Environmental Microbiology
Course 9

Bioremediation

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BIOREMEDIATION

Bioremediation →"Remediate" means to solve a problem, and "bio-remediate" means to use biological organisms to solve an environmental problem such as contaminated soil or groundwater.

Bioremediation

The process of cleaning up the hazardous substances into non-toxic compounds is called the Bioremediation process.

This process is majorly used for any kind of technology clean up that uses the natural microorganisms.

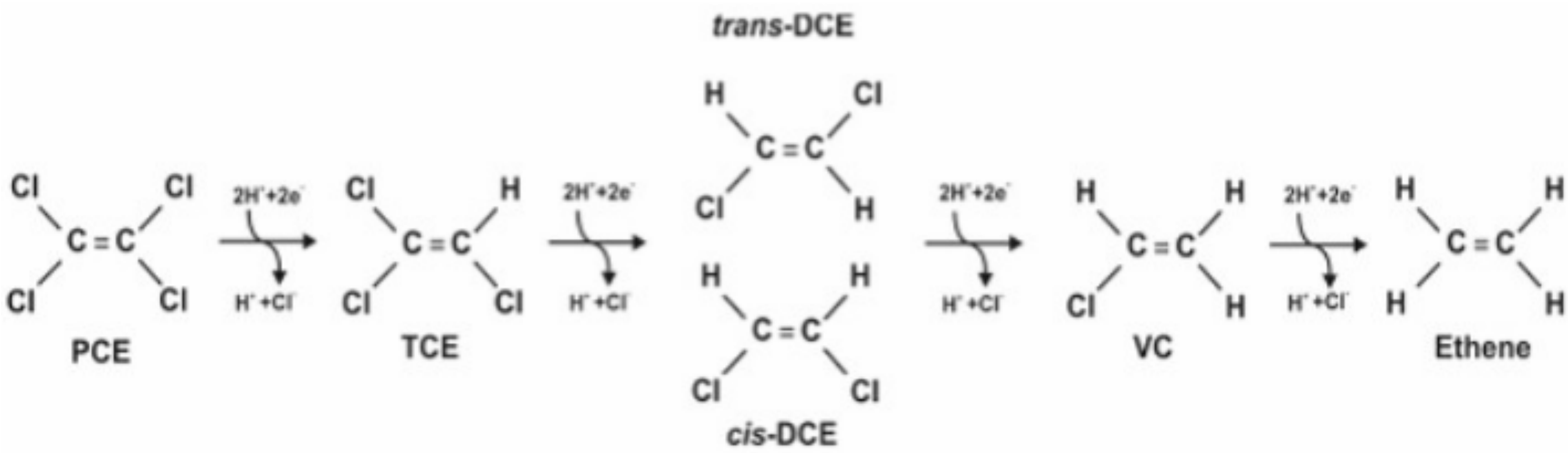
Bioremediation

- Bioremediation works by providing these pollution-eating organisms with **fertilizer, oxygen and other conditions encourage their rapid growth.**
- These organisms would then be able to break down the organic pollutant at a correspondsdingly faster rate.

BIOREMEDIATION

- Bioremediation of a contaminated site typically works in one of two ways.
- In the 1st case described above, ways are found to enhance the growth of whatever pollution-eating microbes might already be living at the contaminated site.
- In the second, less common case, specialized microbes are added to degrade the contaminants.

Bioremediation of chlorinated ethenes uses microorganisms to break down contaminants to less toxic end products



PCE (Tetrachloroethene), which contains four chlorine atoms, is degraded from PCE to TCE to DCE (dichloroethene) to VC (vinyl chloride) to ethene. DCE and VC are toxic but ethene is not toxic.

TYPES OF POLLUTION

1 Air pollution

2 Water pollution

3 Soil pollution

4 Noise pollution

Soil Pollution

- **Soil pollution**, also called **soil contamination**, refers to the degradation of land due to the presence of chemicals or other man-made substances in the soil. The xenobiotic substances alter the natural composition of soil and affect it negatively. These can drastically impact life directly or indirectly.

Soil Pollution

- **For instance, any toxic chemicals present in the soil will get absorbed by the plants. Since plants are producers in an environment, it gets passed up through the food chain.** Compared to the other types of pollution, the effects of soil pollution are a little more obscured, but their implications are very noticeable.

Environmental Contaminants

- **Environmental contaminants are chemicals that accidentally or deliberately enter the environment, often, but not always, as a result of human activities.**
- Some of these contaminants may have been manufactured for industrial use and because they are very stable, they do not break down easily.

Sources of Contamination

- Industrial spills and leaks
- Surface impoundments
- Storage tanks and pipes
- Landfills
- Burial areas and dumps
- BTEX- Benzene, Toluene, Ethyl-benzene, Xylene
- Herbicides and pesticides
- Nitroaromatic explosives and plasticizers
- Nuclear wastes
- Agricultural chemical wastes(Triazine, DDT)
- Inorganics (Uranium, sulfur, sulfuric acid)
- Explosives (RDX, TNT)
- Polyaromatic hydrocarbons (creosote)
- Chlorinated hydrocarbons (Trichlorethylene, PCBs, pentachlorophenol)
- Petroleum hydrocarbons (Gas, deisel)
- Heavy metals (Cd, Cu, Pb)
- PCB (Polychlorinated biphenyls)

What are environmental contaminants?

- pollutants
  - naturally-occurring compounds in the environment that are present in unnaturally high concentrations.
  - Examples:
    - crude oil
    - refined oil
    - phosphates
    - heavy metals

- Xenobiotics
  - chemically synthesized compounds that have never occurred in nature.
  - Examples:
    - pesticides
    - herbicides
    - plastics

Contaminants Potentially Amenable to Bioremediation

Readily degradable	Somewhat degradable	Difficult to degrade	Generally recalcitrant
fuel oils, gasoline	creosote, coal tars	chlorinated solvents (TCE)	dioxins
ketones and alcohols	pentachloro-phenol (PCP)	some pesticides and herbicides	polychlorinated biphenyls (PCB)
monocyclic aromatics			
bicyclic aromatics (naphthalene)			

What Is Bioremediation?

- **Biodegradation** - the use of living organisms such as bacteria, fungi, and plants to degrade chemical compounds
- **Bioremediation** – process of cleaning up environmental sites contaminated with chemical pollutants by using living organisms to degrade hazardous materials into less toxic substances

REQUIREMENTS FOR BIOREMEDIATION

MICROORGANISMS

ENERGY SOURCE | ELECTRON ACCEPTOR

MOISTURE | pH

NUTRIENTS | TEMPERATURE

ABSENCE OF TOXICITY | REMOVAL OF METABOLITIES | ABSENCE OF COMPETITIVE ORGANISMS

BIOREMEDIATION

- **Bacteria mainly produce ATP by :**

1) **Aerobic respiration** : is an oxidative process which uses oxygen as a final electron acceptor.

- Aerobic respiration produces 36-38 ATP per glucose molecule, and is the most efficient form of energy production.

2) **Fermentation** : uses an organic molecule as a final electron acceptor.

- Fermentation is the least efficient means of energy production; it produces only two ATP per glucose molecule.
- organic molecule are mainly carbohydrates (glucose, lactose, maltose, sucrose …etc)

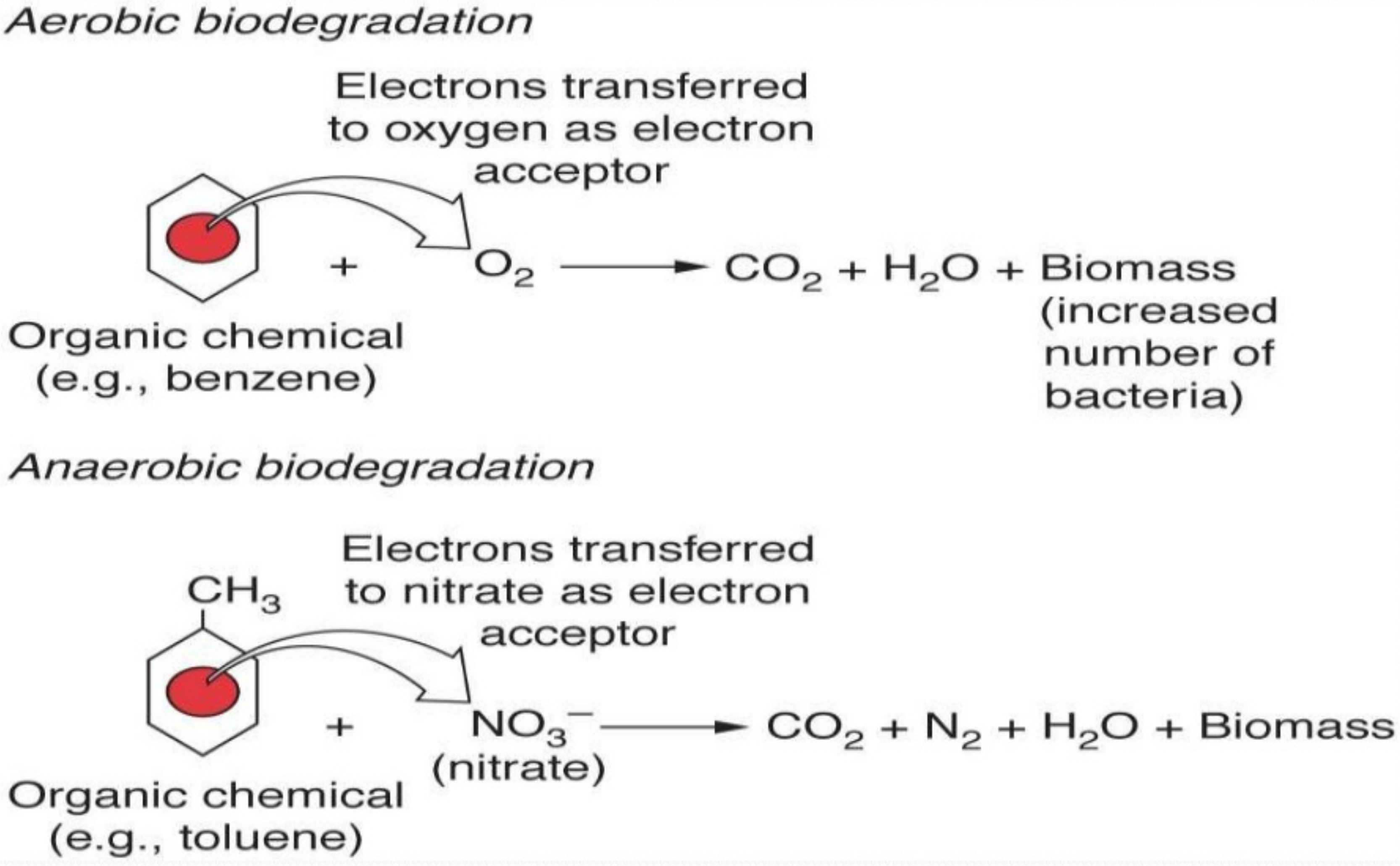
3) **Anaerobic respiration** : is similar to aerobic respiration, but it uses an inorganic molecule other than oxygen as the final electron acceptor.

- The ATP yield per glucose molecule varies, depending on the final electron acceptor used.
- inorganic molecule may be : Fe, Mn, Co, NO3, sulfate or others.
- all fermentative bacteria can ferment the simplest sugar {dextrose (D-glucose)}.

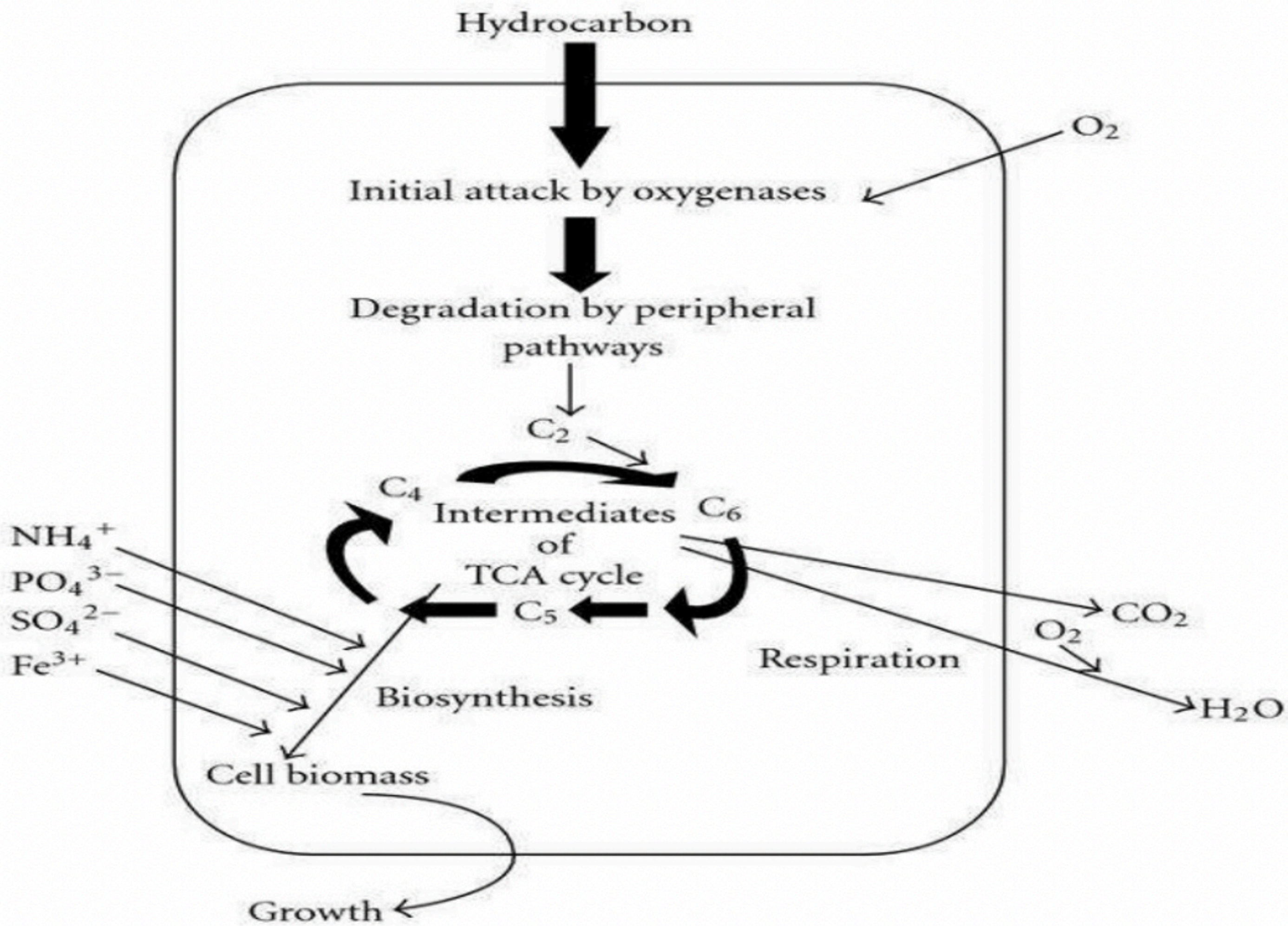
Microorganisms

- Aerobic bacteria:
  - Examples include: *Pseudomonas*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*
  - Shown to degrade pesticides and hydrocarbons; alkanes and polyaromatics
  - May be able to use the contaminant as sole source of carbon and energy.
- Anaerobic bacteria:
  - Not used as frequently as aerobic bacteria
  - Can often be applied to bioremediation of polychlorinated biphenyls (PCBs) in river sediments, trichloroethylene (TCE), and chloroform
- Fungi:
  - Able to degrade a diverse range of persistent or toxic environmental pollutants

