BIODEGRADATION OF HYDROCARBONS

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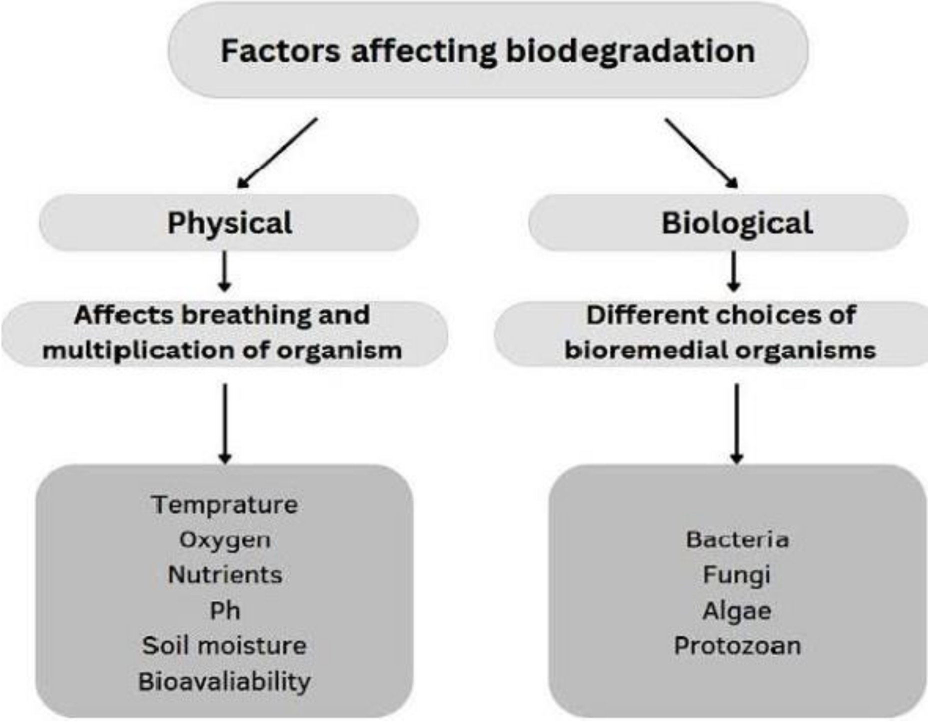
Introduction

- Hydrocarbons are compounds composed entirely of carbon and hydrogen. Aromatic hydrocarbons, alkanes, alkenes, cycloalkanes, alkynes, and combinations of these compounds comprise different types of hydrocarbons.
- Complex mixtures of hydrocarbons occur naturally in crude oil and gasoline. Most can be used as substrates in metabolism by bacteria, archaea, fungi, and algae.
- While fungi and algae degrade hydrocarbons aerobically, bacteria and archaea are capable of both aerobic and anaerobic degradation.
- Aerobic degradation occurs with molecular oxygen (O_2) as both a reactant to oxidize the substrate and an electron acceptor for microbial respiration.
- In contrast, anaerobic degradation uses different biotransformation pathways that don’t depend on oxygen, coupled to microbial respiration of a variety of electron acceptors. Under anaerobic conditions, biodegradation often results from the stepwise concerted action of many different microbes in a process called syntrophy.



Biodegradation of hydrocarbons is the process where microorganisms, like bacteria and fungi, break down hydrocarbons into simpler, non-toxic compounds such as carbon dioxide and water, often using the hydrocarbons as an energy source. A suitable example is the breakdown of crude oil in soil by ***Pseudomonas aeruginosa***, where the bacteria use oxygen to initiate the breakdown of hydrocarbon chains and aromatic rings into harmless substances, a process crucial for cleaning up oil spills.

The process of hydrocarbon biodegradation

- **Aerobic degradation**: This is the more efficient method where oxygen is required. Microorganisms use oxygenase enzymes to add oxygen atoms to the hydrocarbon molecule, a key initial step in its breakdown.
  - **Alkanes**: Aliphatic hydrocarbons (alkanes) are often the first to be degraded because they are more readily broken down.
  - **Aromatic hydrocarbons**: These are more difficult to degrade but can be broken down by aerobic bacteria through ring cleavage reactions after an initial oxygenation step.
- **Anaerobic degradation**: In the absence of oxygen, microorganisms use other electron acceptors like nitrate or sulfate to break down hydrocarbons. This process is generally slower than aerobic degradation.

Alkanes and Alkenes

- Alkanes containing 14 carbons or fewer are prone to volatilization, while alkanes containing more carbons are less volatile.
- Except cyclic alkanes (alkanes in a ring structure), alkanes and alkenes are the most readily degraded hydrocarbons with degradation of alkanes containing up to 44 carbons. Both alkanes and alkenes are degraded by the addition of molecular oxygen.

