MICROBIAL BIOTECHNOLOGY/ BIOREMEDIATION

Dr. Kiran Shahzadi
Assistant Professor
Department of Botany
LCWU, LHR

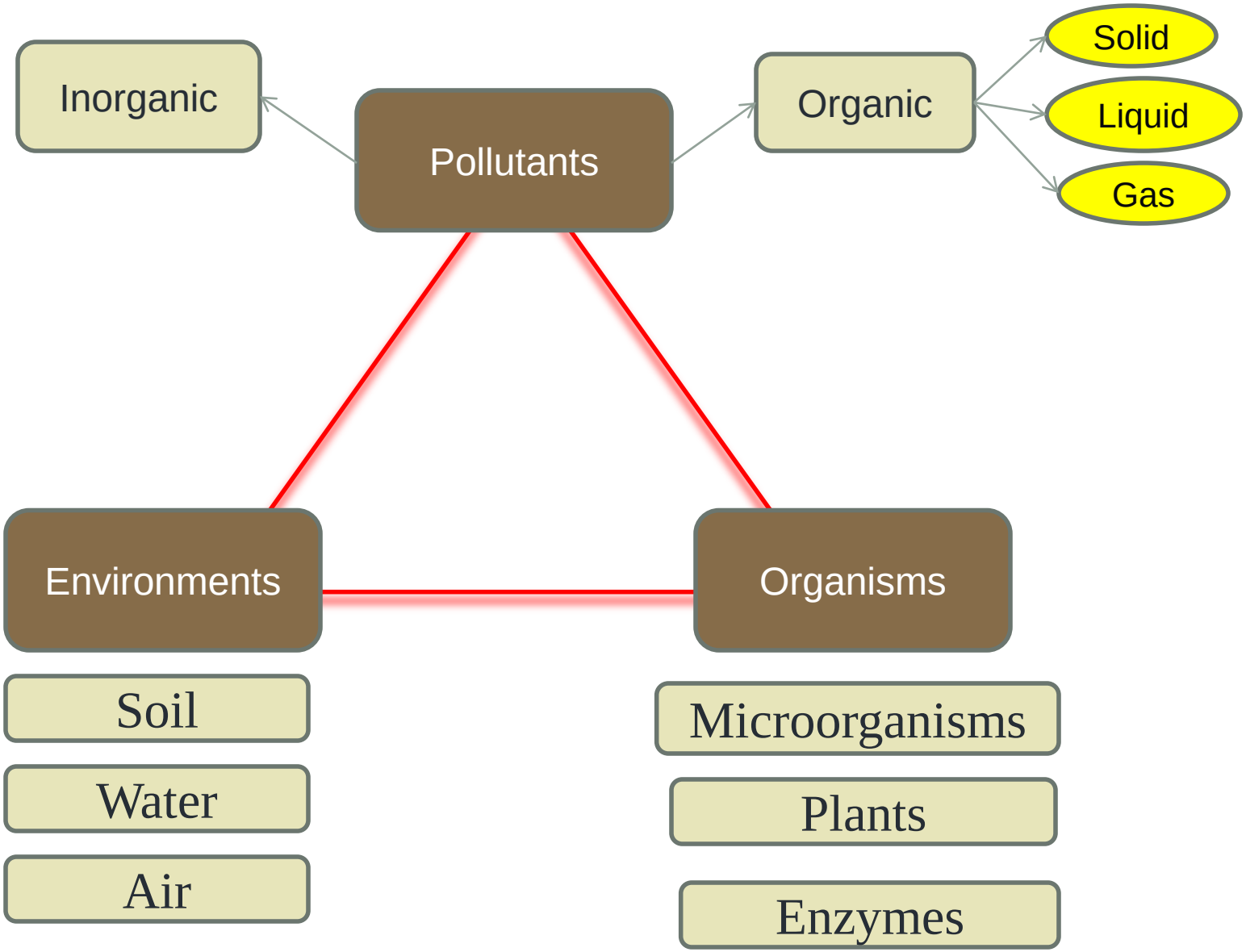
BIOREMEDIATION

- Bioremediation refers to the process of using microorganisms to remove the environmental pollutants or prevent pollution.
- The removal of organic wastes by microbes for environmental clean-up is the essence of bioremediation.
- The other names used for bioremediation are biotreatment, bioreclamation and biorestoration.
- **Xenobiotics** broadly refer to the unnatural, foreign and synthetic chemicals such as pesticide, herbicide & other organic compounds.

