocesses like BDS, BDN, and BDM reduce sulfur, nitrogen, and heavy metals in crude oil and its distillates</p> <p>BDM can be integrated with BDS and can be also combined with BDN to reduce both metal and nitrogen content in a single operation with an enhancing efficiency</p> <p>Thermophilic microorganisms (e.g., <i>Mycobacterium</i>, <i>Bacillus</i>) achieved > 90% sulfur removal from DBT and other sulfur compounds</p> <p>BDS emits approximately 80% fewer GHG emissions than HDS</p>	<p>Operates under milder conditions, reducing energy and infrastructure costs</p> <p>Can be integrated with conventional refining processes (e.g., HDS)</p> <p>Suitable for meeting ultra-low-sulfur diesel (ULSD) standards</p>	<p>Produces cleaner fuels with reduced environmental impact</p> <p>Preserves fuel calorific value and quality</p> <p>BDS is estimated to have 70 to 80% lower carbon dioxide emissions and energy consumption than HDS</p> <p>Lower operational and capital costs compared to HDS</p> <p>Meets stringent environmental regulations, including sulfur content limits (e.g., < 10 ppm for diesel)</p>



Table 9 (continued)

Biotechnological application	Existing data	Estimated practicality	Benefits
Bioremediation of petroleum hydrocarbon pollution	<p>Bioremediation strategies, including biostimulation and bioaugmentation, have been successfully applied to manage petroleum hydrocarbon pollution in various environments</p> <p>Some field trials in India, Spain, and Canada achieved > 90% degradation of petroleum pollutants</p> <p>Bacteria producing biosurfactants (e.g. <i>Pseudomonas aeruginosa</i>) improved pollutant bioavailability and degradation efficiency</p> <p>Costs: \$5–\$300 per cubic meter for bioremediation versus \$600–\$2000 for physico-chemical methods</p>	<p>Effective for managing oil spills, refinery wastewater, and sludge</p> <p>Engineered bioremediation processes offer flexibility for both in-situ and ex-situ applications, depending on site-specific conditions</p> <p>Leverages sustainable, low-cost organic wastes as nutrient sources (e.g., molasses, corn steep liquor) further improves economic feasibility</p>	<p>Cost-effective and environmentally friendly</p> <p>Complete degradation of pollutants, including recalcitrant compounds (e.g., PAHs, PASHs)</p> <p>Restores ecosystems with minimal biodiversity impact</p> <p>Facilitates compliance with sustainability goals and environmental regulations</p>
Nanobiotechnology integration	<p>Nanoscale adsorbents: Magnetic nanoparticles (MNPs) enhance BDS, BDN, and bioremediation</p> <p>Nanoscale materials facilitate better mass transfer, selective adsorption, and biocatalyst stability, leading to higher reaction rates and yields</p> <p>Green-synthesized nanoparticles using agro-industrial wastes reduce costs and environmental impact</p>	<p>Reusable nanomaterials reduce operational costs and minimize waste generation</p> <p>Scalable and adaptable for multiple applications (e.g., bioremediation, bio-upgrading)</p> <p>Facilitates integration into existing bioprocesses</p>	<p>Enhanced process scalability and reproductibility</p> <p>Improved reaction rates and yields in BDS and bioremediation</p> <p>Improved selectivity and efficiency in the removal of sulfur, nitrogen, and heavy metals from petroleum fractions</p> <p>Enhanced stability, reusability, and selectivity of biocatalysts</p> <p>Reduced GHG emissions and energy consumption compared to traditional methods</p> <p>The utilization of the green-synthesized nanoparticles using agro-industrial wastes aligns with green and circular economy goals</p>
Agro-industrial waste utilization	<p>Wastes like molasses, corn steep liquor, and lignocellulosic residues serve as low-cost nutrient sources</p> <p>Improves MEOR, BDS, and bioremediation efficiency</p>	<p>Readily available and sustainable</p> <p>Reduces process costs while promoting waste valorization</p>	<p>Aligns with circular economy principles by minimizing waste and enhancing resource efficiency</p> <p>Reduces costs of biotechnological processes</p> <p>Mitigates methane emissions from natural waste degradation</p>



The integration of MEOR, bio-upgrading, and bioremediation techniques with existing chemical and physical methods offers a pathway to integrate “lean and green” in the petroleum industry, via optimizing the oil production, reducing environmental footprints, and meeting the stringent global regulations. Thus, specialists should focus on hybrid strategies that combine the efficiency of conventional approaches with the sustainability of biotechnological solutions. Not only that, but, recycling and reusing wastewater generated during petroleum processes should also become standard practice to reduce the industry's water footprint.

