PETROLEUM MICROBIOLOGY

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INTRODUCTION

- Petroleum is a complex mixture of hydrocarbons and other organic compounds, including some organo-metallo- constituents
- Petroleum recovered from different reservoirs varies widely in compositional and physical properties
- These hydrocarbons are both a target and a product of microbial metabolism

INTRODUCTION

- A wide range of studies involves biotransformation, biodegradation, and bioremediation of petroleum hydrocarbons.
- Interest in exploiting petroleum-degrading organisms for environmental clean-up has become central to petroleum microbiology.
- The vast range of substrates and metabolites present in hydrocarbon-impacted soils provides an environment for the development of a quite complex microbial community.

INTRODUCTION

- Various microorganisms responsible for hydrocarbon transformations have been isolated and identified
- List of hydrocarbon-degrading organisms include:
  - mesophilic and thermophilic sulfate-reducing bacteria
  - methanogens
  - mesophilic and thermophilic fermentative bacteria
  - iron-reducing bacteria

INTRODUCTION

- Current applied research in petroleum microbiology encompasses:
- oil spill remediation
- fermentor- and wetland-based hydrocarbon treatment
- biofiltration of volatile hydrocarbons
- microbial enhanced oil recovery
- oil and fuel upgrading through:
  - deemulsification, desulfurization, denitrogenation and de-metallation
  - coal processing
  - fine-chemical production
  - microbial community-based site assessment

Microbial Enhanced Oil Recovery (MEOR)

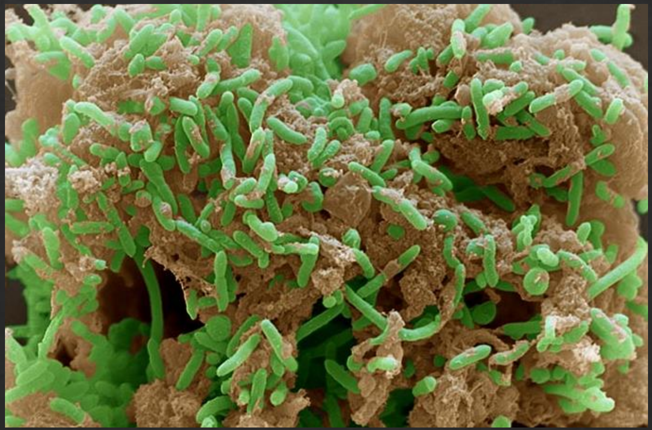
- Involves exploitation of microbial technology in oil reservoirs to improve recovery.
- MEOR processes are somewhat similar to *in situ* bioremediation processes.
- Involves injection of nutrients, together with indigenous or added microbes
  - promote *in situ* microbial growth and/or
  - generation of products which;

Microbial Enhanced Oil Recovery (MEOR)

- mobilize additional oil and;
- move it to producing wells through;
  - **reservoir re-pressurization,**
  - **interfacial tension/oil viscosity reduction, and**
  - **selective plugging of the most permeable zones**
- Alternatively, the oil-mobilizing microbial products may be produced by fermentation and injected into the reservoir

Microbial Enhanced Oil Recovery (MEOR)

- Physicochemical properties of the reservoir affecting MEOR technology include:
  - salinity
  - pH
  - temperature
  - pressure
  - nutrient availability



Microbial Enhanced Oil Recovery (MEOR)

- Molds, yeasts, algae, and protozoa are NOT suitable for MEOR

- Only bacteria are considered promising candidates for MEOR owing to the following characteristics:
  - their small size
  - ability to grow under the conditions present in reservoirs e.g. many petroleum reservoirs have high NaCl conc.
  - Bacteria produce biosurfactants and polymers which can grow at NaCl conc. of up to 8% and selectively plug sandstone to create a biowall to recover additional oil.
  - Ability to tolerate high temperature in the oil wells

Microbial Enhanced Oil Recovery (MEOR)

- Thermophilic isolates useful for MEOR have been described.
- Extremely thermophilic anaerobes that grow at 80 to 110°C have been isolated and cultured in the laboratory.
- All of these organisms belonged to the archaebacteria, living:
  - autotrophically on sulfur, hydrogen, and carbon dioxide by methanogenesis and
  - heterotrophically on organic substrates by sulfur respiration or anaerobic fermentation.

Microbial Enhanced Oil Recovery (MEOR)

- In MEOR technology, oil recovery was found to be sensitive to:
  - variations in the concentration of injected bacteria
  - size of the bacterial culture plug
  - incubation time
  - residual oil saturation
- MEOR-participating MOS produce a variety of fermentation products from crude oil, pure hydrocarbons, and a variety of non-hydrocarbon substrates
- Examples are shown in Table 6

TABLE 6. Microbial products and their applications in enhanced oil recovery[a]

Product	Microorganism	Application in oil recovery
Biomass	*Bacillus licheniformis* *Leuconostoc mesenteroides* *Xanthomonas campestris*	Selective biomass plugging Viscosity reduction Oil degradation, wetability alteration
Biosurfactants (emulsan, sophorolipids, peptidolipid, rhamnolipid)	*Acinetobacter calcoaceticus* *Arthrobacter paraffineus* *Bacillus licheniformis* *Clostridium pasteurianum* *Corynebacterium fasciens* *Pseudomonas rubescens*	Emulsification, decrease of interfacial tension, viscosity reduction
Biopolymers (alginate, xanthan, dextran, pullulan)	*Bacillus polymyxa* *Brevibacterium viscogenes* *Leuconostoc mesenteroides* *Xanthomonas campestris*	Injectivity profile modification, mobility control
Solvents (*n*-butanol, acetone, ethanol)	*Clostridium acetobutylicum* *Clostridium pasteurianum* *Zymomonas mobilis*	Oil dissolution, viscosity reduction
Acids (acetate, butyrate)	*Clostridium* spp. *Enterobacter aerogenes*	Permeability increase, emulsification
Gases (CO_2, CH_4, H_2)	*Clostridium acetobutylicum* *Clostridium acetobutylicum* *Enterobacter aerogenes* *Methanobacterium* sp.	Increased pressure, oil swelling, decrease of interfacial tension, viscosity reduction, permeability increase

Microbial Enhanced Oil Recovery (MEOR)

- **Organic acids** produced through fermentation readily dissolve carbonates and can greatly **enhance permeability** in limestone reservoirs.

- **Organic solvents** and **dissolved CO_2** can **decrease oil viscosity.**

- **Fermentation gases** can **repressurize wells**, leading to displacement and production of light or conventional crude oil through a revitalized gas-driven mechanism

Desirable properties of polymers for MEOR

- Shear stability - a measure of whether and how much of the oil's viscosity is lost
- High solution viscosity- a measure of the resistance of a fluid which is being deformed by either shear stress or tensile stress
- Compatibility with reservoir brine
- Stable viscosity over a wide range of pH, temperature, and pressure
- Resistance to biodegradation in the reservoir environment

Recovery of residual oil in reservoirs

- When highly permeable watered-out regions of oil reservoirs are plugged with bacterial cells and biopolymers.

- Bacteria and nutrients are injected into the reservoir, and the system is shut to allow the biomass to plug the more permeable region as it grows.

- Water is then injected (**water flooding**) to force oil trapped in less permeable regions of the reservoir out into the recovery well.

Recovery of residual oil in reservoirs

- The residual oil remaining after water flooding is a potential target for selective reservoir plugging of porous rocks with in situ bacterial growth on injected nutrients.

- Bacteria may exert a much greater plugging effect when they multiply within the reservoir rock rather than when they are injected and accumulate at the surface.

Recovery of residual oil in reservoirs

- Added or in situ-produced biosurfactants, which aid oil emulsification and detachment of oil films from rocks, have considerable potential in MEOR.