TYPES OF BIOREMEDIATION :

1. Biostimulation
2. Bioaugmentation
3. Intrinsic bioremediation

BIOREMEDIATION IS A TRIPLE-CORNERS PROCESS



REDOX CLEAN-UP REACTIONS

- Anaerobic or aerobic metabolism involve oxidation and reduction reactions or Redox reactions for detoxification.
- Oxygen could be reduced to water and oxidize organic compounds. Anaerobic reaction can use nitrate.
- In return, biomass is gained for bacterial or fungal growth.
- In many cases, combined efforts are needed, indigenous microbes found naturally in polluted sites are useful.

USE OF BACTERIA IN BIOREMEDIATION

- Greatly affected by unstable climatic and environmental factors from moisture to temperature.
- For examples, pH in soil is slightly acidic; petroleum hydrocarbon degrading bacteria do not work well < 10º C.
- These microbes are usually **thermophilic anaerobic**.
- Fertilizers are needed. Seeding or bioaugmentation could be useful too.
- They contain monooxygenases and dehydrogenases to break down organic matters including most toxic substances.

PSEUDOMONAS

- Genetically engineered bacteria (*Pseudomonas*) with plasmid producing enzymes to degrade octane and many different organic compounds from crude oil.

A selected list of genetically engineered microorganisms

GEMs	XENOBIOTICS
Pseudomonas putida	Mono-and dichloro aromatic compounds
P.diminuta	Parathion
P.oleovorans	Alkane
P.cepacia	2,4,5-Trichlorophenol
Acinetobacter species	4-Chlorobenzene
Alcaligenes species	2,4-Dichlorophenoxy acetic acid

USE OF FUNGI IN BIOREMEDIATION

- *Candida* can degrade formaldehyde.
- *Gibeberella* can degrade cyanide.
- Slurry-phase bioremediation is useful too but only for small amounts of contaminated soil.
- Composting can be used to degrade household wastes.

WHITE ROT FUNGI

- White rot fungi can degrade organic pollutants in soil and effluent and decolorize kraft black liquor, e.g. *Phanerochaete chrysosporium* can produce aromatic mixtures with its lignolytic system.
- Pentachlorophenol, dichlorodiphenyltrichloroethane (e.g. DDT), even TNT (trinitrotoluene) can be degraded by white rot fungi.

ENVIRONMENTAL CLEAN-UP PROCESS

- The basis of removal and transportation of wastes for treatment, basically there are two methods.

1.Insitu bioremediation

2.Ex situ bioremediation

➢ **INSITU BIOREMEDIATION**:

* It involves direct approach for the microbial degradation of xenobiotics at the sites of pollution (soil, ground water).

* It has been successfully applied for clan-up oil spillages, beaches etc.

* **There are 2 types of insitu bioremediation** ,

‡ Intrinsic bioremediation

‡ Engineered bioremediation

➢EXSITU BIOREMEDIATION

* The waste or toxic materials can be collected from the polluted sites and the bioremediation with the requisite microorganisms can be carried out at designed places.

METABOLIC EFFECT OF MO'S ON XENOBIOTICS:

¤ Detoxification ¤ Activation

¤ Degradation ¤ Conjugation

TYPES OF REACTIONS IN BIOREMEDIATION:

» Aerobic bioremediation

» Anaerobic bioremediation

» Sequential bioremediation

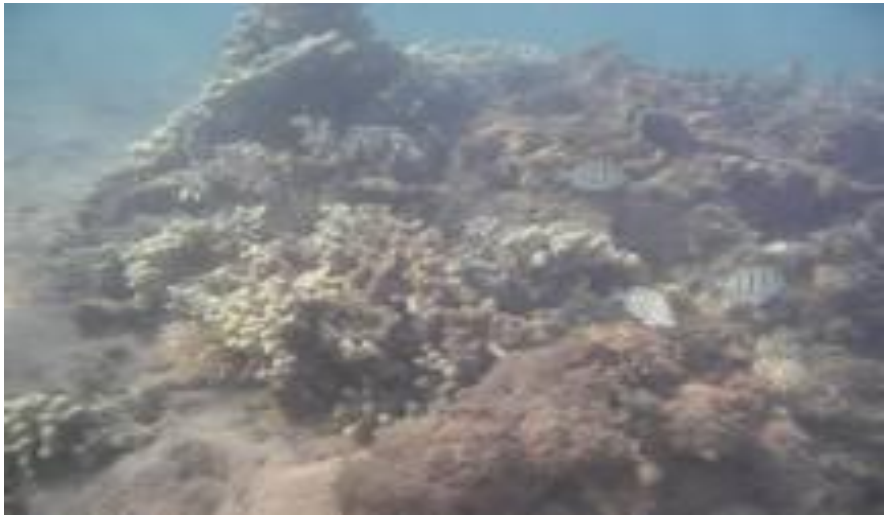
BIOREMEDIATION OF HYDROCARBONS

- Petroleum and its products are hydrocarbons. Oil constitute a variety of hydrocarbons (xylanes, naphthalenes, octane's, camphor etc,).
- The pollutants can be degraded by a consortium of microorganisms, e.g *Pseudomoas*, *Corynebacterium*, *Arthrobacter*, *Mycobacterium* and *Nocardia*.
- Genetically engineered bacterial strains are used to enhance bioremediation.
- In 1979, Anand Mohan Chakrabarty - obtained a strain of *Pseudomonas putida* that contained the XYL and NAH plasmid as well as a hybrid plasmid derived by recombinating parts of CAM and OCT.
- In 1990, the USA Govt allowed him to use this superbug for cleaning up of an oil spill in water of State of Texas. It was produced on a large scale in laboratory, mixed with straw and dried.

MARINE POLLUTION

- Sewage from cities
- Industrial effluents
- Oil slick
- Pollution from warships
- Military activities