Aerobic and Anaerobic Biodegradation



MECHANISM OF AEROBIC DEGRADATION



Metabolism Modes

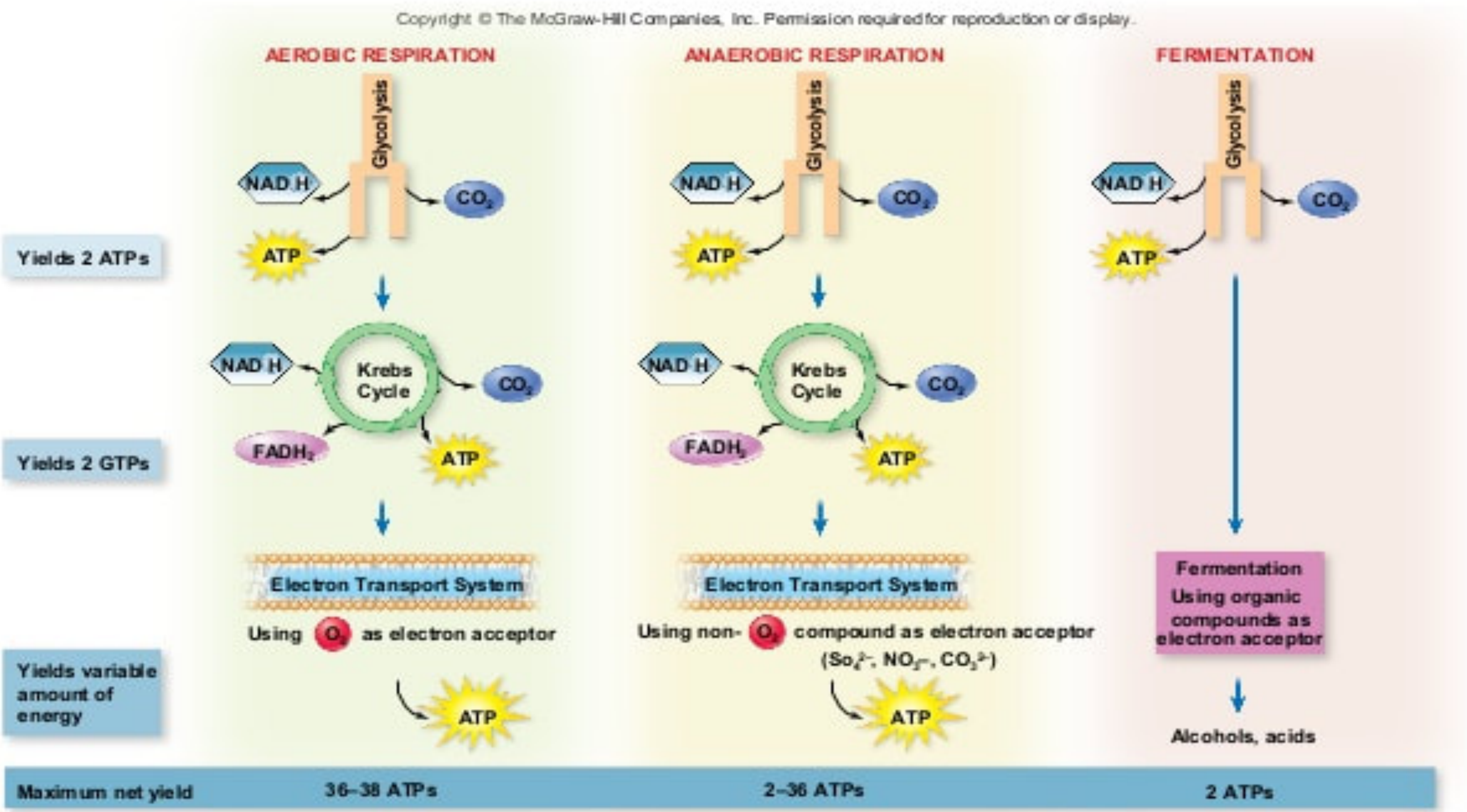
- Aerobic: transformations occur in the presence of molecular oxygen (as electron acceptor), known as aerobic respiration
- Anaerobic: reactions occur only in the absence of molecular oxygen, subdivided into:

– Anaerobic respiration
– Fermentation
– Methane fermentation

Metabolism Modes

- Anaerobic respiration
- – Nitrate as an electron acceptor - denitrifying and nitratereducing
- organisms
- – Sulfate and thiosulfate as electron acceptors - by sulfatereducing
- organisms
- – CO_2 as an electron acceptor, by methanogenic organisms
- – Chlorinated organic compounds as electron acceptors
- • Fermentation - organic compounds serve as both
- electron donors and electron acceptors
- • Methane fermentation - consecutive biochemical
- breakdown of organic compounds to CH_4 and CO_2

Overview of the Three Main Catabolic Pathways



Anaerobic respiration

- Almost the same as aerobic respiration, except replace O_2 with another molecule in electron transport

Metabolic pathways:

1. Glycolysis or alternatives
2. TCA cycle
3. Electron transport – chain different

Example Rxn:

$$C_6H_{12}O_6 + 6\ NO_2^-, \rightarrow 6\ CO_2 + 3\ N_2 + 6\ H_2O$$

– Can generates up to 36 ATPs/glucose (depends on electron acceptor used)

Anaerobic respiration

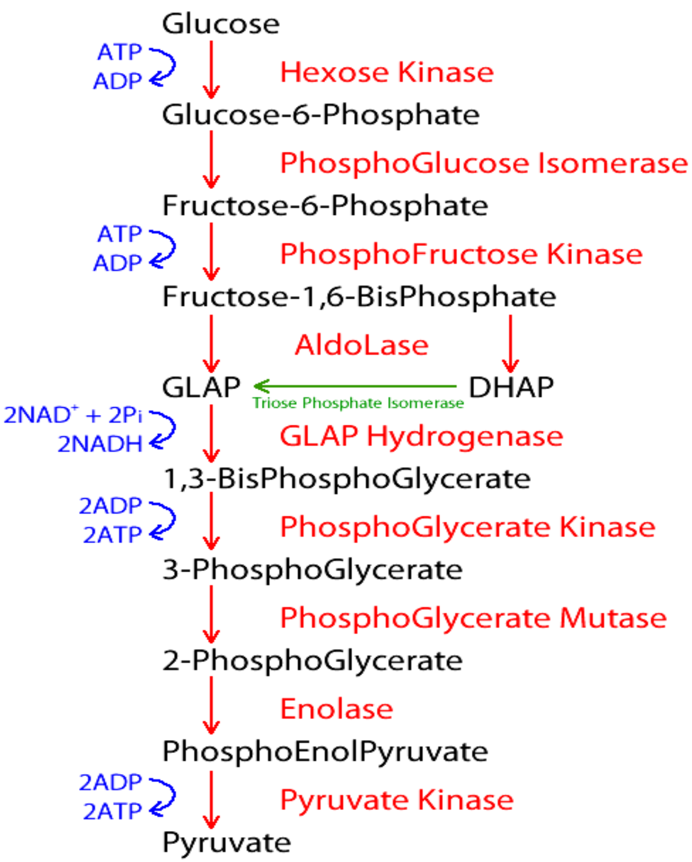
- Compounds that can serve as electron acceptors

Table 6.3 Compounds that can serve as electron acceptors in anaerobic respiration, replacing oxygen

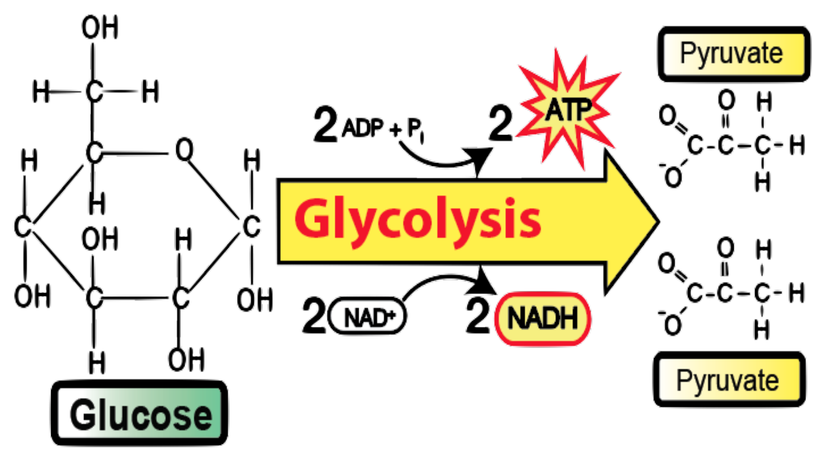
Organic compounds	Inorganic compounds
Fumarate	Nitrate (NO_3^-)
Dimethylsulfoxide (DMSO)	Nitrite (NO_2^-)
Trimethylamine *N*-oxide (TMAO)	Nitrous oxide (N_2O)
	Chlorate (ClO_3^-)
	Perchlorate (ClO_4^-)
	Manganic ion (Mn^{4+})
	Ferric ion (Fe^{3+})
	Gold (Au^{3+})
	Selenate (SeO_4^{2-})
	Arsenate (AsO_4^{3-})
	Sulfate (SO_4^{2-})
	Sulfur (S^0)

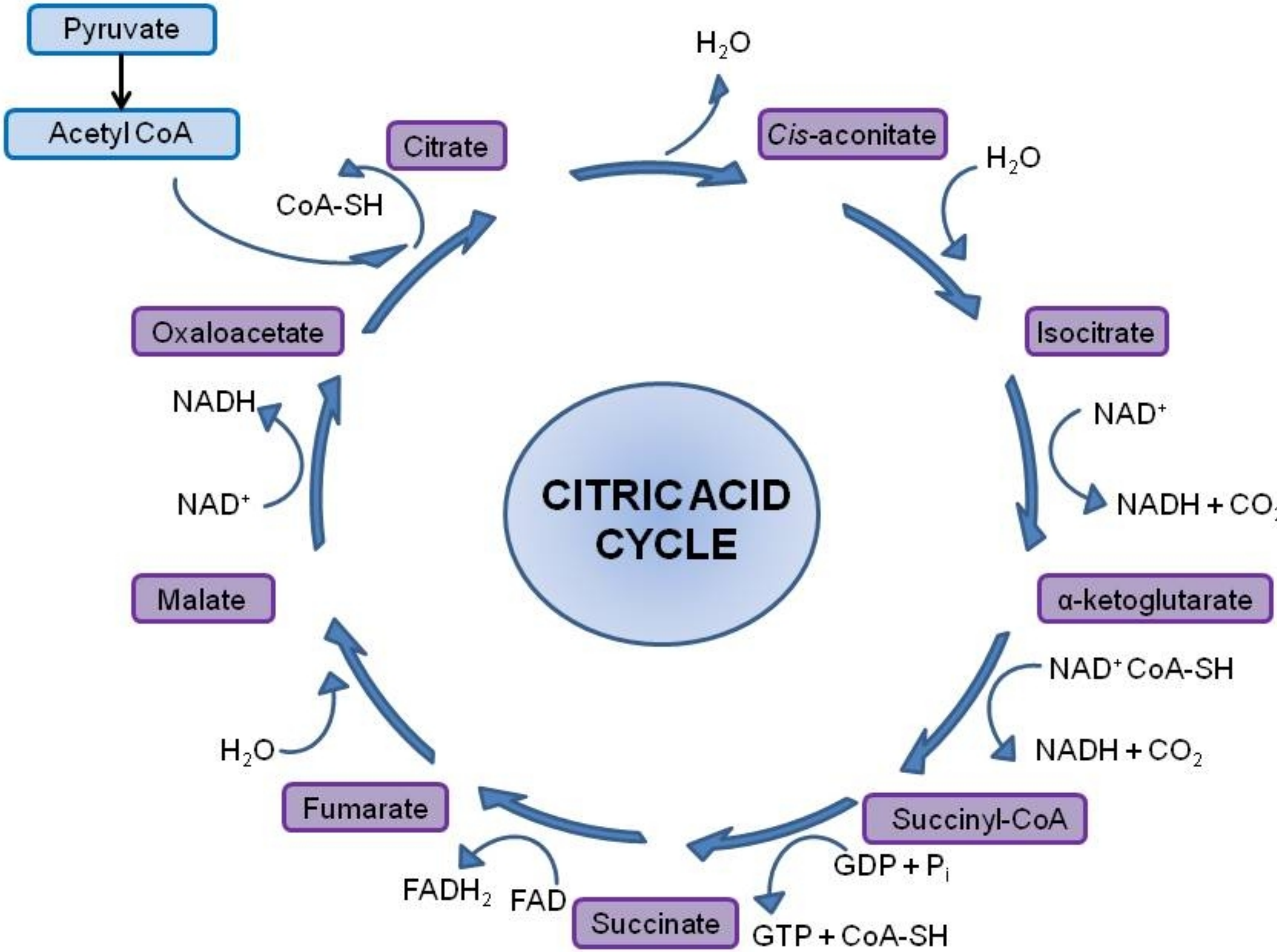
Table 6.3 Microbe

Glycolysis



Simplified Glycolysis diagram. Molecule names contain extra capitals to illustrate components. 21/02/2010 followchemistry.wordpress.com