- Emulsan reduced the viscosity of Boscon heavy crude oil from 200,000 cP to 100 cP, facilitating heavy oil pumping. (cP = Centiposes)

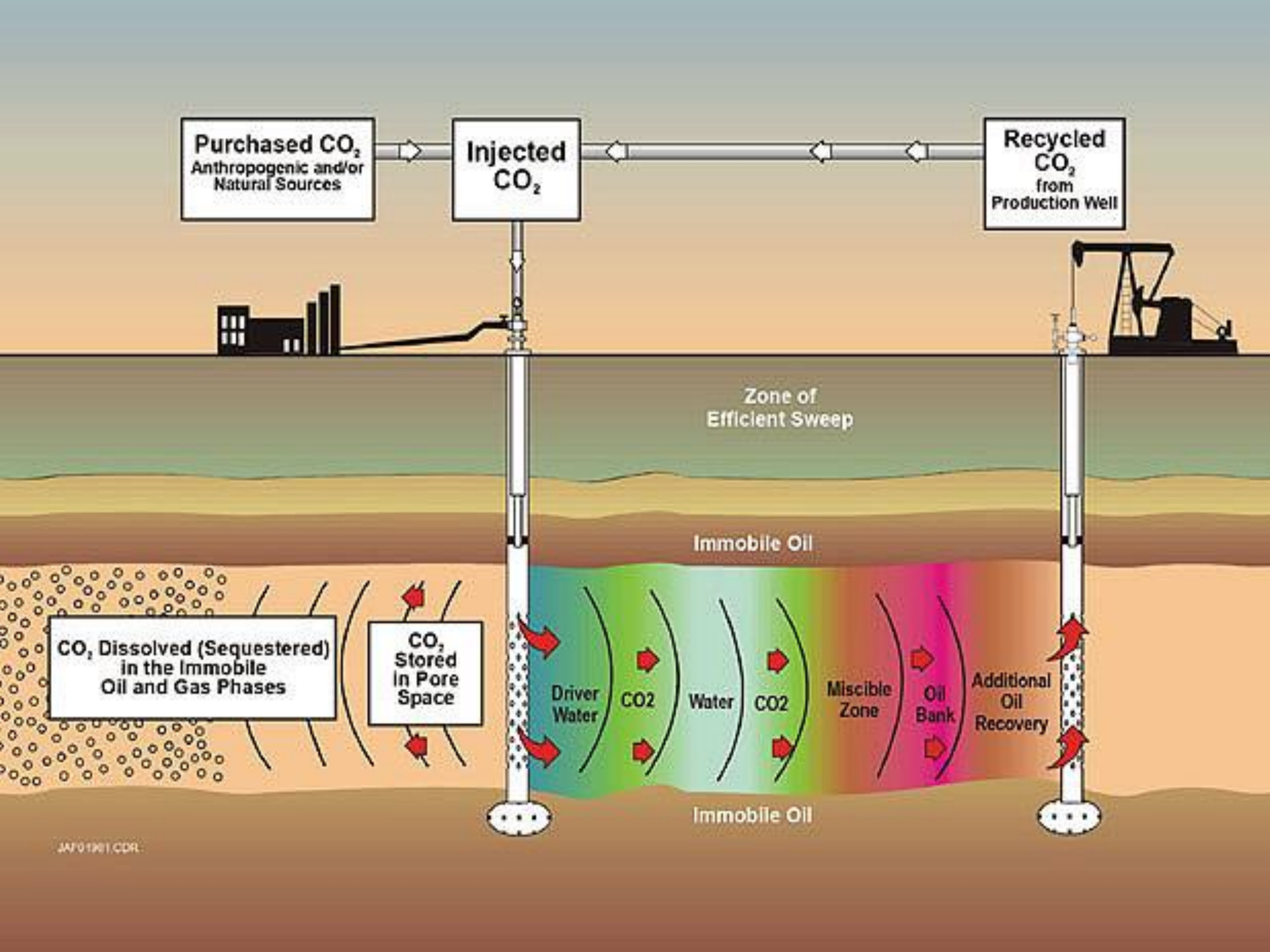
- Biosurfactant from the thermo- and halotolerant species, Bacillus licheniformis isolates and thermotolerant Bacillus subtilis strains have been tested for with various levels of success in reservoirs and in laboratory simulations.

Overall Mechanisms of Stimulation of Oil Production

- **Improvement of the relative mobility of oil to water** by biosurfactants and biopolymers
- **Partial repressurization of the reservoir** by gases (methane and CO_2)
- **Reduction of oil viscosity** through the dissolution of organic solvents in the oil phase
- **Increase of reservoir permeability and widening of the fissures and channels** through the etching of carbonaceous rocks in limestone reservoirs by organic acids produced by anaerobic bacteria

Overall Mechanisms of Stimulation of Oil Production

- **Cleaning of the wellbore region through the acids and gas from *in situ* fermentation.**
  - The gas serves to push oil from dead space and dislodge debris that plugs the pores.
  - The average pore size is increased
  - The capillary pressure near the wellbore is made more favorable for the flow of oil
- **Selective plugging of highly permeable zones by injecting slime-forming bacteria followed by sucrose solution**
  - "turns on" the production of extracellular slimes
  - Aerial sweep efficiency is improved


Purchased CO_2
Anthropogenic and/or Natural Sources
Injected CO_2
Recycled CO_2 from Production Well
Zone of Efficient Sweep
Immobile Oil
CO_2 Dissolved (Sequestered) in the Immobile Oil and Gas Phases
CO_2 Stored in Pore Space
Driver Water
CO2
Water
CO2
Miscible Zone
Oil Bank
Additional Oil Recovery
Immobile Oil

CASE STUDY

- In a field MEOR study in the Southeast Vassar Vertz Sand Unit salt-containing reservoir in Oklahoma
- Nutrient injection stimulated growth of the microbial populations, including several aerobic and anaerobic heterotrophic bacteria, sulfate-reducing bacteria, and methanogenic halophiles.
- Nutrient-stimulated microbial growth produced a 33% drop in the effective permeability in an injection well at North Burbank Unit in Oklahoma, plugging off high-permeability layers and diverting injection fluid to zones of lower permeability and higher oil saturation.

CASE STUDY

- In contrast to the poor experience with exogenous organisms for bioremediation (bioaugmentation),

- **Injection of selected microbial species into oil field plots in Japan and China resulted in improved oil recoveries of 15 to 23%.**

- In one case, microbial treatment caused some degradation of long-chain aliphatic hydrocarbon chains but with no apparent degradation of aromatic ring structures.

Challenges of MEOR

- Despite numerous MEOR tests, considerable **uncertainty remains regarding process performance**.

- Ensuring success requires an ability to manipulate environmental conditions to promote growth and/or product formation by the participating microorganisms.

- Exerting such control over the microbial system in the subsurface is a serious challenge.