Aromatic Hydrocarbons

- Aromatic hydrocarbons are generally more difficult to degrade than shorter alkanes and alkenes due to their greater toxicity, yet they are readily degraded aerobically by many bacteria and fungi. Degradability of aromatic hydrocarbons decreases with increasing number of rings and increased molecular size, due to increased hydrophobicity and sorption capacity.

Example: Degradation of crude oil

- **Organisms**: Bacteria such as *Pseudomonas aeruginosa* and *Alcanivorax borkumensis*, along with certain fungi and algae, are effective at breaking down hydrocarbons in crude oil.
- **Mechanism**:

1. **Initial oxidation**: Enzymes like monooxygenases and dioxygenases add oxygen to the hydrocarbon molecule, converting it into an alcohol.
2. **Pathway conversion**: These alcohols are further oxidized and enter a metabolic pathway within the bacteria.
3. **Carbon chain cleavage**: The carbon-carbon bonds of the hydrocarbon are broken down in a series of steps, often via beta-oxidation.
4. **Mineralization**: Eventually, the process results in the complete breakdown of the hydrocarbon into carbon dioxide, water, and other inorganic compounds.

Biodegradation of hydrocarbons is the natural process by which microorganisms such as **bacteria, fungi, and algae** break down complex hydrocarbon molecules into simpler, less harmful substances, ultimately converting them into carbon dioxide, water, and biomass. This process is the primary mechanism for the natural cleanup of petroleum-contaminated environments.

Detailed Process of Biodegradation

Microbial degradation of hydrocarbons generally occurs through two main pathways, aerobic (with oxygen) and anaerobic (without oxygen), with aerobic degradation being significantly more efficient.

1. Aerobic Degradation

Hydrocarbons are readily degraded under aerobic conditions. Bacteria, fungi, and algae are all capable of aerobic hydrocarbon degradation. In general, alkenes (hydrocarbons containing double bonds) and short-chain alkanes (hydrocarbons containing only single bonds) are the most easily degraded, followed by branched alkanes (alkanes with side chains) and then aromatics (hydrocarbons in a stable ring structure).

Degradation rates by fungal species reportedly range from ~ 30-100% degradation over 28 days or less. The primary rate-limiting factor in aerobic biodegradation is delivery of oxygen.

Oxygen availability is dependent on the ability of oxygen to move or diffuse through the site environment as well as on the uptake rate by microorganisms. Addition of oxygen can increase degradation rates several orders of magnitude over naturally occurring rates.

In aerobic conditions, the initial step involves the activation of the hydrocarbon molecule through the addition of oxygen catalyzed by enzymes called **oxygenases**.

- **Initial Step:** For linear alkanes (e.g., n-hexadecane), an alkane monooxygenase enzyme adds an oxygen atom to the terminal carbon to form a primary alcohol.
- **Intermediate Steps:** The alcohol is then sequentially oxidized to an aldehyde and then to a fatty acid.
- **Final Steps:** The resulting fatty acid is metabolized through the **beta-oxidation** pathway, which breaks the chain into acetyl-CoA units. These units then enter the **tricarboxylic acid (TCA) cycle** (or Krebs cycle) to be fully mineralized into CO_2 H_2O, and energy for the cell.

For aromatic hydrocarbons (e.g., benzene, toluene), dioxygenases typically add two oxygen atoms to the ring structure, forming a *cis*-dihydrodiol intermediate. This intermediate then undergoes further reactions, including ring cleavage, to form products like catechol, which are channeled into central metabolic pathways.

2. Anaerobic Degradation (Example: Toluene)

Hydrocarbon degradation under anaerobic conditions is often slower compared to aerobic degradation, due to less favourable reaction energetics with alternate electron acceptors. Despite this limitation, both facultative and obligately anaerobic bacteria and archaea are known to degrade hydrocarbons without oxygen. Anaerobic degradation is a much slower process and uses alternative electron acceptors such as nitrate, sulfate, or ferric iron instead of oxygen.

- **Initial Step:** The general strategy is to add a more reactive group to the hydrocarbon. For example, in the case of toluene, a common pathway is **fumarate addition**, catalyzed by the enzyme benzylsuccinate synthase. Toluene reacts with fumarate to form (R)-benzylsuccinate.
- **Intermediate Steps:** This intermediate is then further processed into various carboxylic acids and ultimately converted to benzoyl-CoA.
- **Final Steps:** Benzoyl-CoA is then degraded through ring reduction and cleavage pathways, eventually entering the central metabolism.