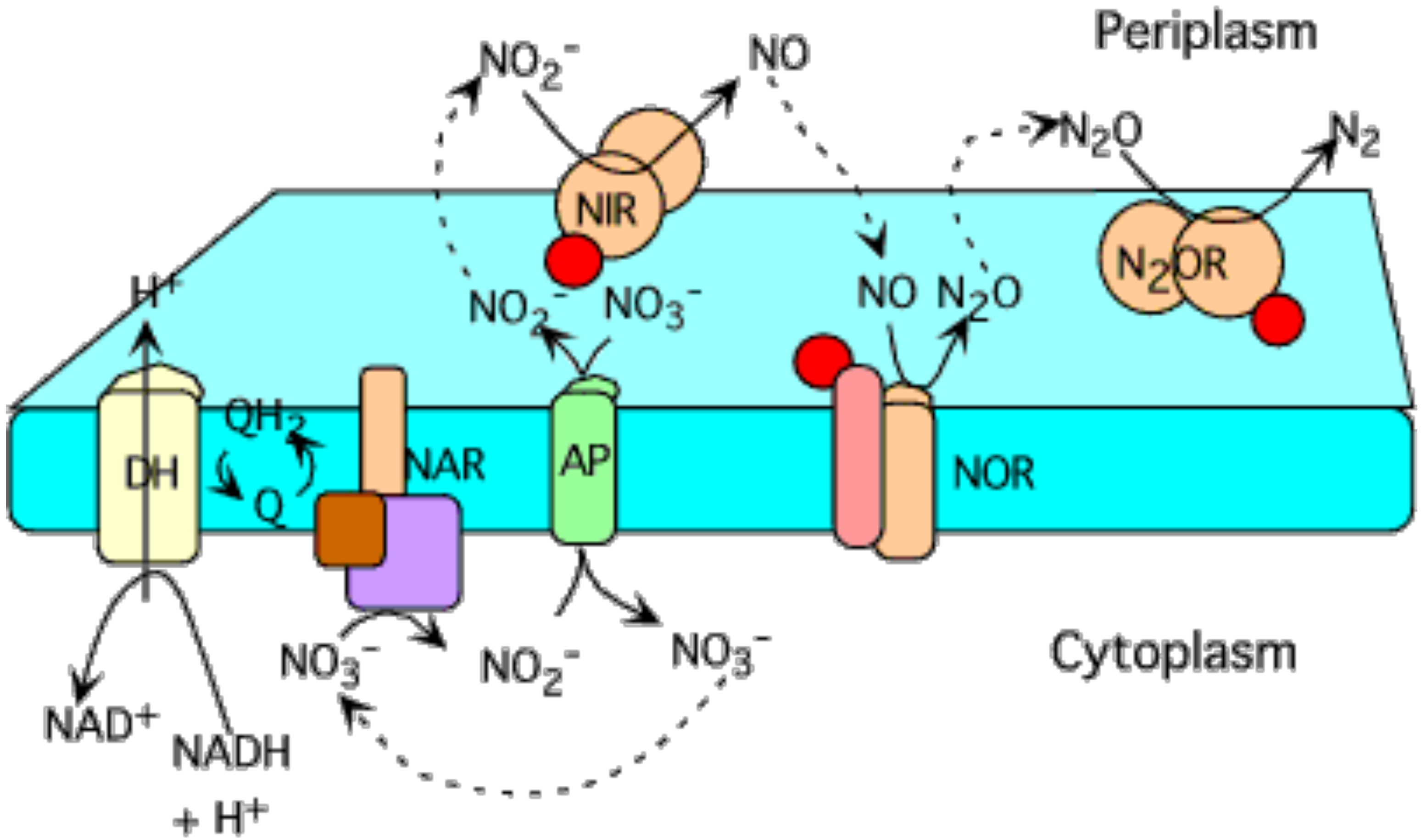
Pyruvate
Acetyl CoA
Citrate
CoA-SH
H_2O
Cis-aconitate
H_2O
Isocitrate
NAD^+
$NADH + CO_2$
α-ketoglutarate
NAD^+ CoA-SH
$NADH + CO_2$
Succinyl-CoA
$GDP + P_i$
GTP + CoA-SH
Succinate
FAD
$FADH_2$
Fumarate
H_2O
Malate
NAD^+
NADH
Oxaloacetate
CITRIC ACID CYCLE

Citric Acid Cycle: Balance Sheet

Citric acid cycle

inputs	outputs
2 acetyl groups	4 CO_2
6 NAD^+	6 NADH
2 FAD	2 $FADH_2$
2 ADP + 2 P	2 ATP

Electron Transport System (ETS)



Anaerobic respiration

A. Denitrification

- Reduction of nitrate or nitrite all the way to dinitrogen (N_2) gas
- Done by some facultative anaerobic chemohetero-trophic bacteria (*Pseudomonas, Ralstonia, Paracoccus*) if oxygen not present

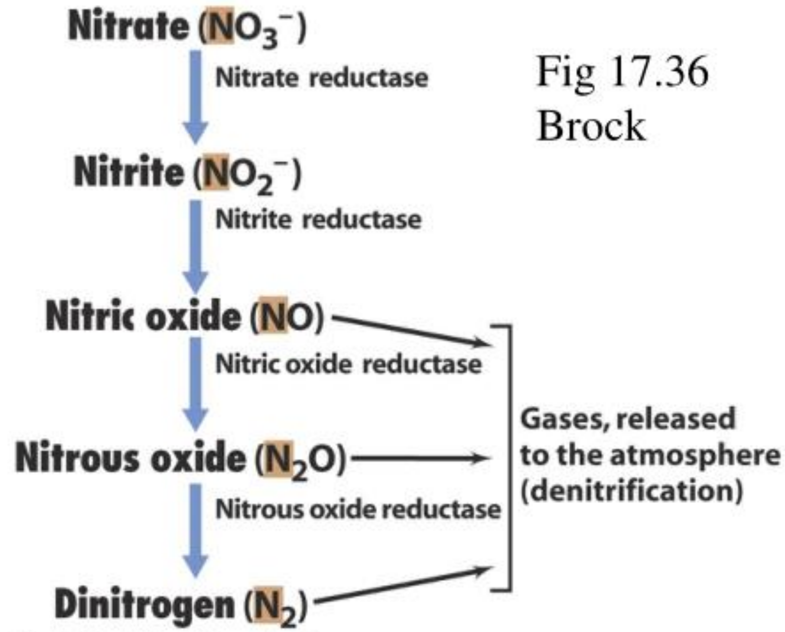


Figure 17-36 Brock Biology of Microorganisms 11/e

Fig 17.36 Brock

- Reduces nitrogen in soil and wastewater in treatment plants

Anaerobic respiration

B. Nitrate reduction

- A few bacteria reduce nitrate to ammonia
- Ammonia can be assimilated by incorporation into amino acids
- Mentioned in Section 9.1.4

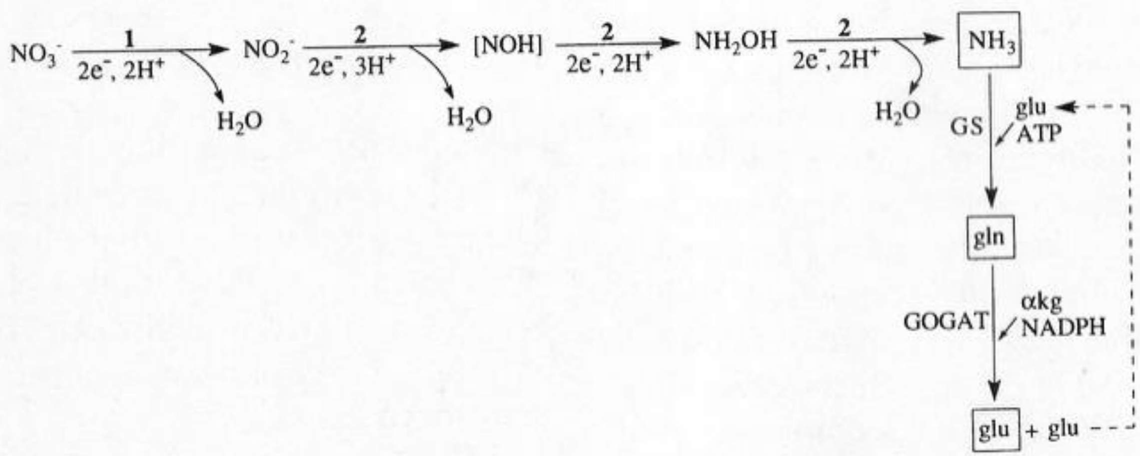


Fig 12.1 White 3rd

Anaerobic respiration

C. Sulfate reduction or sulfidogenesis
   - Electrons reduce sulfate to H_2S
   - Some get electrons from carbon compounds like lactate, ethanol
   - Other (chemoauto-trophics) get from H_2 (hydrogen bacteria)

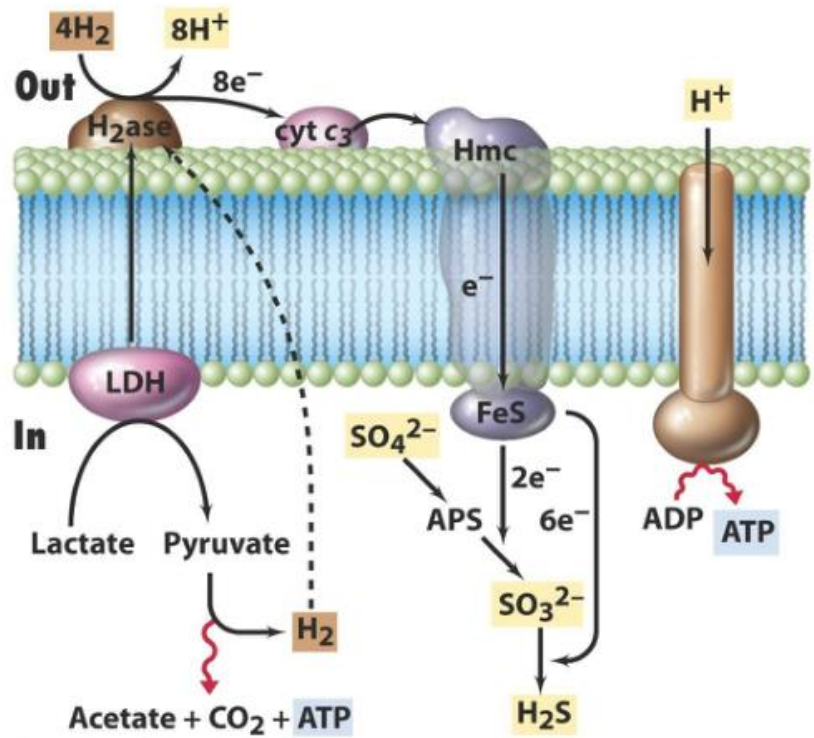


Figure 17-39 Brock Biology of Microorganisms 11/e

Fig 17.39 Brock

Types of bacterial food fermentation

1. Lactic acid fermentation

- most desirable food fermentation, sour dough bread, sorghum beer, fermented milks, fermented vegetables.
- *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*
- Homolactic fermentation:

  $$\text{Glucose} \longrightarrow \text{2 lactic acid}$$

- Heterolactic fermentation:

  $$\text{Glucose} \longrightarrow \text{lactic acid} + \text{ethanol} + CO_2$$

Types of bacterial food fermentation

2. Acetic acid fermentation

- Vinegar from fruit juices and alcohols
- acetic acid bacteria/ acetobacter- convert alcohol to acetic acid in the presence of oxygen