Challenges of MEOR

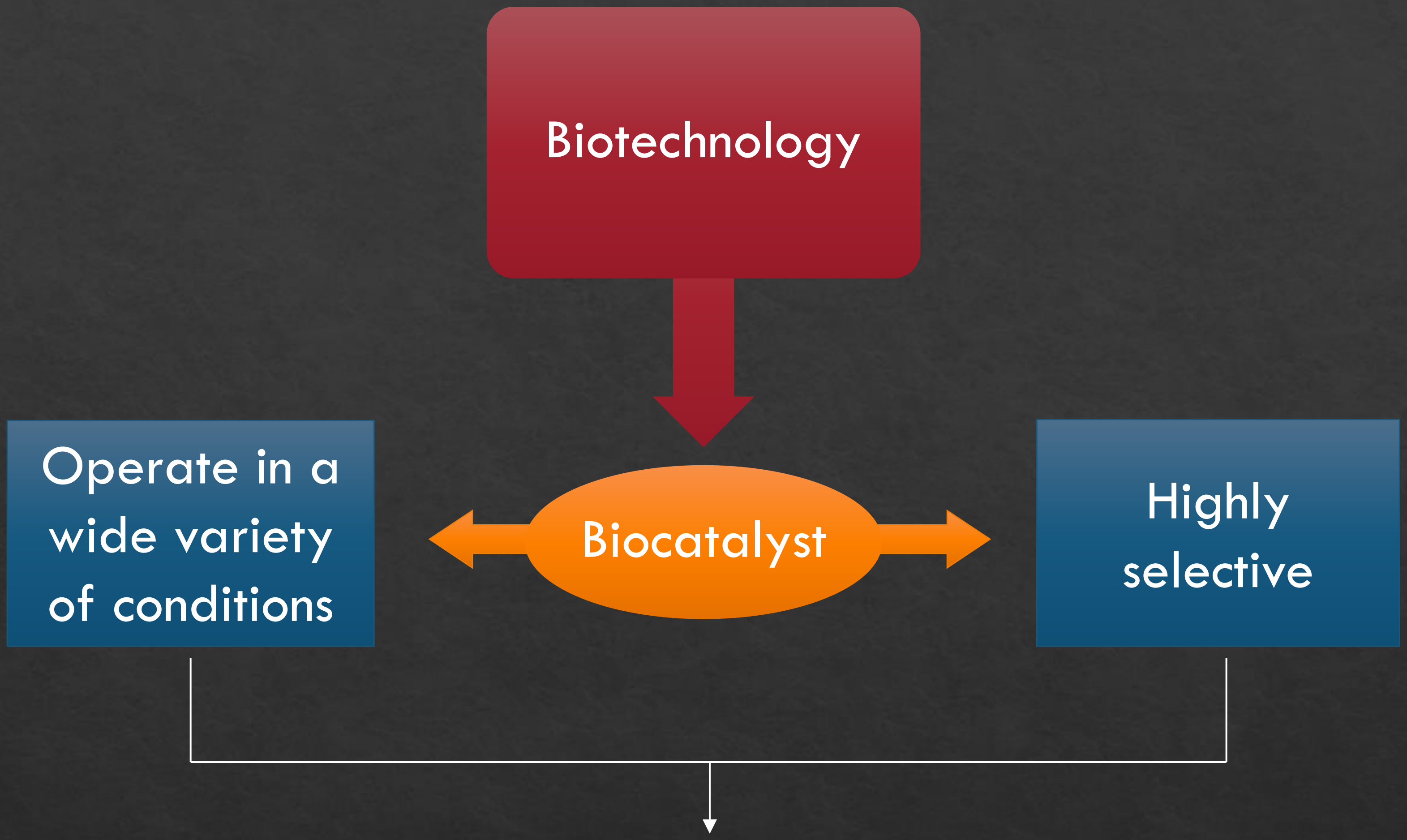
- The MEOR process **may modify the immediate reservoir environment** in a number of ways that could also damage the production hardware or the formation itself.
  - **Certain sulfate reducers can produce H_2S, which can corrode pipeline and other components of the recovery equipment.**
- **Reservoir heterogeneity** significantly affects oil recovery efficiency.
  - Variation of conditions from reservoir to reservoir, which calls for reservoir-specific customization of the MEOR process undermine microbial process economic viability.

Challenges of MEOR

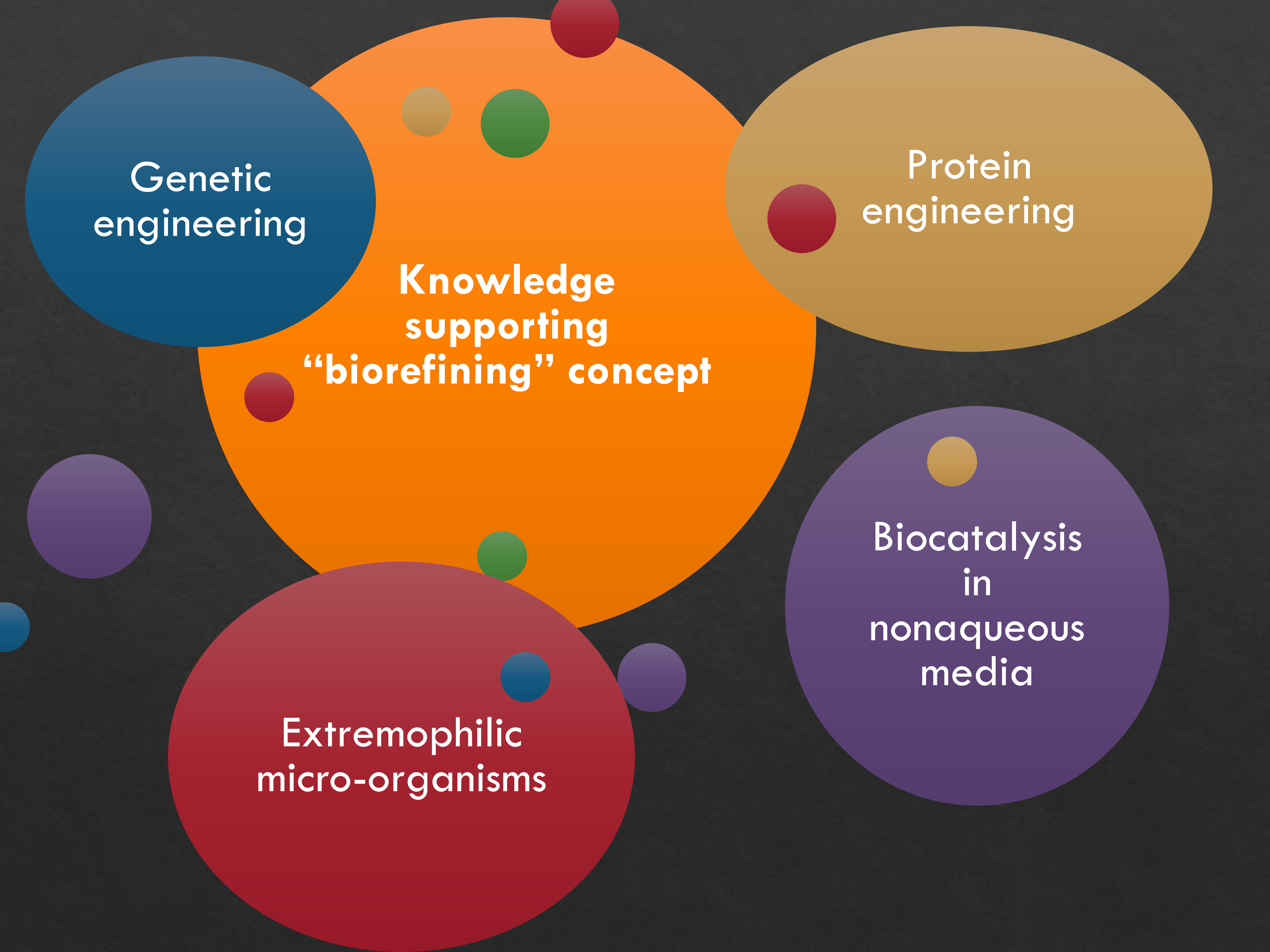
- MEOR systems currently represent high-risk processes to oil producers looking for efficient and predictable oil recovery.

- *Development of a universal additive mixture, consisting of a combination of microbial strains, nutrients, surfactants, and buffering agents in appropriate proportions, may represent a further productive line of research.*

PETROLEUM BIOREFINING



- Decreased energy costs
- Low emissions
- No generation of undesirable by-products


Genetic engineering
Knowledge supporting "biorefining" concept
Protein engineering
Biocatalysis in nonaqueous media
Extremophilic micro-organisms

Genetic engineering

- Modifying the genetic material of MOS of industrial interest to acquire new / enhanced capabilities.

Criteria when designing recombinant microorganisms

- Transform different types of compounds
- Stable under process conditions
- Possess higher activities & secrete biosurfactants

Protein engineering

- Protein engineering creates proteins with new or enhanced properties by direct manipulation of their genes.