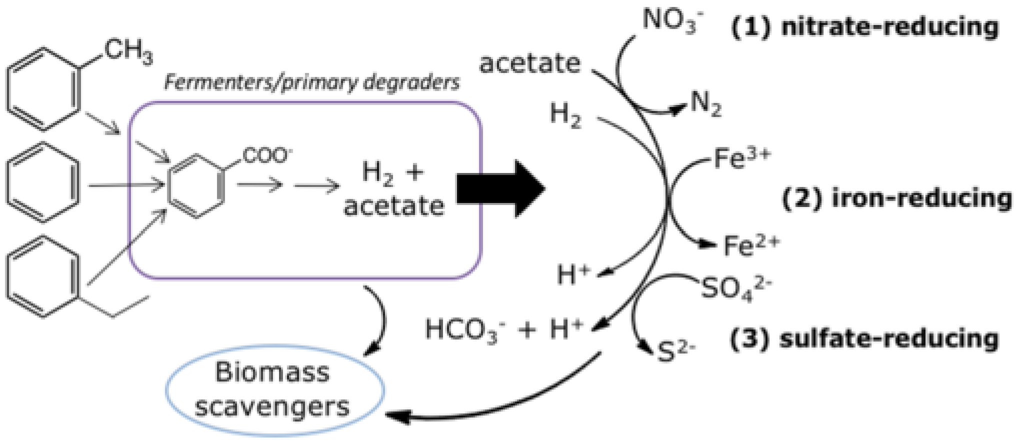


Fig: Conceptual model for syntrophic anaerobic degradation of benzene and alkylbenzenes. Acetate and H_2 are consumed in reactions 1, 2, and 3, keeping the fermentation reaction energetically favorable. When external electron acceptors (e.g., nitrate, iron, or sulphate) are no longer available, methanogens consume acetate and hydrogen.

Strategies For Activating Hydrocarbons Without Oxygen

1. Fumarate Addition

Bacteria use fumarate addition to activate alkanes from 3-20 carbons as well as alkyl-substituted aromatics like toluene, xylenes, or methylnaphthalene. Fumarate is a common cellular metabolite that contains two carboxylic acid groups and a double bond.

2. Oxygen-Independent Hydroxylation

Oxygen-independent hydroxylation can denitrify bacteria metabolizing ethylbenzene. In this pathway, a hydroxyl (-OH) group is added to the C1 carbon (carbon atom closest to the aromatic ring) on the side chain by ethylbenzene dehydrogenase to form S-1-phenylethanol, followed by oxidation to acetophenone and transformation to benzoyl-CoA and acetyl-CoA.

3. Carboxylation

All mechanisms described to date only apply to hydrocarbons with alkyl groups, and not to unsubstituted aromatic hydrocarbons like benzene or naphthalene. In fact, anaerobic degradation of benzene is much slower than that of toluene, or xylenes, and may not occur at all sites. One mechanism for naphthalene degradation and postulated for anaerobic benzene degradation is **carboxylation**. In this process, CO_2 is added directly to aliphatic and aromatic hydrocarbons.

Implications for remediation

Hydrocarbon remediation occurs more quickly under aerobic conditions than anaerobic.

The primary concern in in situ aerobic remediation is oxygen delivery and mixing, which may be achieved by a number of previously-established methods including landfarming, sparging, groundwater recirculation, and peroxide addition.

Aerobic remediation is not feasible in all environments, particularly low permeability soils. In these cases, anaerobic bioremediation may be preferred.

Important considerations for biodegradation include the nature of the suite of hydrocarbons present (light or heavy hydrocarbons), bioavailability, microorganism community composition, nutrient availability, soil permeability, and pH. Biostimulation through addition of nutrients such as nitrogen, phosphorus, and iron is often helpful.

Bioaugmentation by addition of actively degrading microbial cultures and nutrients may accelerate biotransformation of particularly recalcitrant hydrocarbons like benzene and **polycyclic aromatic hydrocarbons (PAHs)**, but site conditions must first be assessed to predict and ensure their effectiveness.

Knowing the degradation pathways and responsible organisms is useful to –

(a) assess potential for natural and enhanced remediation and

(b) track biodegradation at a site by monitoring functional genes biomarkers and degradation intermediates.

Suitable Examples of Microorganisms

A diverse range of bacteria and fungi are capable of hydrocarbon degradation. Often, a mixed microbial community (consortium) is more effective at degrading complex mixtures like crude oil than a single species because different microbes specialize in different hydrocarbon components.

Microorganism Genus	**Example Substrates**	**Pathway**
Pseudomonas spp.	n-alkanes, toluene, phenanthrene, pyrene	Aerobic (oxygenases), biosurfactant production
Alcanivorax spp.	n-alkanes and branched alkanes	Aerobic, obligate hydrocarbon degrader
Rhodococcus spp.	n-alkanes, cycloalkanes, mono-/polyaromatics	Aerobic (P450 oxygenases)
Mycobacterium spp.	Monoaromatics, PAHs	Aerobic (dioxygenases)
Marinobacter spp.	Crude oil, various hydrocarbons	Aerobic
Thauera spp.	Toluene, xylene	Anaerobic (fumarate addition)

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Importance of biodegradation

- **Bioremediation**: It is a sustainable and cost-effective method for cleaning up environmental pollution, such as oil spills, as it uses natural processes to remove harmful substances from the environment.
- **Enhanced efficiency**: A combination of different microbial groups is often needed to degrade the complex mixture of hydrocarbons found in products like crude oil.