$$\text{Alcohol} \longrightarrow \text{acetic acid + water}$$

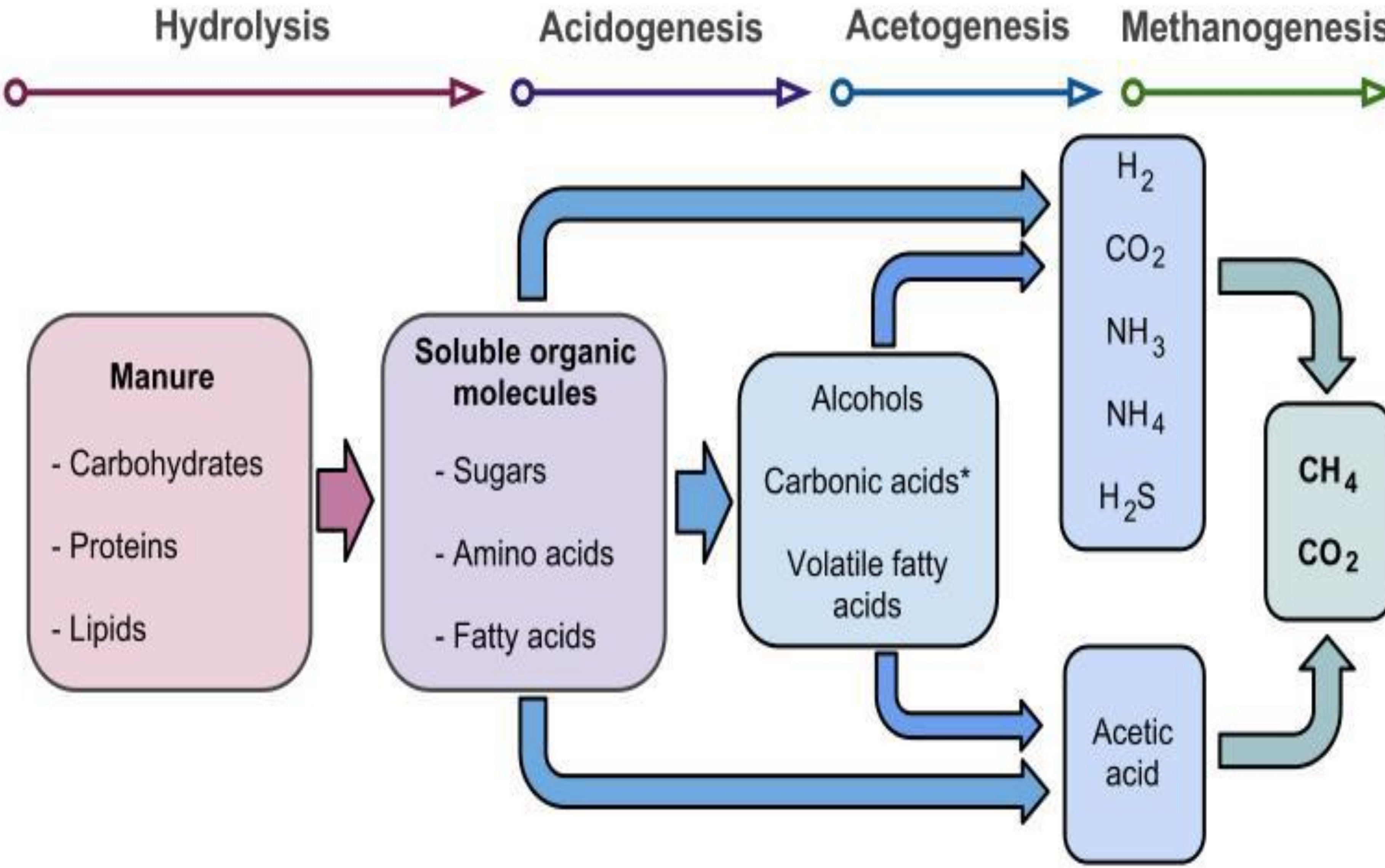
Methane Fermentation

- Methane fermentation is a versatile biotechnology capable of converting almost all types of polymeric materials to methane and carbon dioxide under anaerobic conditions.
- This is achieved as a result of the consecutive biochemical breakdown of polymers to methane and carbon dioxide in an environment in which a variety of microorganisms which include fermentative microbes (acidogens); hydrogen-producing, acetate-forming microbes (acetogens); and methane-producing microbes (methanogens) harmoniously grow and produce reduced end-products.
- Anaerobes play important roles in establishing a stable environment at various stages of methane fermentation.

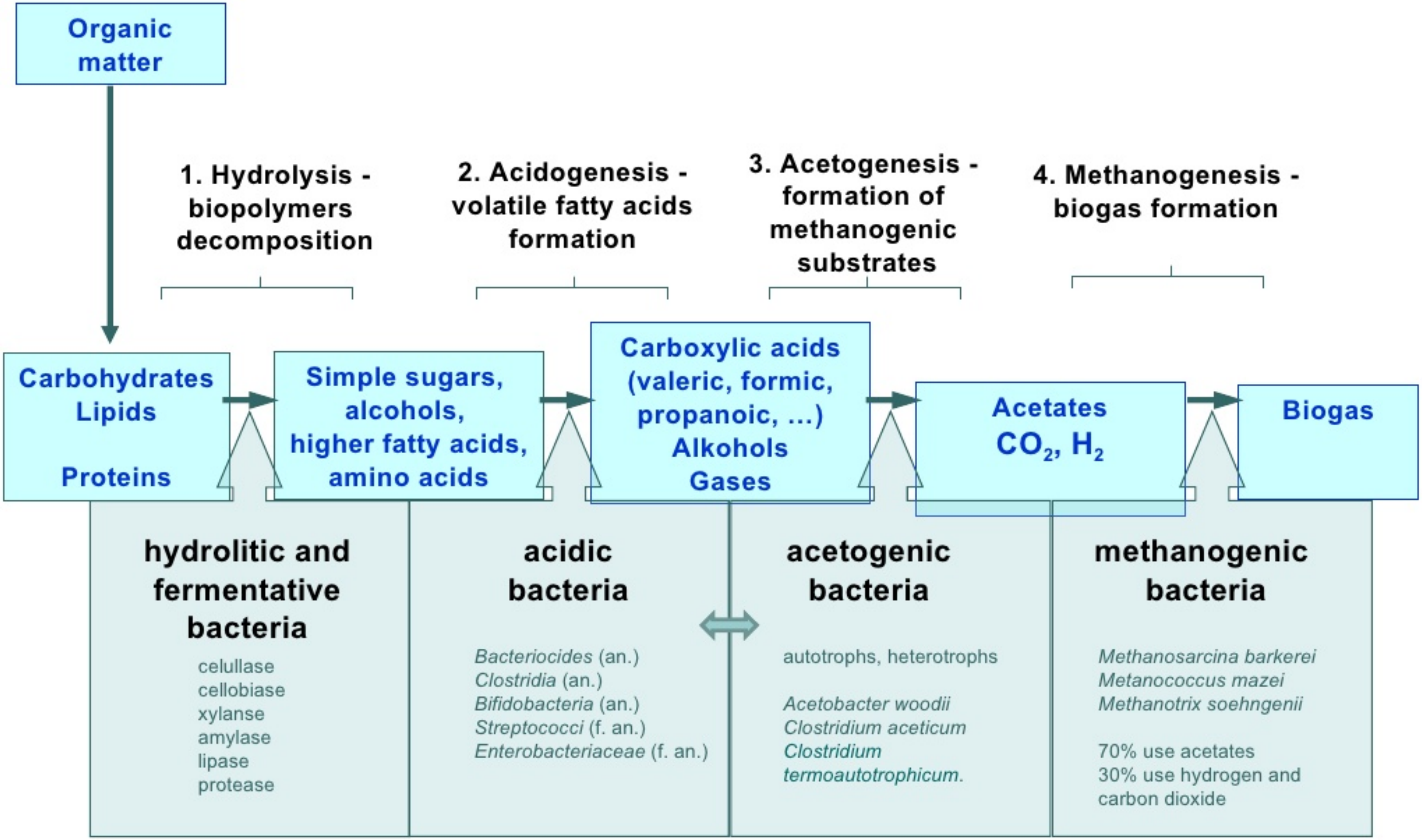
PROCESS

There are four key biological and chemical stages of methane fermentation

- **Hydrolysis**
- **Acidogenesis**
- **Acetogenesis**
- **Methanogenesis**


Hydrolysis
Acidogenesis
Acetogenesis
Methanogenesis
Manure
- Carbohydrates
- Proteins
- Lipids
Soluble organic molecules
- Sugars
- Amino acids
- Fatty acids
Alcohols
Carbonic acids*
Volatile fatty acids
H_2
CO_2
NH_3
NH_4
H_2S
Acetic acid
CH_4
CO_2

Bioprocesses in biogas production



The Biochemistry

1 Hydrolysis

Complex organic molecules to soluble

2 Acidogenesis

Small organic molecules to fatty acids

3 Acetogenesis

Conversion to acetic acid & CO_2

4 Methanogenesis

Final conversion to methane [CH_4]

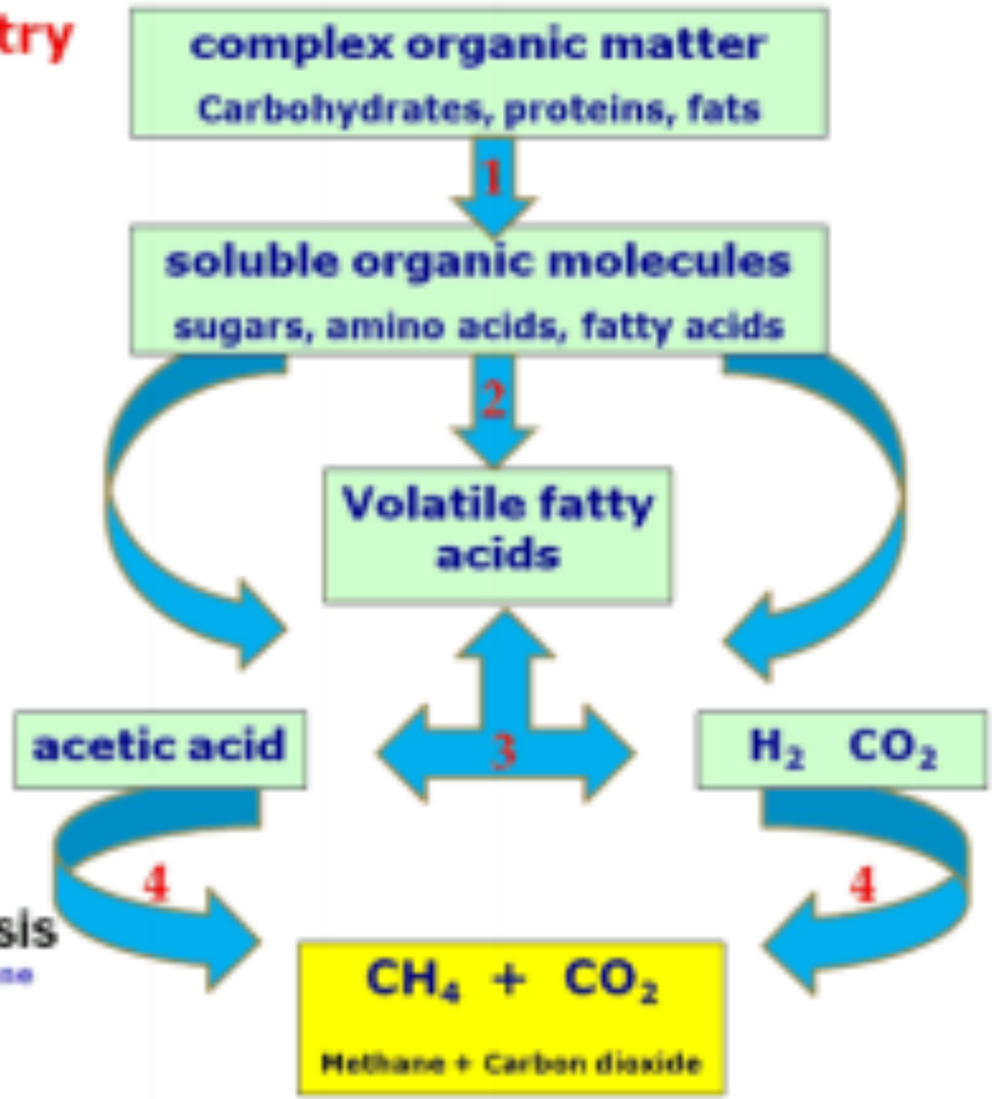


TABLE 24.4. The most common types of anaerobic respiration

	Type	Overall process	Microorganisms involved
1.	Nitrate respiration (denitrification)	$NO_3^- \longrightarrow NO_2^-, N_2O, N_2$	Facultative aerobes (e.g., *Escherichia coli, Paracoccus denitrificans, Pseudomonas stutzeri*)
2.	Sulphate respiration (Sulphate reduction)	$SO_4^{-2} \longrightarrow H_2S$	Obligate anaerobes (e.g., *Desulfovibrio desulfuricans, Desulfobacter*)
3.	Carbonate respiration	$CO_2 \longrightarrow CH_3COOH$ (Acetic acid)	Obligate anaerobes (homoacetogenic bacteria such as *Acetobacterium woodii* and *Clostridium aceticum*)
		$CO_2 \longrightarrow CH_4$(Methane)	Obligate anaerobes (methanogenic archae bacteria such as *Methanobacterium, Methanobrevibacter, Methanococcus*, etc.)
4.	Sulphur respiration	$S^0 \longrightarrow H_2S$	Obligate anaerobes (e.g., *Desulfurococcus, Pyrodictium*) and facultative aerobes.
5.	Iron respiration	$Fe^{3+} \longrightarrow Fe^{2+}$ (Ferric) (Ferrous)	Obligate anaerobes and facultative aerobes (*Shewanella, Geobacter, Geospirillum*, and *Geovibrio*)
6.	Fumarate respiration	Fumarate $\longrightarrow$ Succinate	Facultative aerobes (*Wolinella succinogenes, Desulfovibrio gigas*, some clostridia, and *Escherichia coli*)