Criteria for designing enzymes in PB

- Highly active & stable
- Able to accommodate hydrophobic substrates
- Able to transform a range of hydrocarbons
- Soluble and stable in organic media

Biocatalysis in non-aqueous media

- Previously, only aqeous media was thought to support biocatalytic reactions.
- Advances in research – realized biocatalysis in non aqeous media.
- These systems are advantageous for the development of industrial biotech processes e.g. petroleum

- Advantages of non-aqueous media:

  Low H_2O potential increases solubility & bioavailability of hydrophobic substrates

Extremophilic microorganisms

- Survive extreme conditions
- Their enzymes possess unique properties
- Petroleum industry is an area that displays an extreme environment
- Hence, extremozymes are attractive catalysts for petroleum refining
- Properties of extremozymes:
  - Extremely thermostable
  - Resistant to organic solvents
  - Resistant to extreme pH
- Genes encoding extremozymes are transferred to mesophilic hosts e.g. *E. coli* thus large quantities of enzymes produced
- *Understanding amino acid sequences of extremozymes can be used to redesign conventional enzymes to work in extreme conditions*

Microbial Deemulsification

- Oilfield emulsions, both oil-in-water and water-in-oil, formed at various stages of exploration, production and oil recovery and processing, represent a major problem for the petroleum industry.

- These emulsions are characterized according to their stability as:
  - tight (microemulsion, very fine droplets of around 100 Å, hard to break)
  - loose (coarse droplets, size around 5 μm, unstable, easily broken).

Microbial Deemulsification

- Water and dirt in crude oil cause corrosion and scaling in tanks, pipelines and reactors

- A maximum sediment and water content of 0.5 to 2.0% has been specified to be allowable in crude oil for transportation through the existing pipelines

- A process of de-emulsification is required to recover oil from these emulsions

- Factors that influence the stability of emulsions include viscosity, droplet size, phase volume ratio, temperature, pH, age of emulsion, type of emulsifying agent present, density difference and agitation.

Microbial Deemulsification

- Traditional de-emulsification methods include centrifugation, heat treatment, electrical treatment and use of chemicals containing soap, fatty acids and long-chain alcohols

- However, physico-chemical de-emulsification processes are capital intensive and emulsions often generated at the wellhead have to be transported to central processing facilities.

- An effective microbial de-emulsification process could be used directly to treat emulsions at the wellhead, thus saving on transport and high capital equipment costs.

TABLE 7. Potential microorganisms with petroleum deemulsification properties

Microorganism	Petroleum oil emulsion tested	Emulsion type	Reference(s)
Acinetobacter calcoaceticus	Kerosene-water model; oilfield emulsion	Water-in-oil; oil-in-water	449
Acinetobacter radioresistans	Kerosene-water model	Water-in-oil	449
Aeromonas sp.	Kerosene-water model	Oil-in-water	455
Alteromonas sp.	Kerosene-water model	Oil-in-water	455
Alcaligenes latus	Kerosene-water model	Water-in-oil	449
Corynebacterium petrophilum	Kerosene-water model; crude oil-water	Water-in-oil	161, 583
Bacillus subtilis	Crude oil-water model	Oil-in-water	283
Micrococcus sp.	Kerosene-water	Oil-in-water; water-in-oil	141
Nocardia amarae	Kerosene-water model; oilfield emulsion	Water-in-oil; oil-in-water	95, 346
Pseudomonas carboxydohydrogena	Kerosene-water model	Water-in-oil; oil-in-water	449
Rhodococcus aurantiacus	Kerosene-water model	Water-in-oil; oil-in-water	503
Rhodococcus rhodochrous	Kerosene-water model	Water-in-oil; oil-in-water	667
Rhodococcus ruber	Kerosene-water model	Water-in-oil; oil-in-water	345
Torulopsis bombicola	Oilfield emulsions	Water-in-oil	161
Mixed bacterial culture	Kerosene-water model; oilfield emulsion	Water-in-oil; oil-in-water	448

Microbial Deemulsification

- Microbes exploit the;

- Petroleum hydrocarbon-induced hydrophobic cell surfaces

OR

- the dual hydrophobic/hydrophilic nature of biosurfactants

- **To displace or alter the emulsifiers that are present at the oil–water interface**

- Some biologically-produced agents such as acetoin, polysaccharides, glycolipids, glycoproteins, phospholipids and rhamnolipids exhibit de-emulsification properties.

Microbial Deemulsification

- Different physiological properties support deemulsification of **oil-in-water** and **water-in-oil** emulsions.

- Deemulsification of **water-in-oil emulsions** requires the **hydrophilic cell surfaces** which exist around cells growing **exponentially** and in **early stationary** phase

- Deemulsification of **oil-in-water emulsions** requires **hydrophobic surfaces** produced during the **endogenous metabolic phase**

Microbial Deemulsification

- The de-emulsifying activity depends on the species, growth medium, culture age and post-harvest treatment.
- The microbial deemulsification rate varies with differences in emulsion composition
- The high viscosity of the emulsion prevents significant deemulsification

Microbial Deemulsification

- **Elevating the incubation temperature** generally accelerates de-emulsification of emulsions by:
  - **reducing the viscosity of the oil phase,**
  - **increases density difference between the phases,**
  - **weakens the stabilizing interfacial film,**
  - **causes an increased rate of droplet collision leading to coalescence (merging of 2 or more droplets).**

- However, variations in the properties of crude oil emulsions results in inconsistencies in the performance of all deemulsification processes (physical, chemical, and biological).

Biodesulfurization of fuels

- Sulfur is usually the third most abundant element in crude oil, normally accounting for 0.05 to 5%, but up to 14% in heavier oils.
- Refiners use expensive physicochemical methods, including hydro-desulfurization to remove sulfur from crude oil.
- The high cost is driving the search for more efficient desulfurization methods, including biodesulfurization.

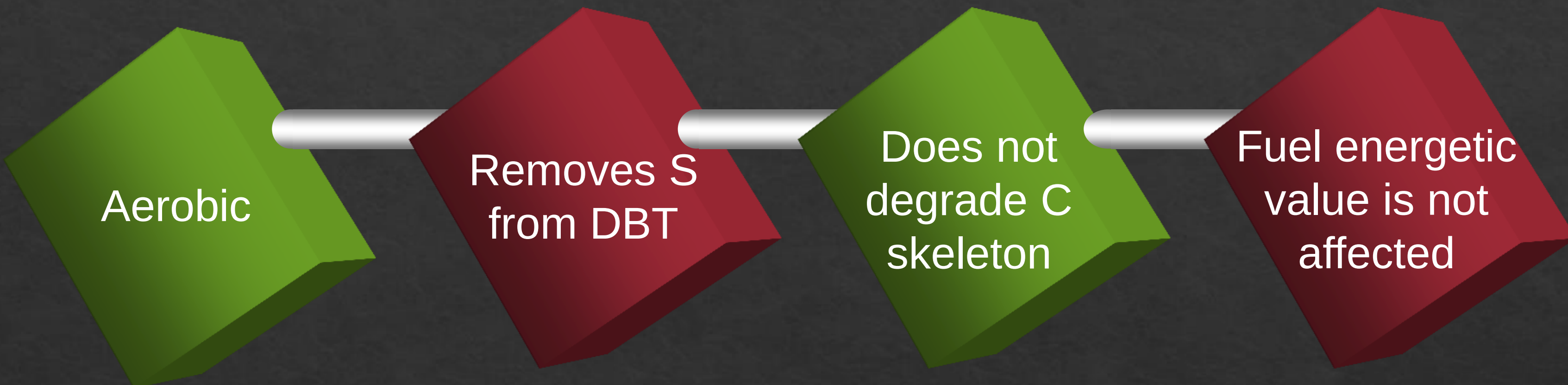
Biodesulfurization of fuels

- Most of the sulfur in crude oil is organically bound, mainly in the form of condensed thiopenes
- Organic sulfur in fossil fuels is one of the major sources of environmental pollution
- Emissions of sulfur oxides during combustion of diesel oil results in:
  - Acid rain
  - Atmospheric contamination
- 70% of S in petroleum is found as **dibenzothiophene (DBT)**
- Selective oxidation of the sulfur component is important