MARINE POLLUTION

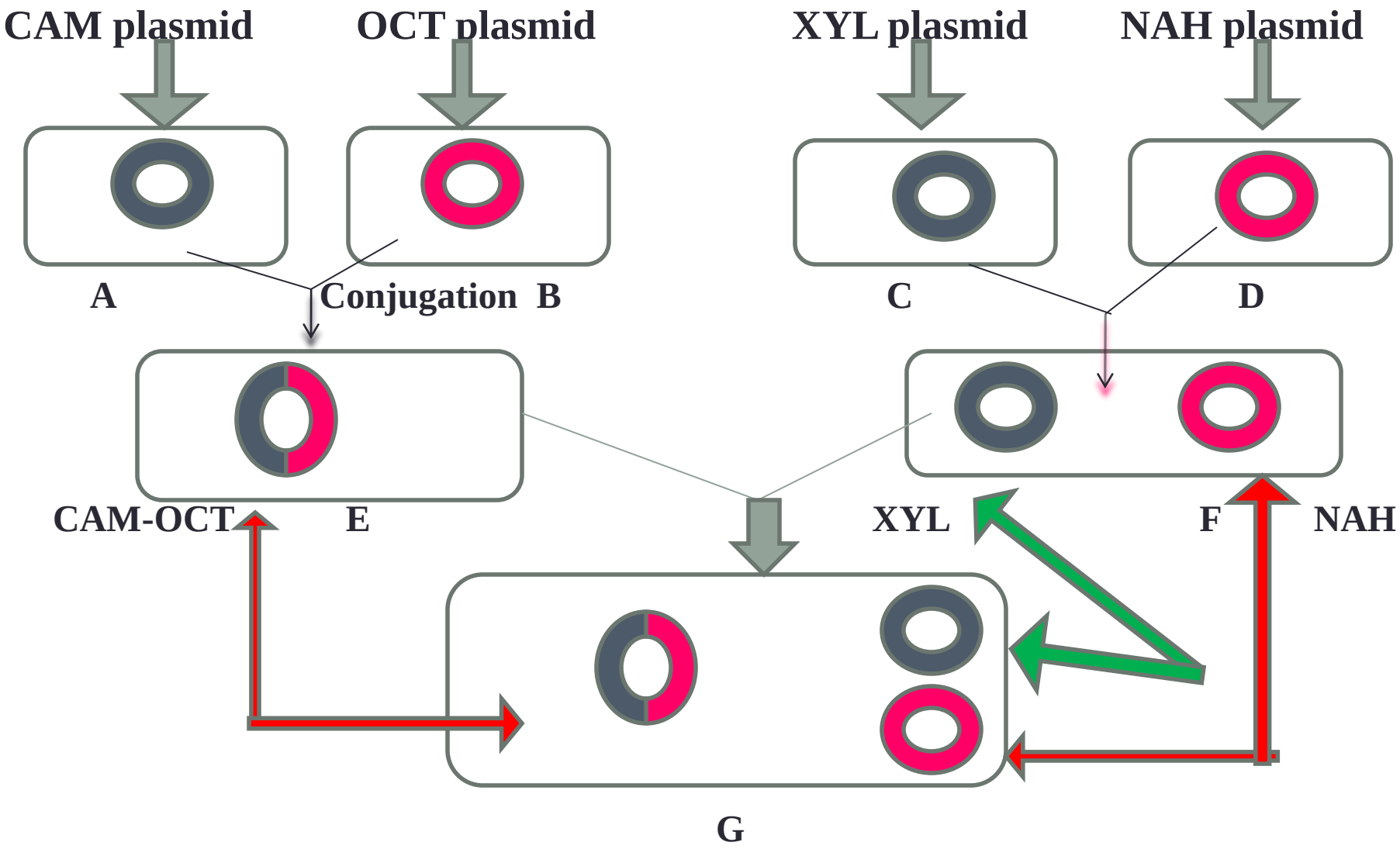


Oil spill

11 million gallon oil spill from the supertanker *EXXON VALDEZ*-ALASKA in March-1989.

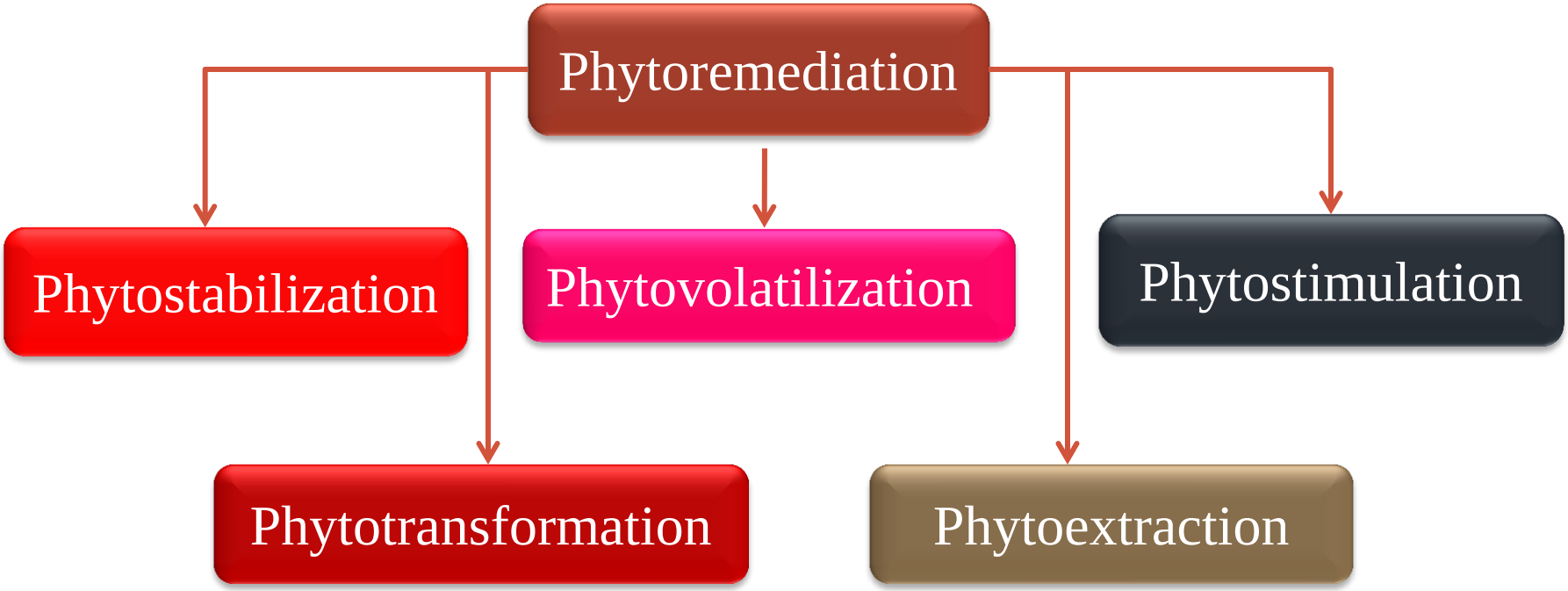
- straw was spread over oil slicks, the straw soaked up the oil and bacteria broke up the oil into non-polluting and harmless products.