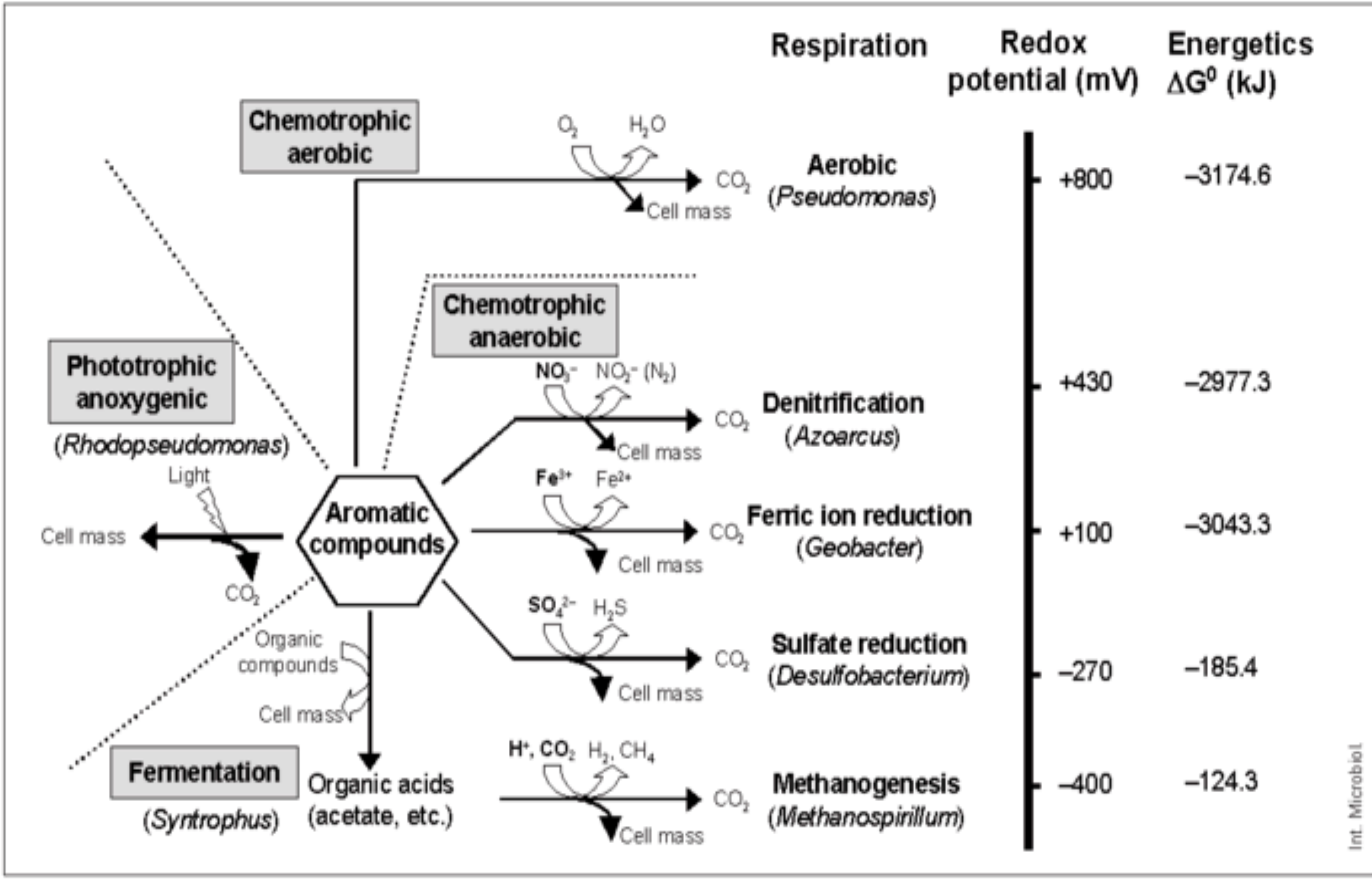
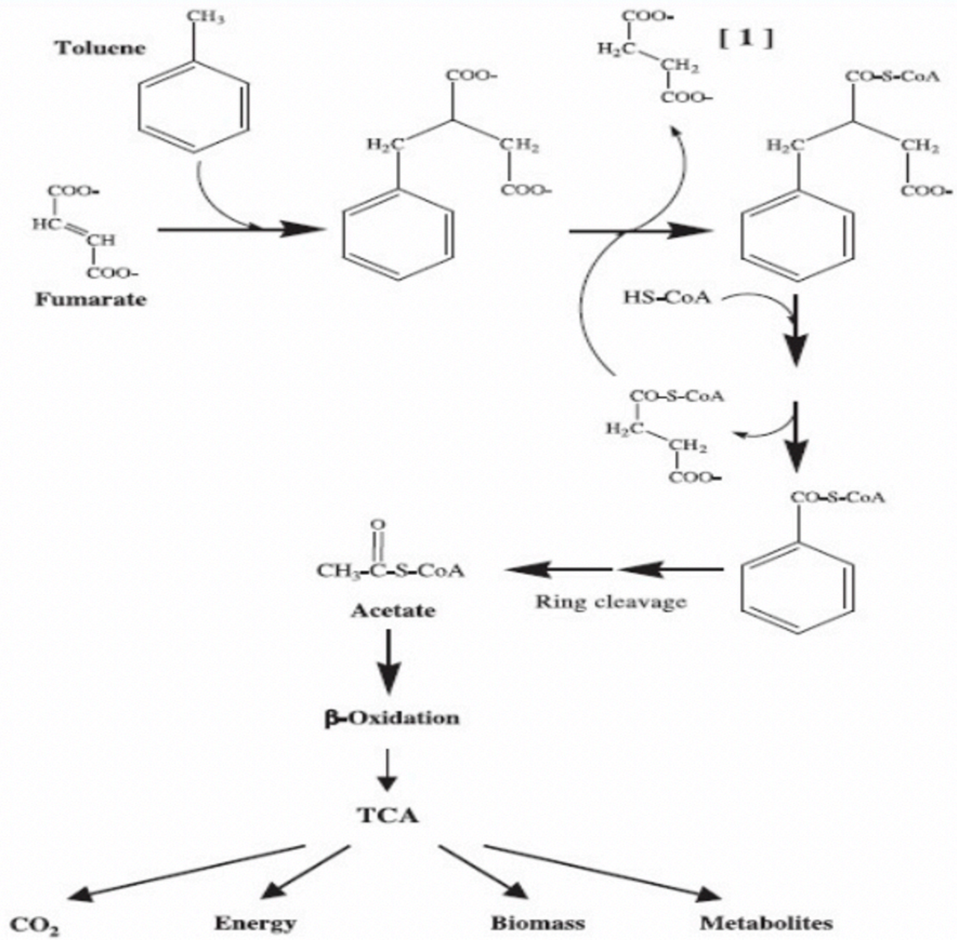


Fig. 2. Microbial utilization of aromatic compounds. The different terminal electron acceptors in respiration are indicated in bold and they are aligned with the redox potential bar. The energetics (free-energy changes) of the aerobic and anaerobic degradation of a model aromatic compound, benzoate, are indicated on the right. Methanogenesis needs to be coupled to fermentation reactions. Bacterial genera representative of each type of metabolism are shown in parentheses. Modified from [35].

DEGRADATION OF AROMATIC HYDROCARBONS

- It can be converted to the natural intermediates that is catechol and protocatechuate
- Some Gram negative bacteia have plasmids that encode enzymes for degradation of aromatics called TOL plasmids
- It mainly involves hydroxylation catalysed by dioxygenase

DEGRADATION OF TOULENE



ANAEROBIC DEGRADATION OF AROMATIC HYDROCARBONS

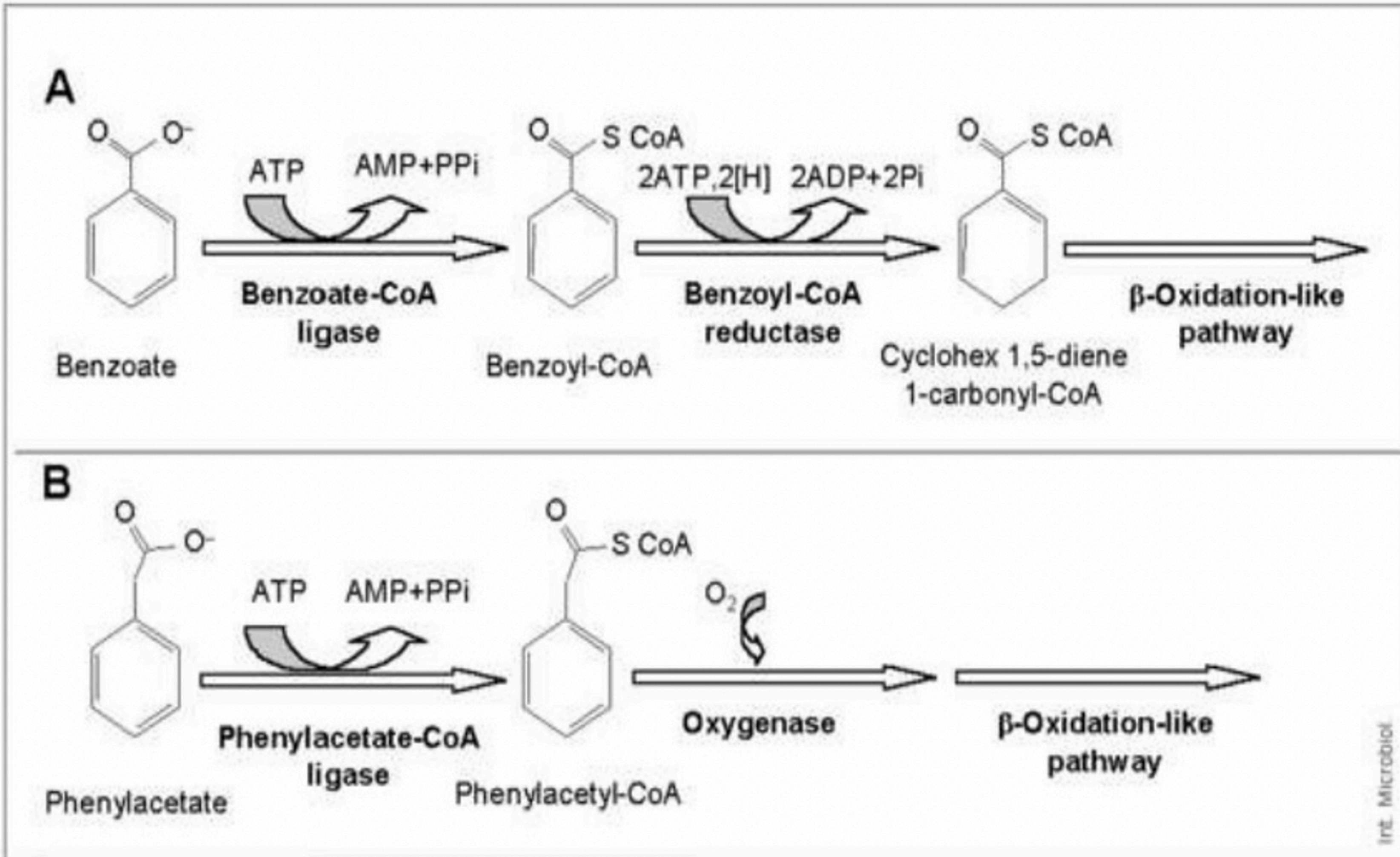
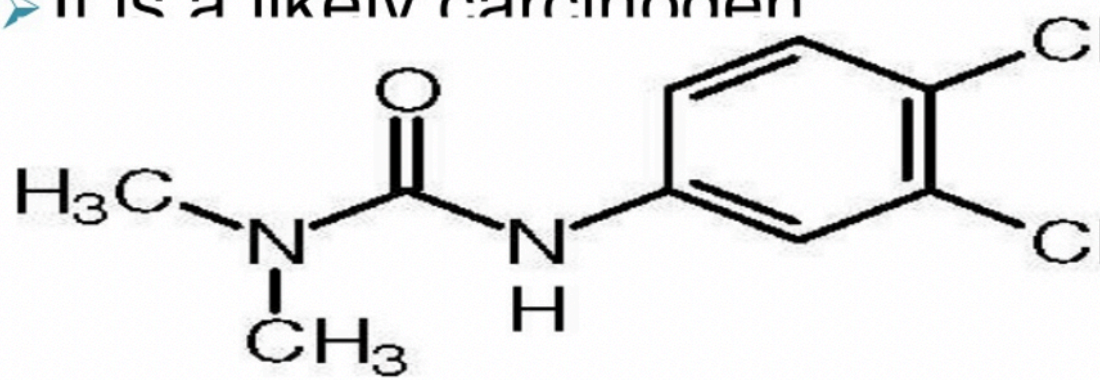


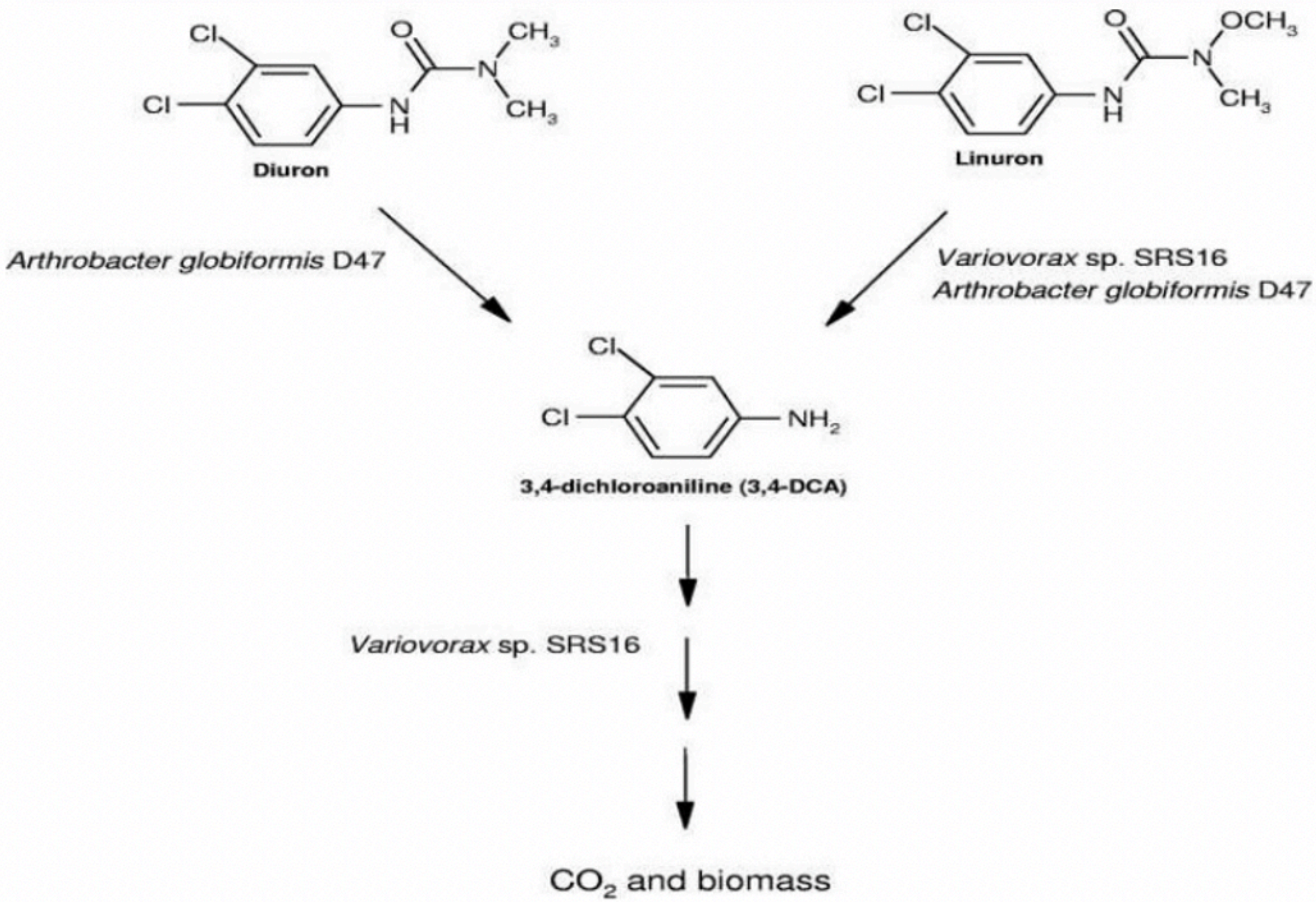
Fig. 4. (A) Anaerobic degradation of benzoate. (B) Aerobic hybrid pathway for the catabolism of phenylacetate.