Hydrodesulfurization

- Chemical process for S removal
  - Requires high temps & pressures
  - Expensive & leads to atmospheric emissions
- Hence, biodesulfurization of fuels is a preferred choice
- Microbes can liberate sulfur from sulfur-containing heteroaromatic compounds through a series of enzyme-catalyzed reactions, without attacking the hydrocarbon

Rhodococcus erythropolis IGTS8



DBT is not degraded but only transformed to 2-hydroxybiphenyl (2-HBP) which is recycled to the organic phase

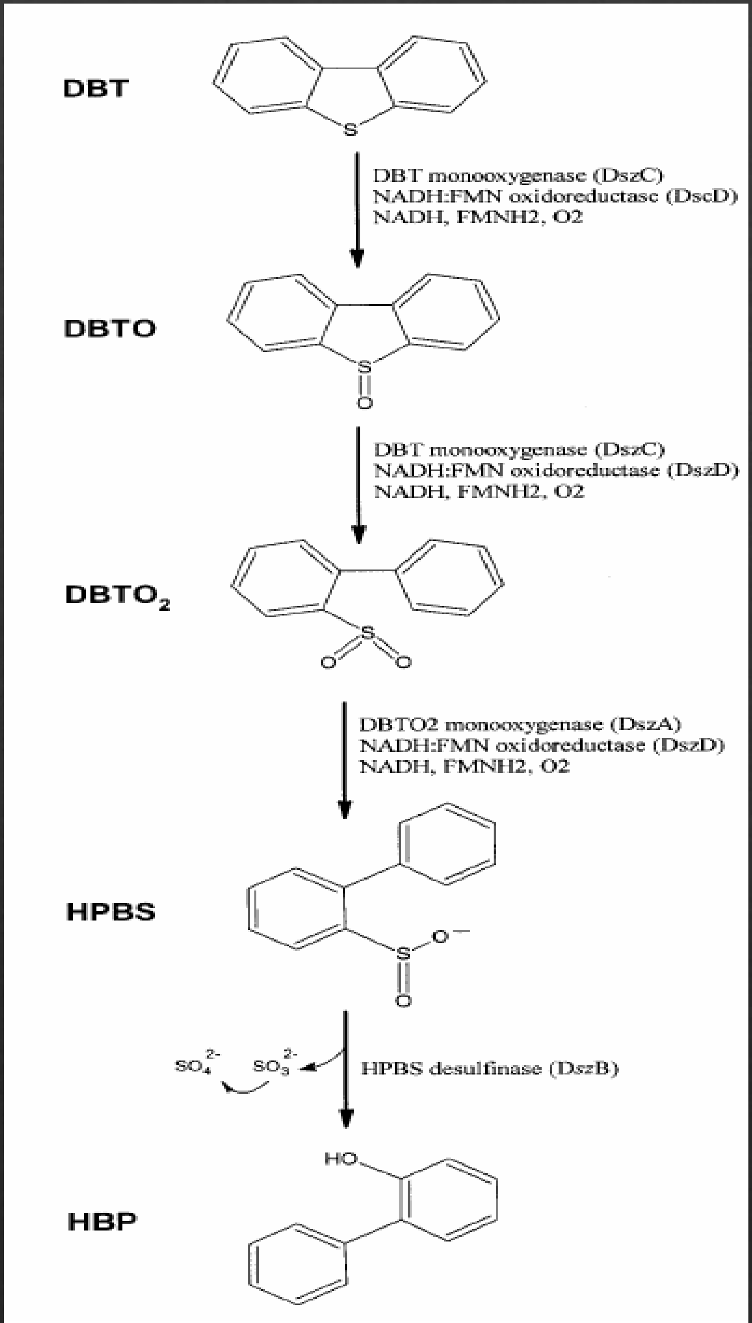
- Dsz pathway is responsible for desulfurization
- This system involves 4 different enzymes, *viz.*

1. DszA
2. DszC

DszA and DszC → Monooxygenases

3. DszD — flavin reductase enzyme
4. DszB — desulfinase

- Studies have shown that by eliminating sulfate repression & deleting the last gene of the metabolic pathway, dszB, allows for the accumulation of hydroxybiphenyl sulfinate (HPBS)
- This is a more valuable product than sulfate as it may be used as a surfactant.



Proposed sulfur-specific pathway for dibenzothiophene (DBT) desulfurization by Rhodococcus species.

Abbreviations: **DBTO, dibenzothiophene sulfoxide; $DBTO_2$, dibenzothiophene sulfone; HPBS, hydrophenyl benzene sulfinate; HBP, hydroxy biphenyl.**

The Rhodococcus pathway does not continue to intermediary metabolism and stops with the release of hydroxy biphenyl, and therefore no decrease in carbon content occurs.

The physiological significance of the pathway is to obtain sulfur for growth. DszA, DszB, DszC, and DszD are the catalytic gene products of *dszA*, *dszB*, *dszC*, and *dszD*, respectively.

- The process is highly flammable and explosive
- Consideration of cloning the desulfurization genes into anaerobic hosts, which would hyper-produce the enzymes for addition to the crude oil.
- Critical aspects of the biodesulfurization process development include reactor design, product or by-product recovery, and oil-water separation.
- Possibility of creating **two-phase oil-water systems for biodesulfurization of viscous crude oils** using more refined products, such as diesel or gasoline.

REACTOR DESIGN

Emulsion phase contactor

Creates small water/fuel/ biocatalyst droplets → Designed to Minimize intro of water into fuel → Very low desulfurization rates were observed

Fluidized bed reactor

Biocatalyst immobilized on matrix → Continuous operation & easy Product separation → Biocatalyst requires further development

New developments for petroleum refining

Paenibacillus

- Thermophilic bacterium that functions between 50 – 60 °C
- Selectively desulfurizes DBT & its derivatives
- Follow same metabolic pathway as *R. erythopolis*
- Their enzymes are homologous to *R. erythopolis*
- Their enzymes are active at high temperatures

BDS PROCESS & BIOCATALYSTS

Process

1. Contacting the biocatalyst with fuel
2. Separation of desulfurized product
3. Recovery of biocatalyst
4. Recycling of biocatalyst

Biocatalysts

- Must be whole resting cells
- Possess high desulfurizing activity
- Recyclable
- Have sufficient substrate specificity

Biodenitrogenation

- Crude oil contains about 0.5 to 2.1% nitrogen, with 70 – 75% consisting of pyrroles, indoles, and carbazole non-basic compounds
- Denitrogenation of petroleum might facilitate their desulfurization by hydrodesulfurization (HDS)

- Contributes to acid rain
- Toxic & mutagenic
- Causes atmospheric contamination (Nitric oxides)
- Interferes with refining process
- Poison cracking catalysts
- Inhibits HDS
- Equipment corrosion & catalyst poisoning