Additionally, implementing bioprocesses such as biodesulfurization (BDS) and biotransformation of heavy crude oils (HCO) can significantly lower greenhouse gas (GHG) emissions, contributing to climate change mitigation. However, transitioning bio-upgrading technology of petroleum and its distillates from laboratory small-scale or pilot-scale tests to large-scale commercial operations necessitates significant enhancements in several technical aspects. This includes engineering reactor systems, optimizing operational parameters, and refining downstream processing techniques. Not only that, but it is also critical to improve the performance characteristics of the biocatalysts involved, besides optimizing the bioprocess designs, including reactor configurations, nutrient delivery systems, and operational parameters. This includes designing bioreactors capable of maintaining controlled conditions such as pH, temperature, and oxygen levels, ensuring scalability without compromising efficiency.

The development of tailored microbial consortia, selected based on reservoir or environmental conditions (e.g., temperature, salinity, and pressure), is essential for improving the efficiency and consistency of MEOR and bioremediation. Genetic engineering and adaptive evolution can be used to enhance microbial tolerance to extreme conditions and inhibitory compounds.

Bioremediation should be prioritized as a cost-effective and environmentally friendly method for managing petroleum hydrocarbon pollution. Strategies such as bioaugmentation with hydrocarbon-degrading microorganisms and biostimulation using sustainable nutrient sources should be further explored for large-scale applications.

Nevertheless, the integration of green and sustainable practicing in petroleum industry via MEOR, biotransformation of HCO into LCO through the BDA and BDPA processes, and bio-upgrading of HCO and its distillates through the BDM, BDS, and BDN, as well as biotreatment of polluted effluents and bioremediation of contaminated sites will open a new era for the sustainability of this sector with a decrease in its carbon foot print and environmental impact. Utilizing agro-industrial by-products (such as corn steep liquor, molasses, and lignocellulosic residues) as carbon and nutrient sources not only reduces the cost of

bioprocesses but also promotes circular economy practices. This approach aligns with sustainability goals by minimizing waste and contributing to resource efficiency. Moreover, the integration of biowaste valorization and biorefineries into petroleum processing through zero-waste, low-carbon and water footprints technologies (Fig. 14) is very promising to achieve sustainable strategy and practicing to reduce the cost, maximize the profit, save the resources, reduce the environmental impact, and save the planet. Thus, this integration not only enhances resource efficiency and reduces waste but also contributes significantly to the principles of green and circular economy.

Likewise, the integration of nanobiotechnology within the applied bioprocesses in the petroleum industry presents a transformative opportunity to advance sustainability and align with global development goals. The efficiency of bioprocesses will be enriched, the life-time, operational stability, and activity of the applied biocatalysts will be also enhanced. Thus, the operational and capital cost, energy consumption, and GHG emissions will decrease. This innovative approach not only addresses critical challenges associated with petroleum processing but also aligns with the broader objectives of sustainability, including climate action, responsible consumption, and industry innovation. As the industry strives to meet increasing global energy demands, the application of nanobiotechnology offers a pathway to optimize processes, improve product quality, and mitigate environmental degradation. Thus, future research should focus on optimizing nanoparticle synthesis using green methods and evaluating their long-term environmental impacts.

Furthermore, advanced monitoring tools and real-time data analytics should be employed to evaluate the performance of bioprocesses, such as microbial activity, biosurfactant production, and oil recovery rates. These technologies will enable dynamic adjustments to optimize efficiency and minimize operational risks. Moreover, the petroleum industry must invest in capacity building and training programs to equip engineers, scientists, and practitioners with the skills required to implement these innovative biotechnological solutions effectively. Collaborative research initiatives and knowledge-sharing platforms should be established to accelerate technological advancements.