DEGRADATION OF HERBICIDES

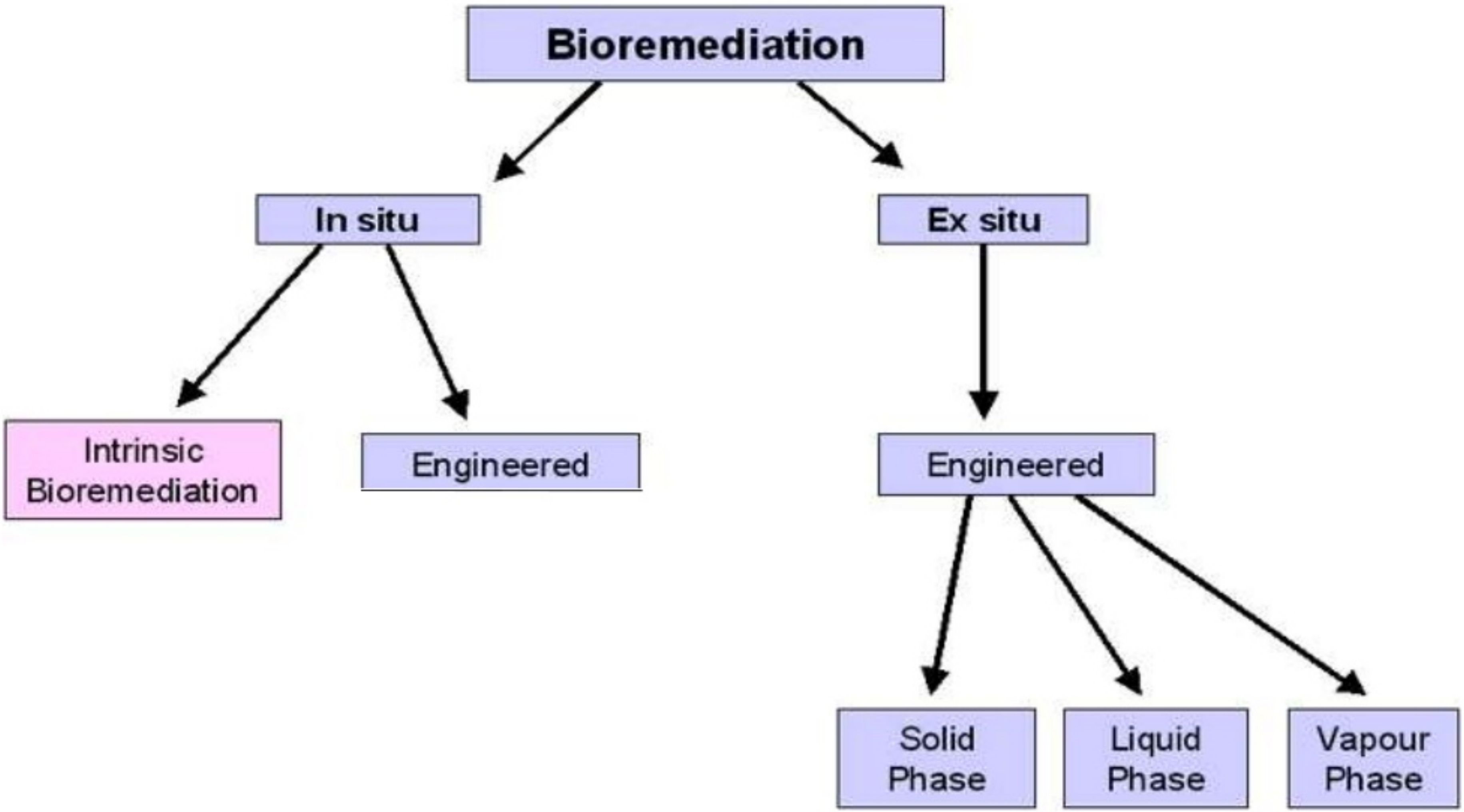
- DIURON is a systemic herbicide derived from urea, relatively persistent in soil,
- half-lives from 90 to 180 days.
- It shows slight acute toxicity
- It is a likely carcinogen



ANAEROBIC DEGRADATION OF DIURON



Bioremediation Strategies



(Barathi S and Vasudevan N, 2001)

Bioremediation Strategies

In situ Bioremediation
(at the site)

Ex situ Bioremediation
(away from the site)

(Barathi S and Vasudevan N, 2001)

How Microbes Use the Contaminant

- Contaminants may serve as:
  - Primary substrate
    - enough available to be the sole energy source.
  - Secondary substrate
    - provides energy, not available in high enough concentration.
  - Co metabolic substrate
    - Utilization of a compound by a microbe relying on some other primary substrate.

(Bodishbaugh, D.F., 2006)

Some m.o. involved in the biodegradation of organic pollutants

Organic Pollutants	Organisms
Phenolic compound	- *Achromobacter, Alcaligenes, Acinetobacter, Arthrobacter, Azotobacter, Flavobacterium, Pseudomonas putida* - *Candida tropicalis Trichosporon cutaneoum* - *Aspergillus, Penicillium*
Benzoate & related compound	*Arthrobacter*, *Bacillus* spp., *Micrococcus*, *P. putida*

Organic Pollutants	Organisms
Hydrocarbon	***E. coli, P. putida, P. Aeruginosa***
Surfactants	***Alcaligenes, Achromobacter, Bacillus, Flavobacterium, Pseudomonas, Candida***
Pesticides	***P. Aeruginosa***
DDT	***Arthrobacter, P. cepacia***
BHC	***P. cepacia***
Parathion	***Pseudomonas spp., E. coli, P. aeruginosa***

(Vidali, 2007)

Criteria for Bioremediation Strategies

i) Organisms must have necessary catabolic activity required for degradation of contaminant at fast rate to bring down the concentration of contaminant.

ii) The target contaminant must have bioavailability.

iii) Soil conditions must be favourable for microbial/plant growth and enzymatic activity.

iv) Cost of bioremediation must be less than other technologies of removal of contaminants.

TYPES OF BIOREMEDIATION

- The two main types of bioremediation are **in situ** bioremediation and **ex situ** bioremediation. In addition, another offshoot of bioremediation is phytoremediation.

Forms of Bioremediation

- In situ Bioremediation
  - Bioventing
  - In situ biodegradation
  - Biostimulation
  - Biosparging
  - Bioaugmentation
  - Natural Attenuation

- Ex situ Bioremediation
  - Land farming
  - Composting
  - Biopiles
  - Bioreactors

In Situ Bioremediation

- In situ bioremediation is when the contaminated site is cleaned up exactly where it occurred.

- It is the most commonly used type of bioremediation because it is the **cheapest** and **most efficient**, so it's generally better to use.

- There are two main types of in situ bioremediation: intrinsic bioremediation and accelerated bioremediation.

Five Steps of In Situ Bioremediation

1. Site investigation
2. Treatability studies
3. Recovery of free product and removal of the contamination source
4. Design and implementation of the in situ bioremediation system
5. Monitoring and performance evaluation of the in situ bioremediation system

Intrinsic Bioremediation

- **Intrinsic bioremediation** uses microorganisms already present in the environment to biodegrade harmful contaminant.

- There is no human intervention involved in this type of bioremediation, and since it is the cheapest means of bioremediation available, it is the most commonly used.

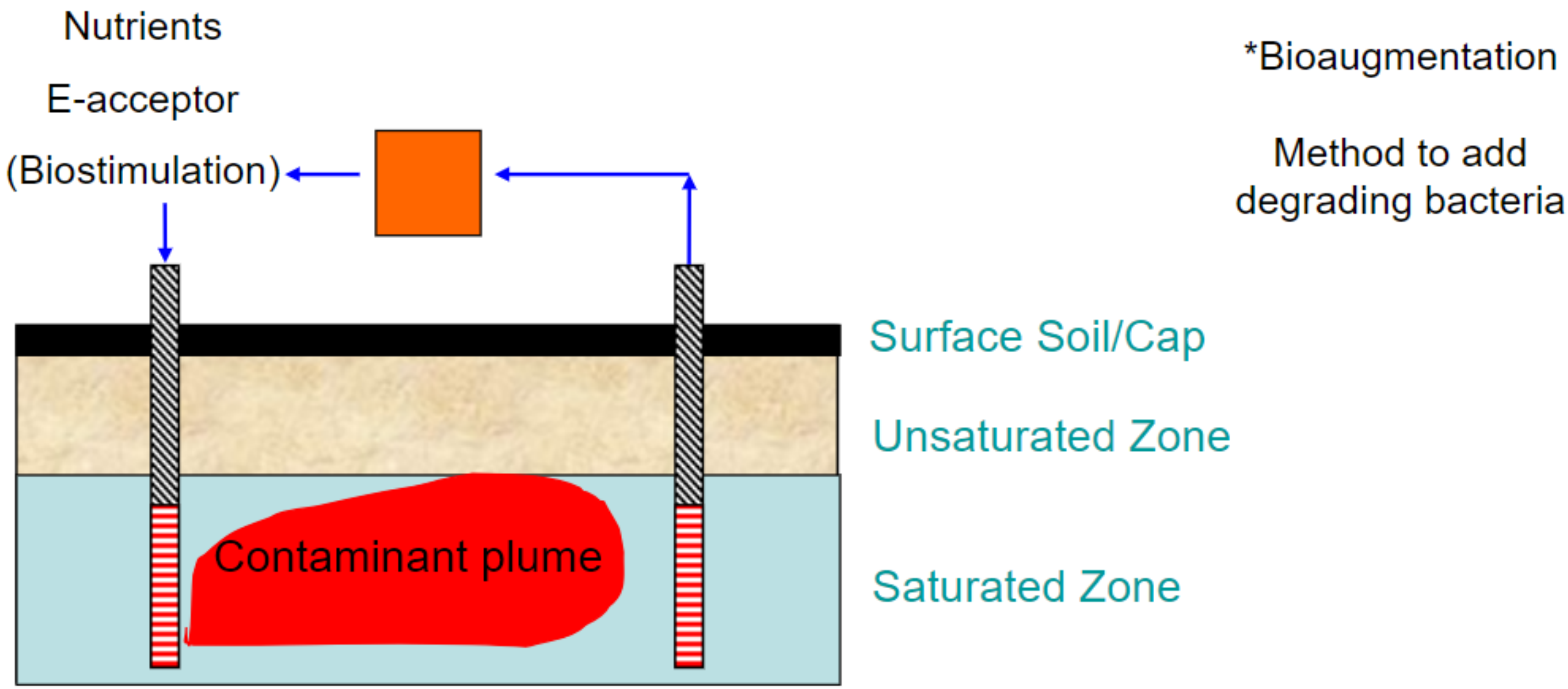
- When intrinsic bioremediation isn't feasible, scientists turn next to accelerated bioremediation.

Accelerated Bioremediation

- **In accelerated bioremediation**, either substrate or nutrients are added to the environment to help break down the toxic spill by making the microorganisms grow more rapidly.
- Usually the microorganisms are indigenous, but occasionally microorganisms that are very efficient at degrading a certain contaminant are additionally added.

- Stimulating Bioremediation
  - *Nutrient enrichment* (fertilization) – fertilizers are added to a contaminated environment to stimulate the growth of indigenous microorganisms that can degrade pollutants
  - *Bioaugmentation* (seeding) –bacteria are added to the contaminated environment to support indigenous microbes with biodegradative processes

In Situ Bioremediation



Biostimulation: addition of nutrients and electron-acceptor into groundwater in order to stimulate subsurface microorganisms (mainly bacteria) to degrade contaminants.

Bioaugmentation: addition of efficiently degrading bacteria into contaminated groundwater with nutrients/electron acceptor to enhance biodegradation of contaminant.

- Main advantage is that site disturbance is minimized, which is particularly important when the contaminated plume has moved under permanent structures.

- Biggest limitation of in situ treatment has been the inability to deal effectively with metal contaminants mixed with organic compounds.