Biodenitrogenation

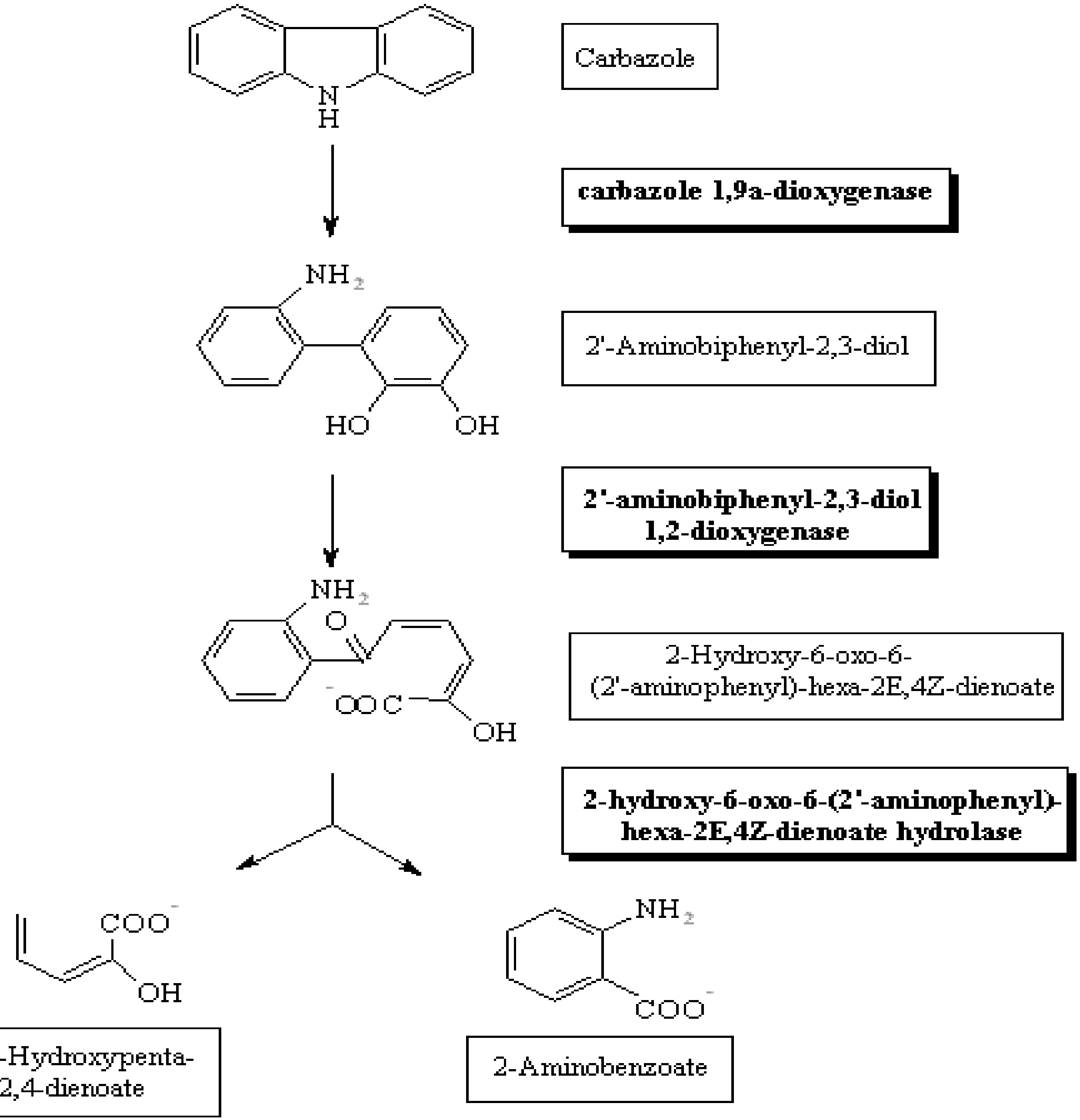
- Several species of bacteria that can utilize indole, pyridine, quinoline, and carbazole and its alkyl derivatives have been isolated and characterized
- Bacteria exhibit some general similarities in the pathways for the transformation of aromatic compounds
- **Oxygenases** play an important role in the initial attack in the transformation of nitrogen compounds
- The initial enzymatic conversion steps yield **dihydroxylated intermediates**
- Followed by either a meta- or an ortho- pathway, leading to intermediates of central metabolic pathways

Pseudomonas sp. CA10;

Pseudomonas stutzeri OM1;

Sphingomonas sp. CB3;