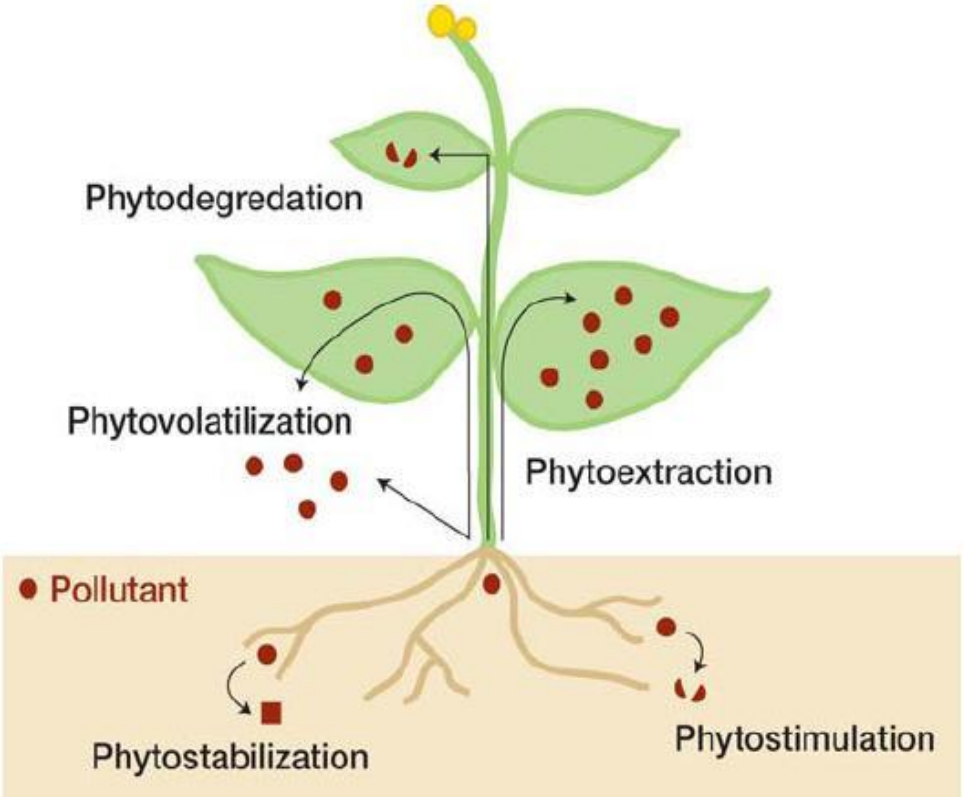
CREATION OF THE SUPERBUG BY TRANSFER OF PLASMIDS.



PHYTOREMEDIATION

- Phytoremediation is use of plants for accumulation, removal or conversion of pollutants.





- Approximately 400 plant species have been classified as hyperaccumulators of heavy metals, such as grasses, sunflower, corn, hemp, flax, alfalfa, tobacco, willow, Indian mustard, poplar, water hyacinth, etc.