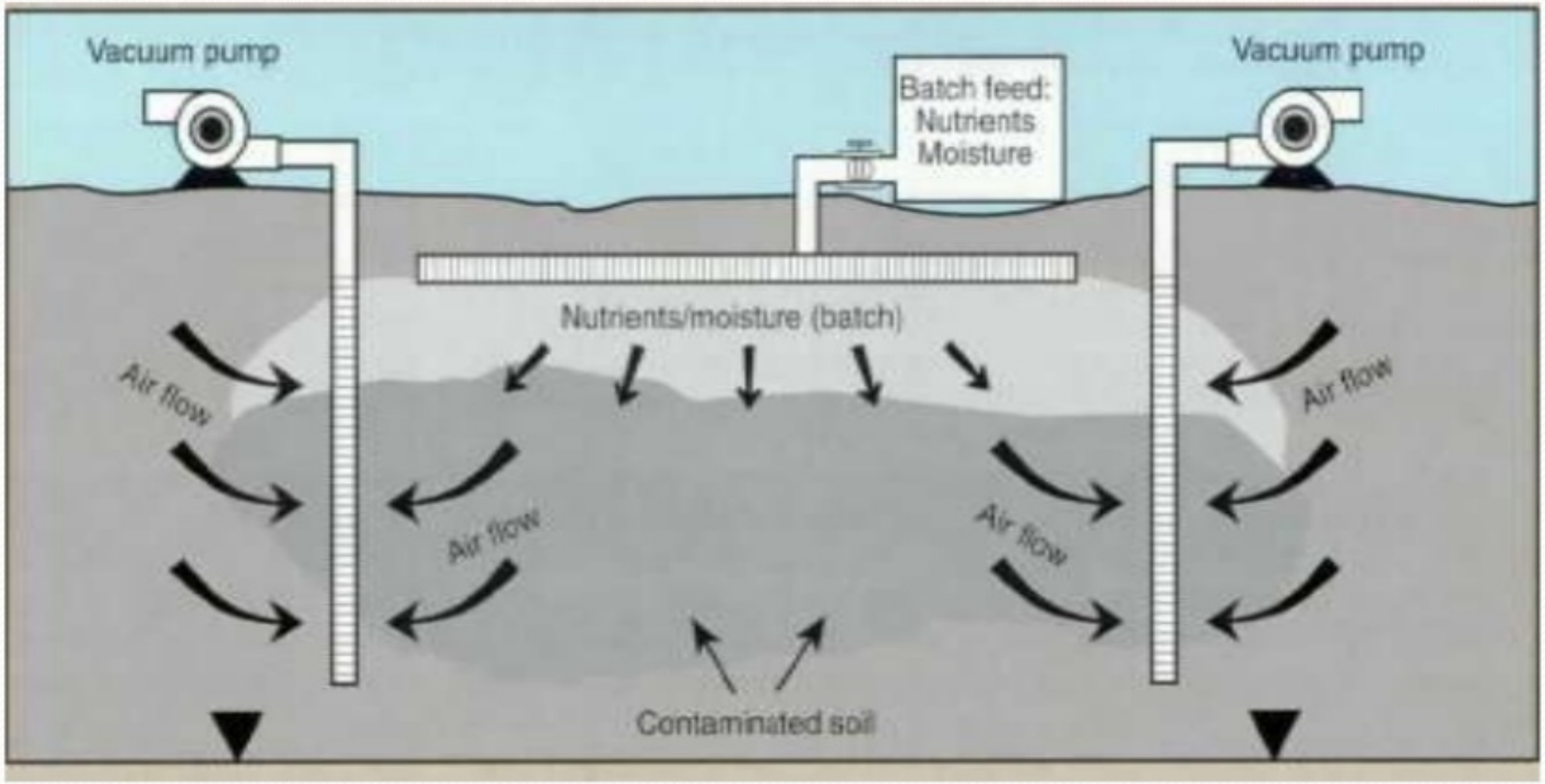
- The goal of in situ treatment is to manage and manipulate the subsurface environment to optimize microbial degradation.

In Situ Bioremediation

- Land treatments:

Bioventing is the most common *in situ* treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria.

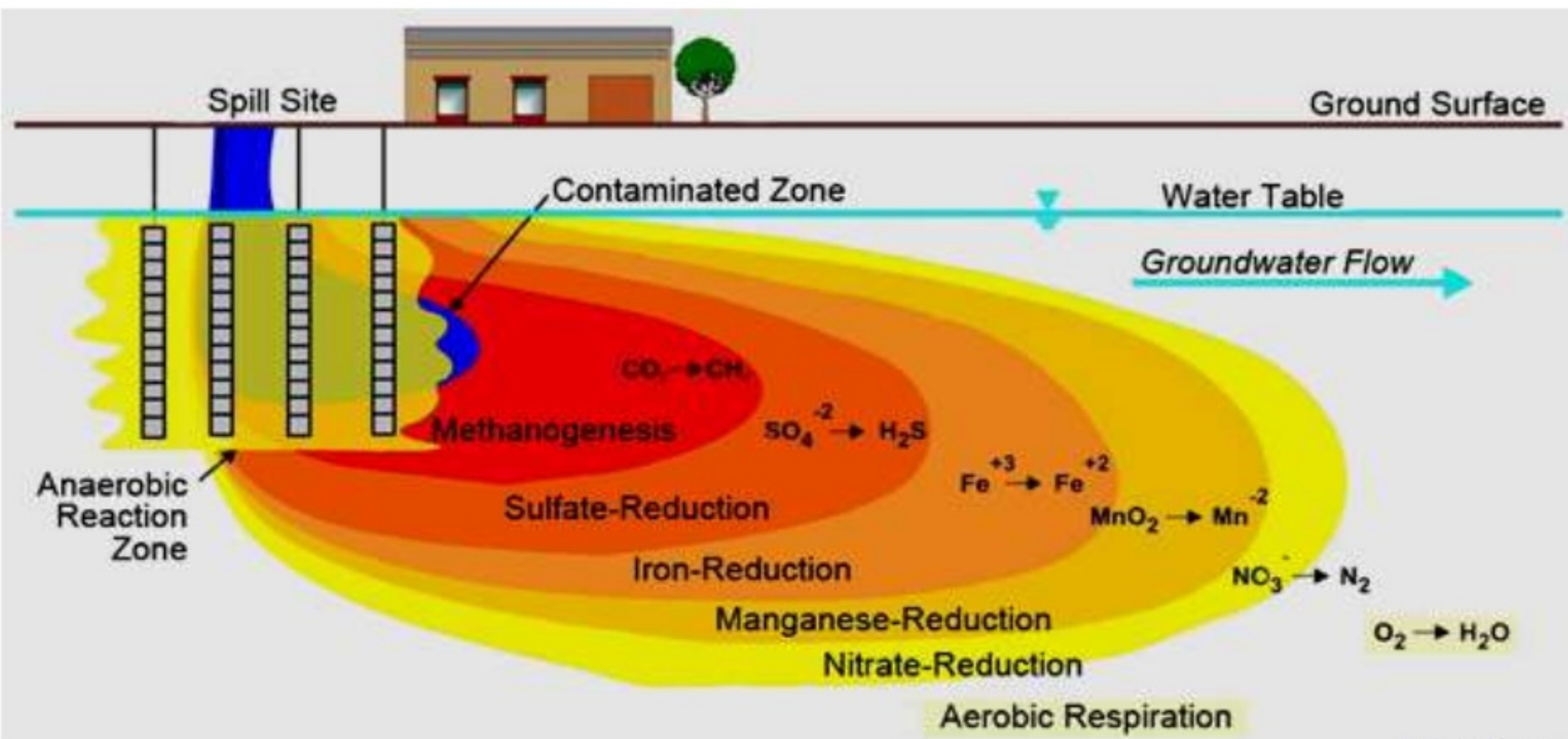
Bioventing



Vacuum pump
Batch feed:
Nutrients
Moisture
Vacuum pump
Nutrients/moisture (batch)
Air flow
Air flow
Air flow
Air flow
Contaminated soil

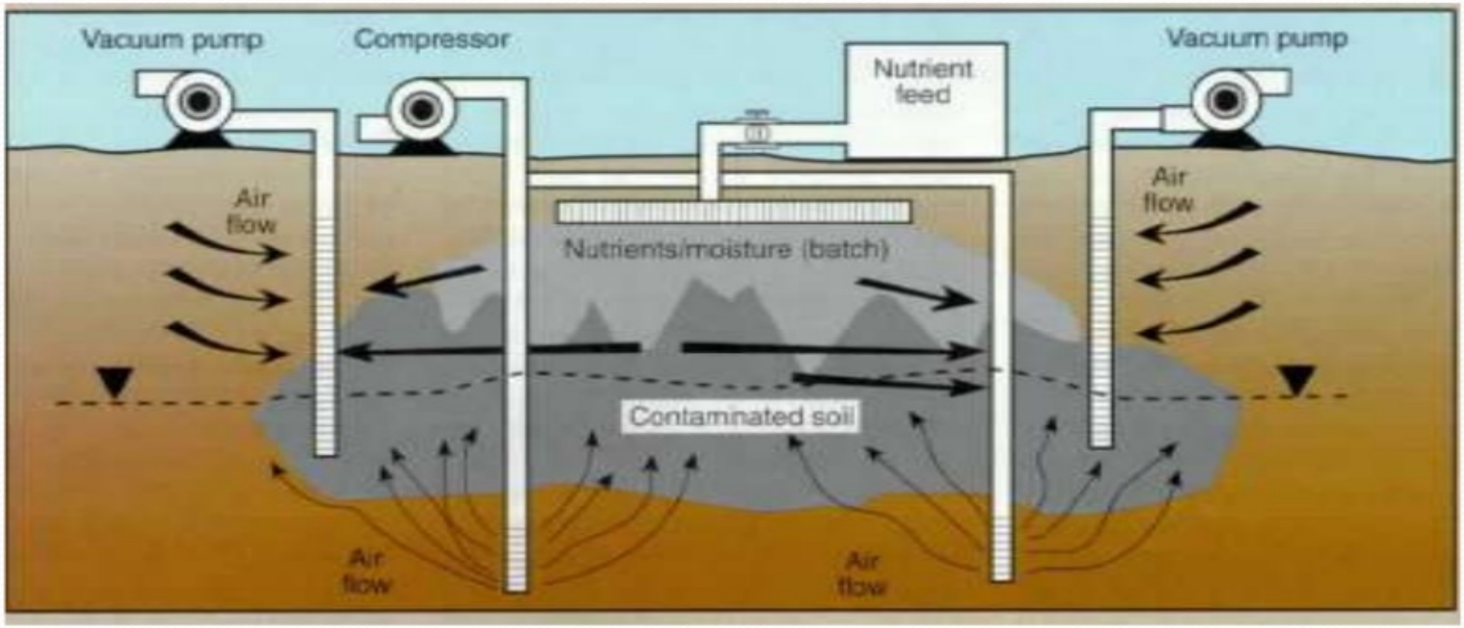
Biosparging

involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria.



(Vidali,M.2001)

Biosparging



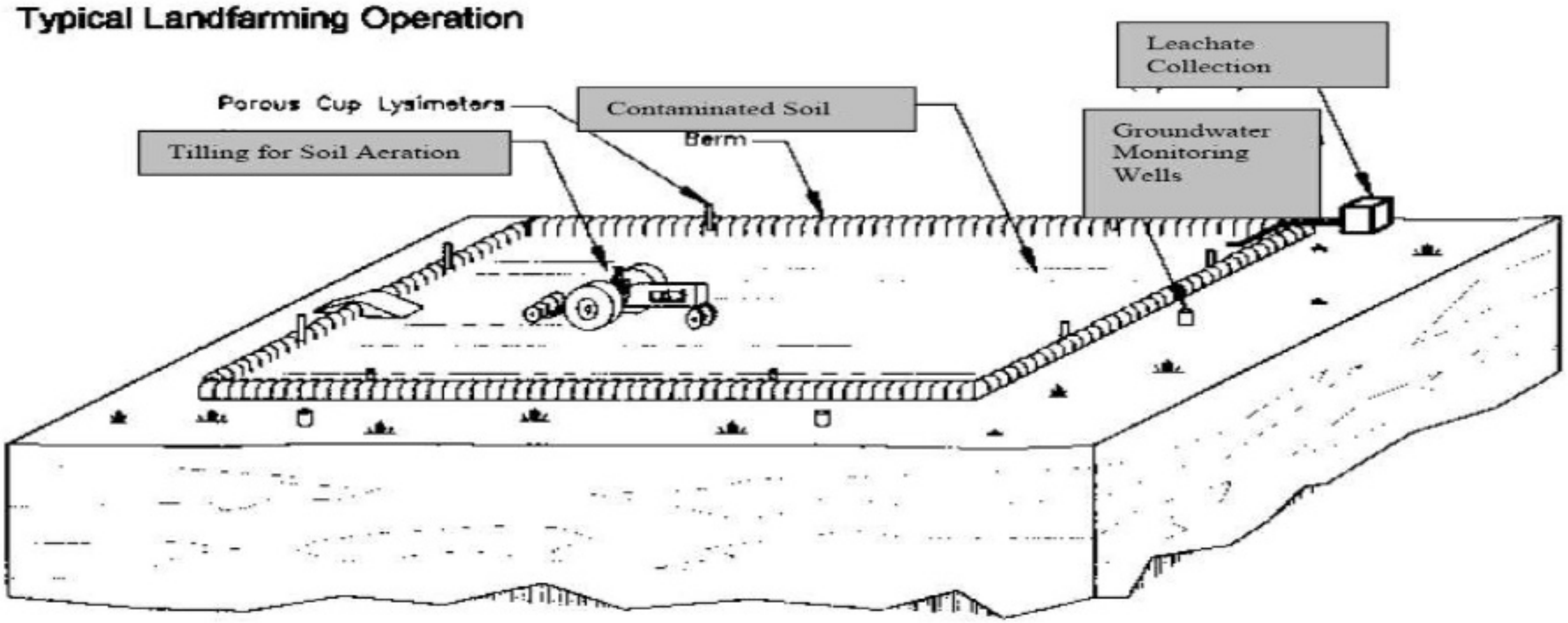
Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater.