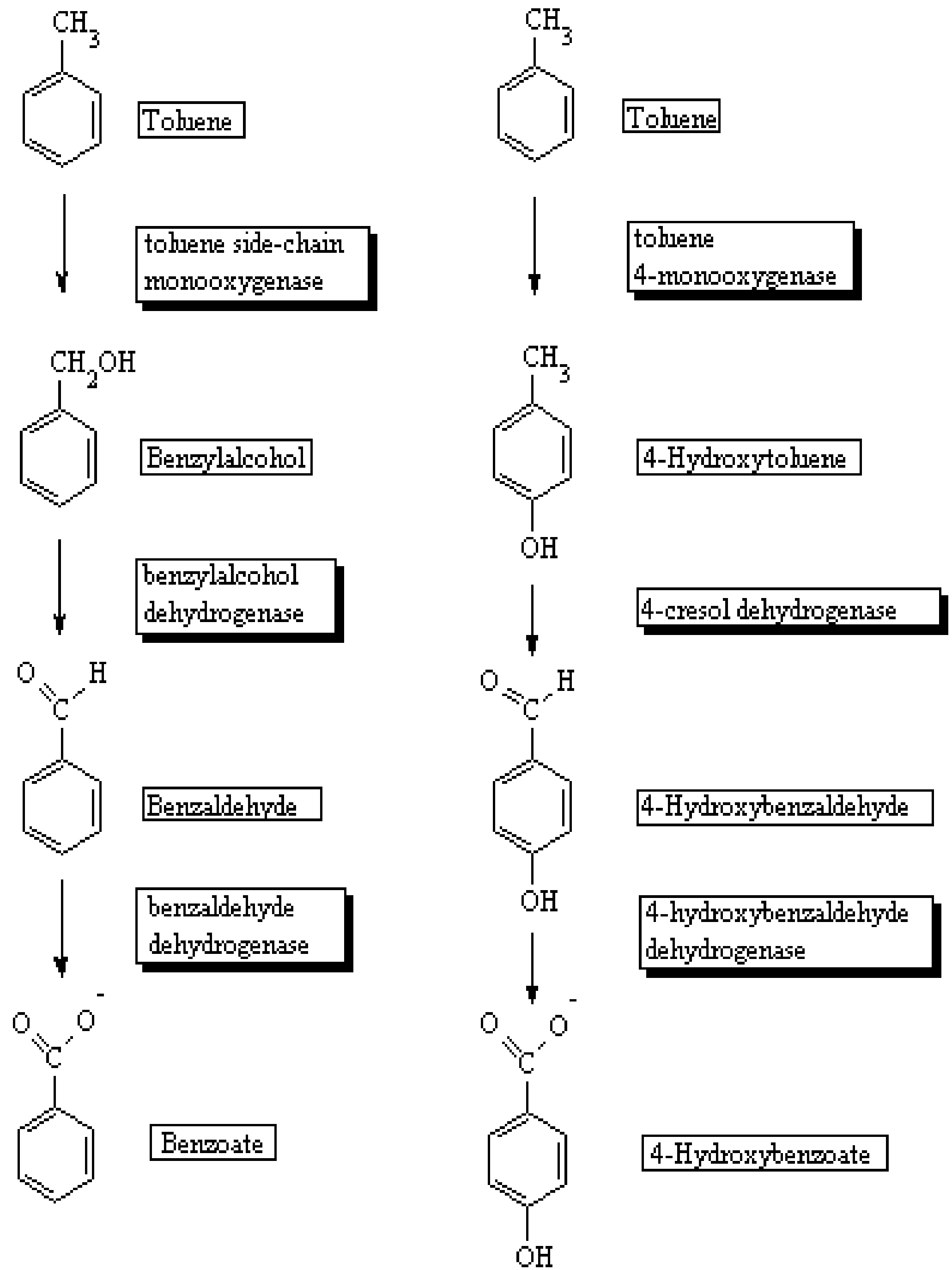
Escherichia coli



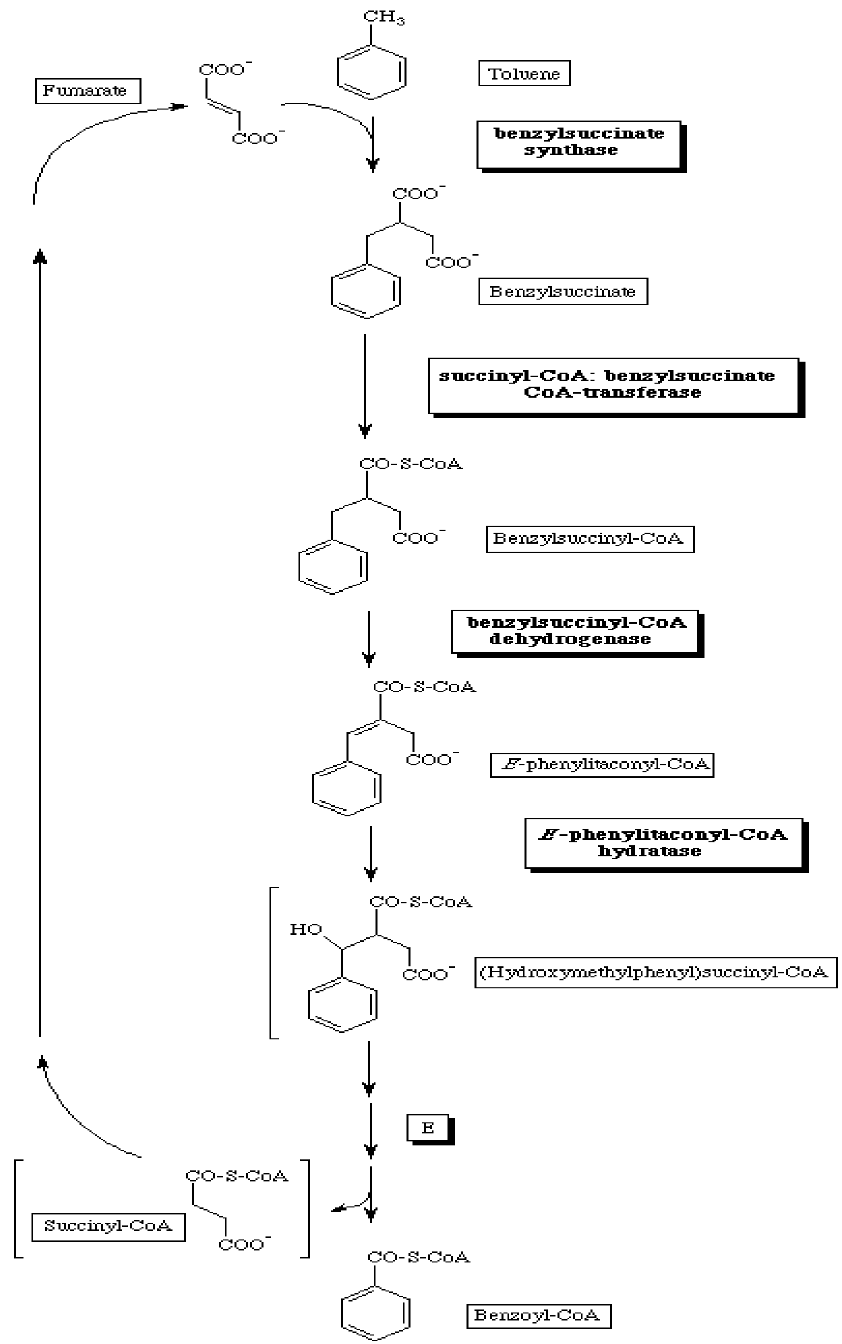
Toluene pathway

2-Aminobenzoate pathway

Toluene pathway
(Aerobic)

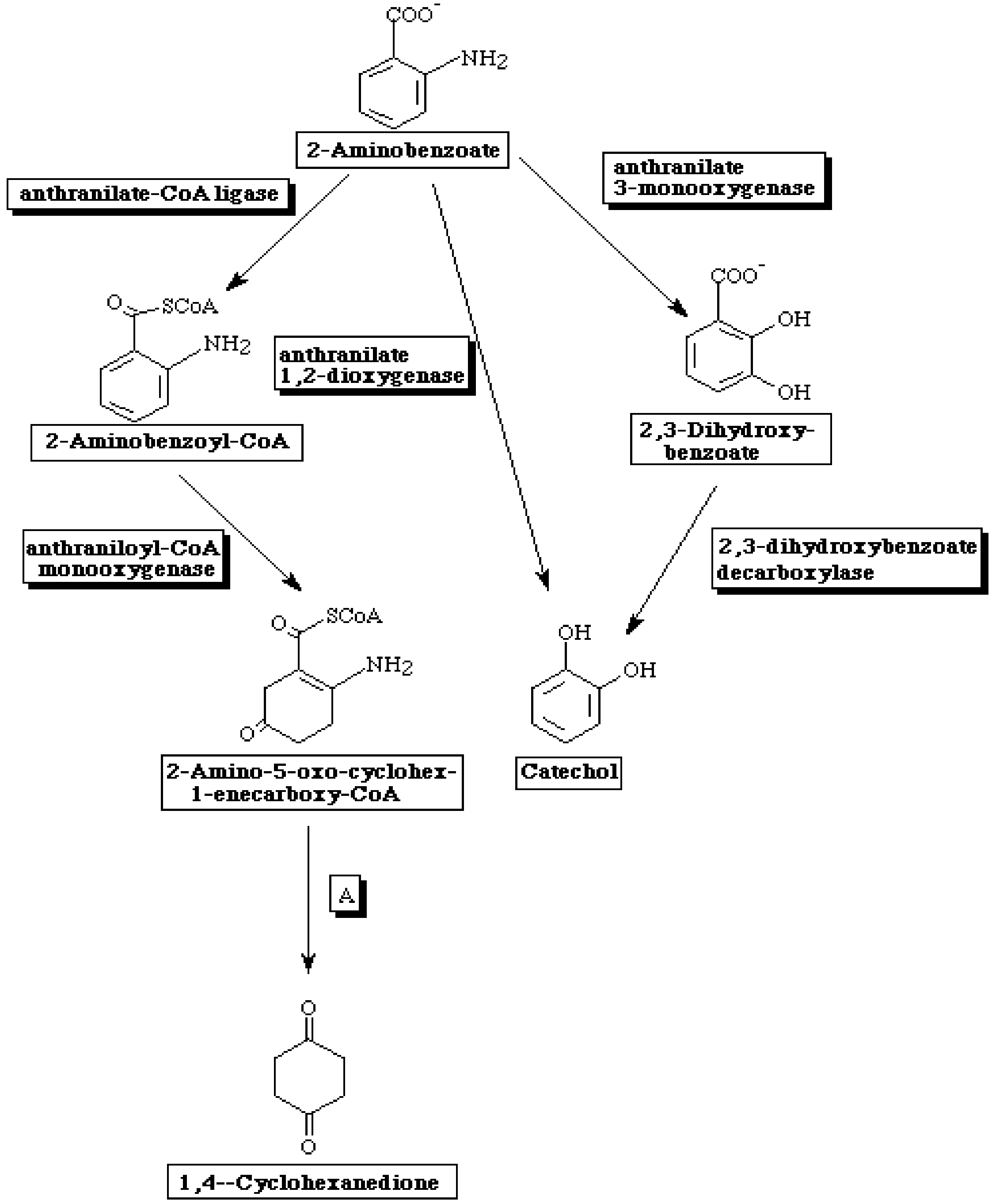


Toluene pathway
(Anaerobic)