Yet, global unrelenting policies, strategies, and investments are still required to develop more and more innovative environmental and petroleum biotechnologies to meet the growing challenges in the petroleum industry and economically fulfill society's needs for affordable energy and petrochemical products and strict global environmental regulations. Thus, governments and industry stakeholders must collaborate to incentivize the adoption of biotechnological innovations in the petroleum industry. This could include financial support for pilot-scale projects, regulatory

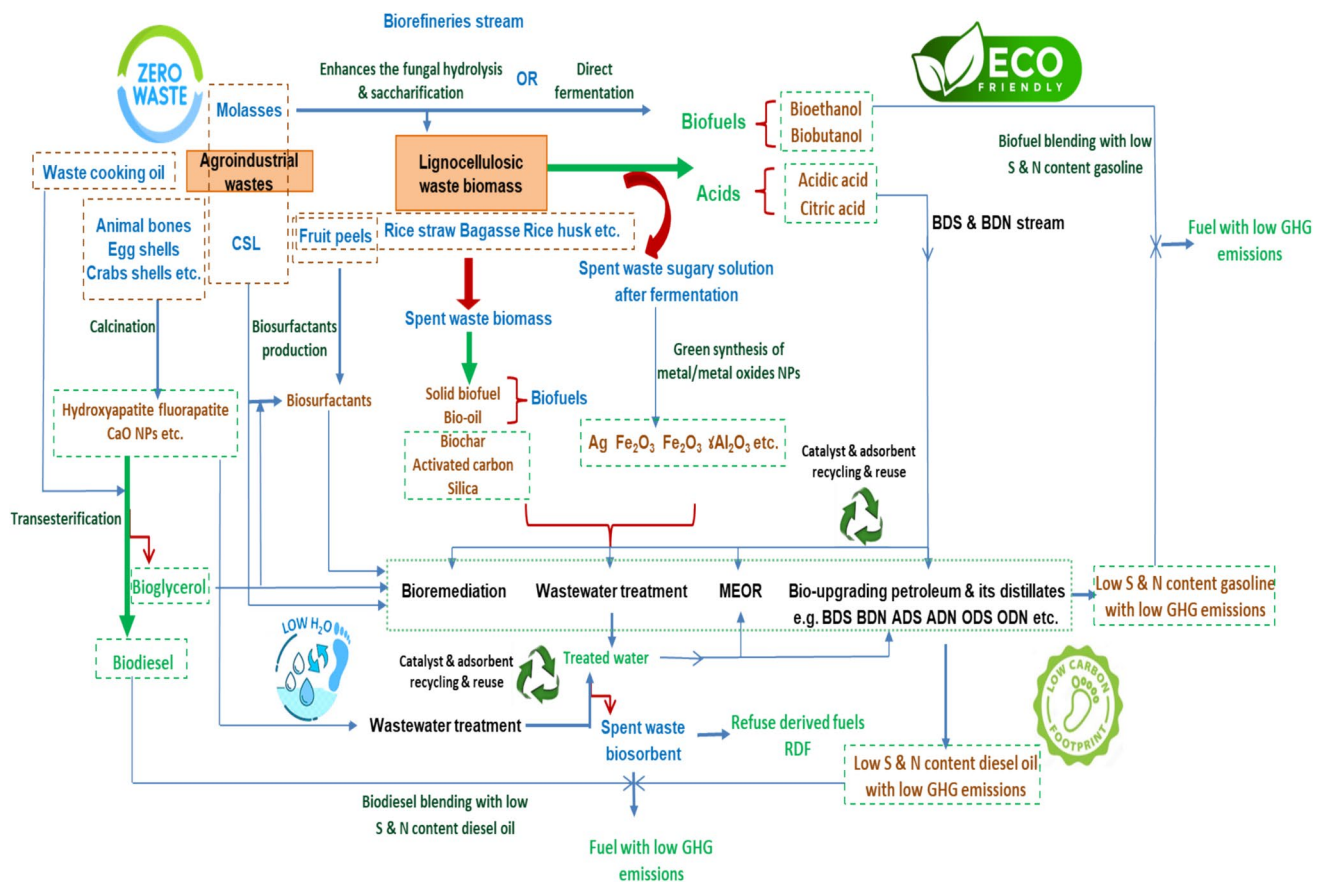


Fig. 14 A suggested ecofriendly, zero-waste, low carbon and water footprints fully integrated process for sustainability in the oil and gas industry

frameworks that prioritize eco-friendly solutions, and public–private partnerships to drive research and development (R&D).

Finally, by integrating these aforementioned practical recommendations into industry practices, the petroleum industry can significantly reduce its environmental footprint, enhance resource efficiency, and align itself with global sustainability goals, ultimately paving the way for a greener and more responsible future.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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