Ex Situ Bioremediation

- which is when contaminated land are taken out of the area to be cleaned up by the organisms.
- This type of bioremediation is generally used only when the site is threatened for some reason, usually by the spill that needs to be cleaned up.
- Ex situ bioremediation is only used when necessary because it's expensive and damaging to the area, since the contaminated land is physically removed.

Land farming Operation

Land farming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The practice is limited to the treatment of superficial 10–35 cm of soil.

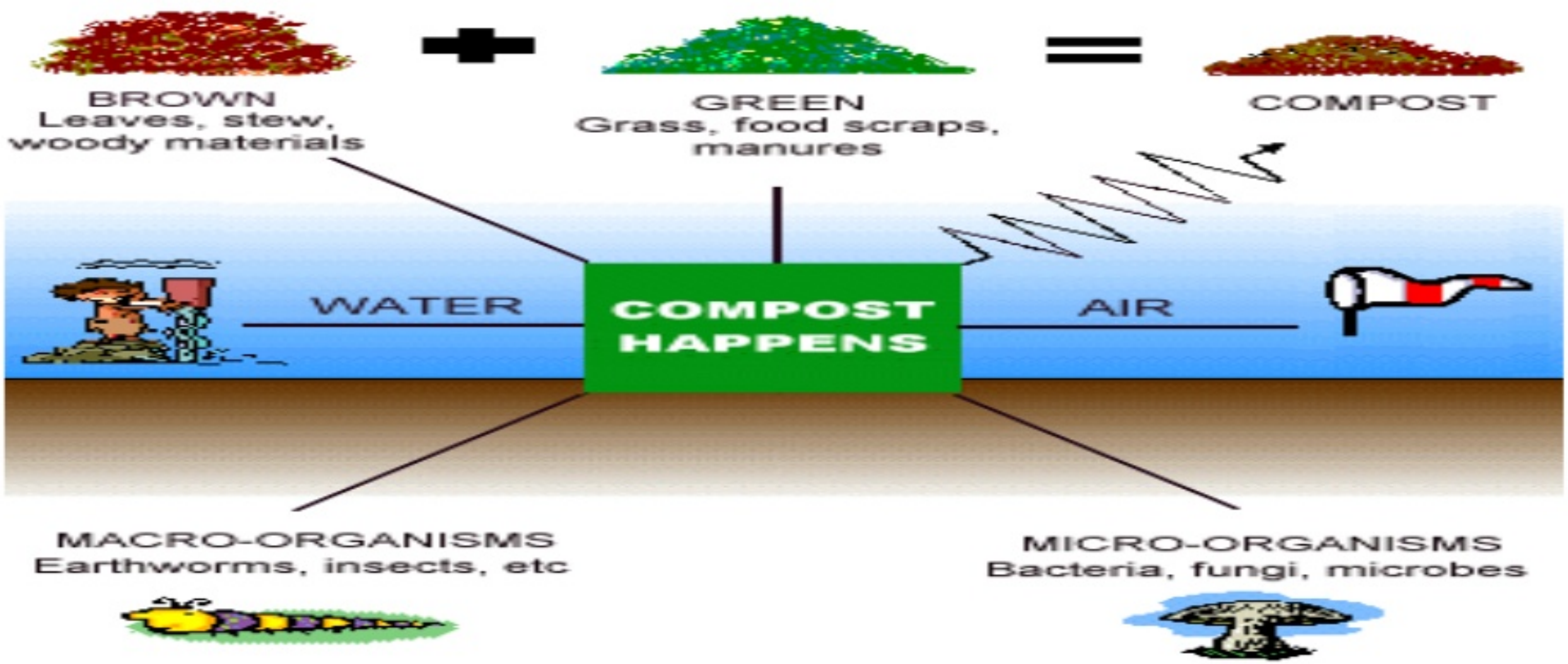


(Rittmann, B.E and McCarty, P.L, 2001)

Solid phase system Ex Situ Bioremediation

Composting is a technique that involves combining contaminated soil with organic compounds such as agricultural wastes.

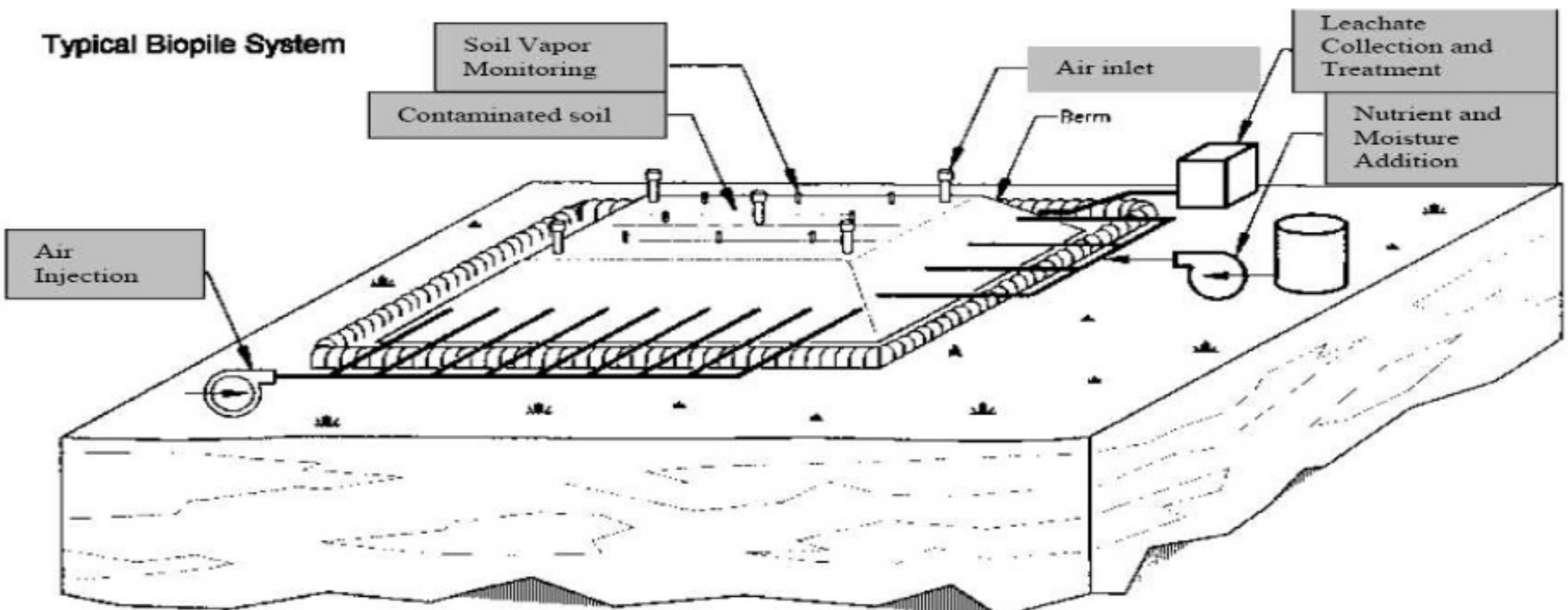
The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting.



(Source: https://www.google.co.in/search?q=bioremediation+images)

Biopile System

Biopiles are a hybrid of land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms.



(Rittmann,B.E and McCarty,P.L.2001)

- ***Bioreactors***-Slurry reactors or aqueous reactors are used for *ex situ* treatment of contaminated soil and water pumped up from a contaminated plume.
- Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system.

Cleanup Sites and Strategies

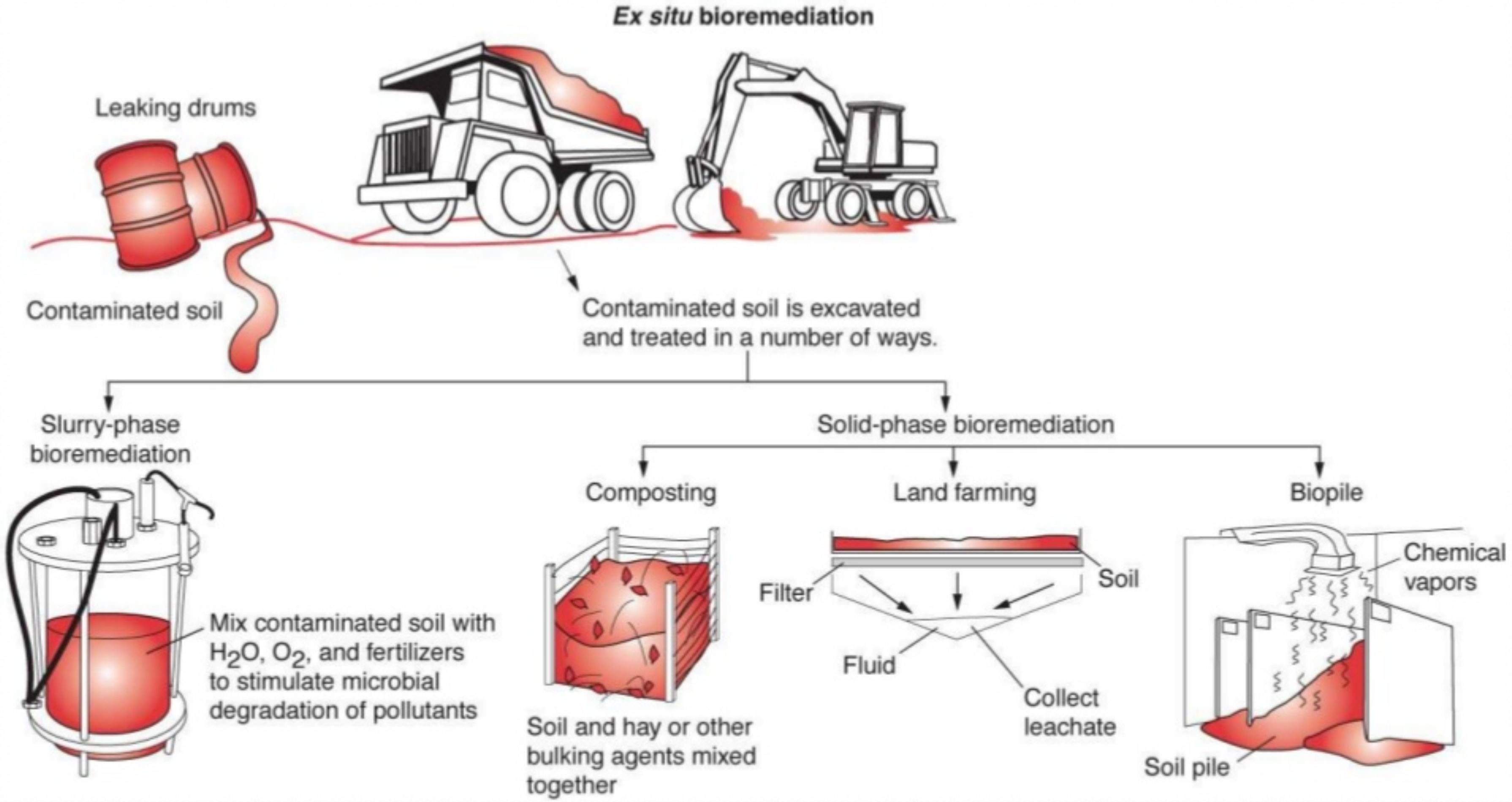


Table 4 Summary of bioremediation strategies.

Technology	Examples	Benefits	Limitations	Factors to consider
In situ	*In situ* bioremediation Biosparging Bioventing Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation processes Treats soil and water	Environmental constraints Extended treatment time Monitoring difficulties	Biodegradative abilities of indigenous microorganisms Presence of metals and other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants
Ex situ	Landfarming Composting Biopiles	Cost efficient Low cost Can be done on site	Space requirements Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation	See above
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactants	Soil requires excavation Relatively high cost capital Relatively high operating cost	See above Bioaugmentation Toxicity of amendments Toxic concentrations of contaminants

Advantages and Disadvantages

Advantages of bioremediation

- Bioremediation is a natural process and is therefore perceived by the public
- Bioremediation is useful for the complete destruction of a wide variety of contaminants.
- Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.

Adv

- Bioremediation can often be carried out on site, often without causing a major disruption of normal activities.

- Bioremediation can prove less expensive than other technologies that are used for cleanup of hazardous waste

Advantages and Disadvantages

Disadvantages of bioremediation

- Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.

- There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.

EXTRACTELLULAR ENZYMES IN BIOREMEDIATION:

- Extracellular enzymes refer to those enzymes that are either secreted by the microbes, such as white rot fungi or those that enter the aqueous phase during an aerobic submerged fermentation process.
- Such enzymes are naturally produced by the microbes and then harvested.
- Enzymes from white rot fungi have been shown to be effective degraders of TNT, phenols, PCBs, PAHs and dyes.

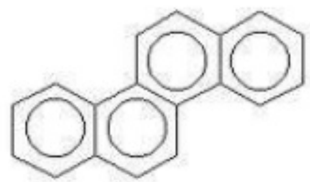
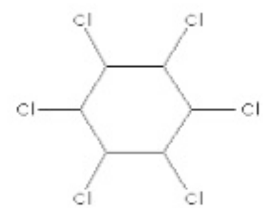
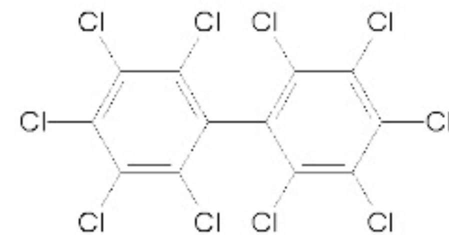
		
Chrysene(PAHs)	**Lindane(pesticide)**	**Polychlorinated biphenyls(PCBs)**

Figure 3: Some xenobiotics amenable to enzymatic bioremediation