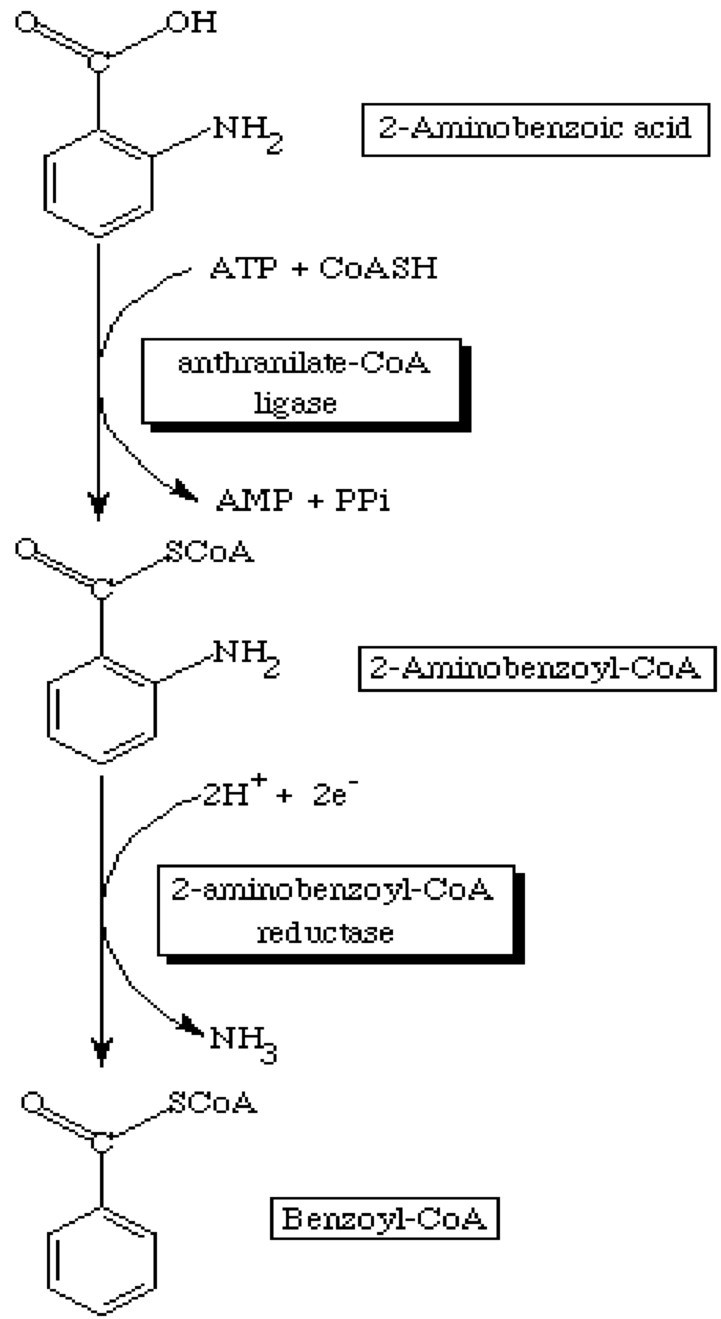


2-Aminobenzoate Pathway

(Aerobic)



2-Aminobenzoate Pathway
(Anaerobic)

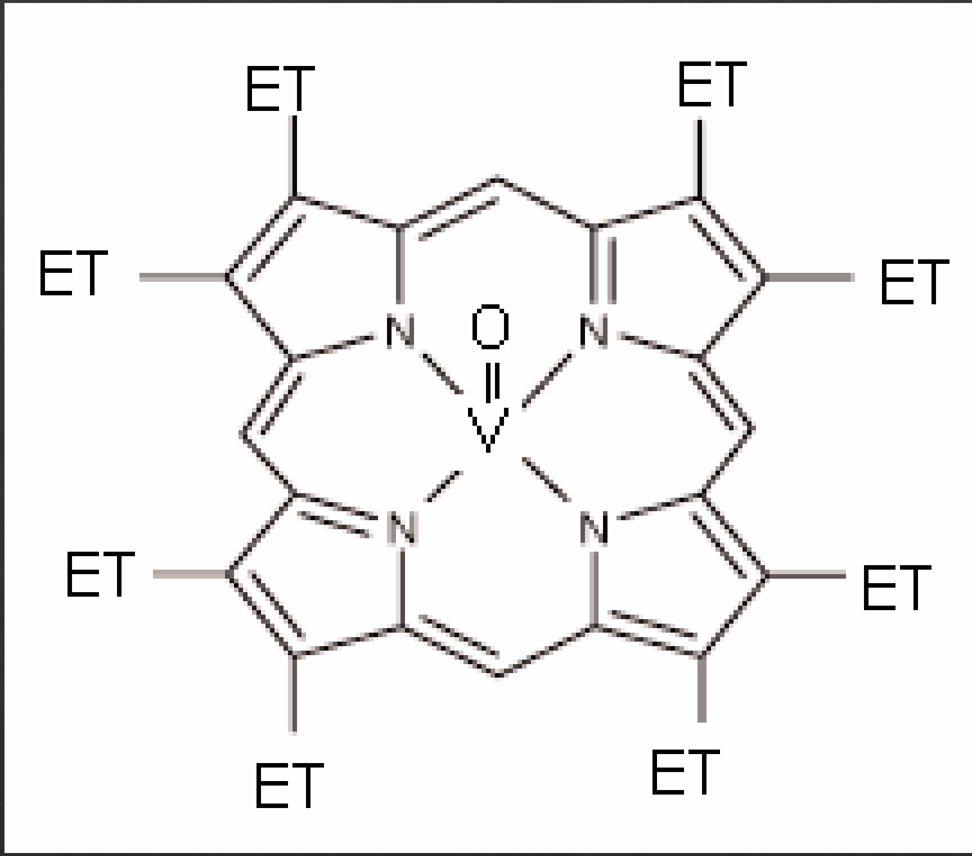


Demetallation

- Crude oil contains metals in the form of:
- **Salts** — Removed during crude oil desalting process
- **Petroporphyrins**
- **Complexes in asphaltenes**

Petroporphyrins and complexes in asphaltenes → Difficult to remove due to complex structures

- Asphaltenes are molecular substances that are found in crude oil, along with resins, aromatic hydrocarbons, and alkanes (i.e., saturated hydrocarbons).
- Asphaltenes consist primarily of carbon, hydrogen, nitrogen, oxygen, and sulfur, as well as trace amounts of vanadium and nickel.



Molecular structure of vanadium oxide octaethyl porphyrin

Microorganisms involved in Demetallation

	1	2
Organism	*Coldariomyces fumago*	*Bacillus megaterium* & C. *roseuse*
Enzyme secreted	Chloroperoxidase	Cytochrome C reductases
Function	Removes metals in petroporphyrin & asphaltenes	Oxidation of porphyrin rings
By-products liberated	Chlorinated products	none

TABLE 8. Microorganisms with potential petroleum-biorefining activities

Biorefining process	Biocatalyst	Microorganism
Desulfurization	Aerobic bacteria	*Rhodococcus erythropolis* H2
		Arthrobacter sp.
		Corynebacterium sp. strain SY1
		Nocardia sp.
		Agrobacterium sp. strain MC501
		Mycobacterium sp. strain G3
		Gordona sp. strain CYKS1
		Klebsiella sp.
		Paenibacillus sp.
		Pseudomonas alcaligenes
		Rhodococcus sp. strain IGTS8
		Rhodococcus sp. strain ECRD-1
		Xanthomonas sp.
	Anaerobic bacteria	*Desulfovibrio desulfuricans* M6
Denitrogenation	Aerobic bacteria	*Pseudomonas ayucida* IGTN9m
		Pseudomonas aeruginosa
		Pseudomonas sp. strain CA10
		Pseudomonas putida 86
		Pseudomonas stutzeri
		Rhodococcus sp. strain B1
		Comamonas acidovorans
		Comamonas testosteroni
		Nocardioides sp.
Demetalation	Chloroperoxidase	*Caldariomyces fumago*
	Cytochrome *c* reductase; heme oxygenase	*Bacillus megaterium*, *Escherichia coli*