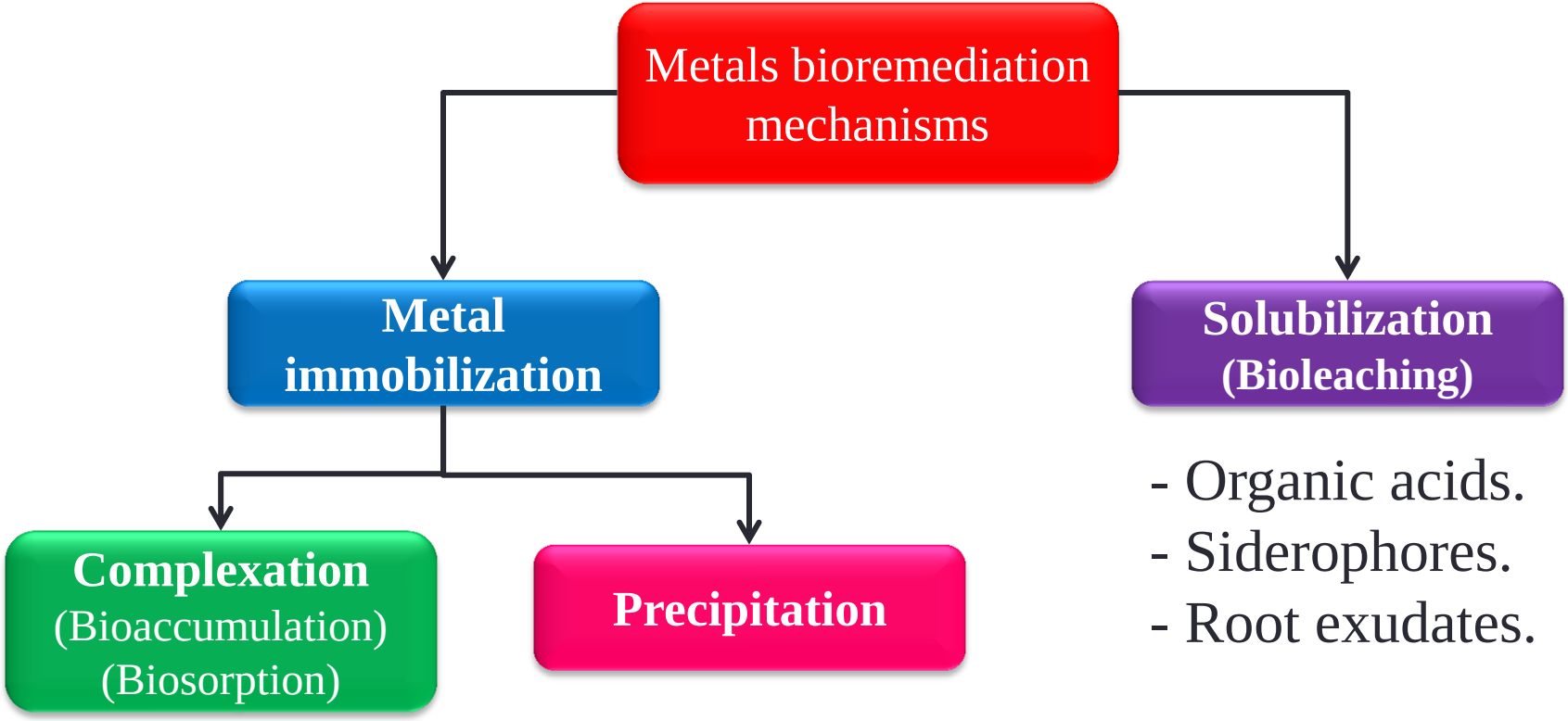
The root exudates of these plants play an important role in phytoremediation as it activate the surrounded microorganisms.

Genetic engineering are used as in case of BT protein or insect pheromones producing plants to reduce the use of pesticides.

PHYTO-REMEDIATION

- Effective and low cost , environmental friendly.
- Soil clean up of heavy metals and organic compounds.
- Pollutants are absorbed in roots, thus plants removed could be disposed or burned.
- Sunflower plants were used to remove cesium and strontium from ponds at the Chernobyl nuclear power plant.
- Transgenic plants with exogenous metallothionein (a metal binding protein) used to remove metals .

Bioremediation of metals contaminated environment:



- Organic acids.
- Siderophores.
- Root exudates.

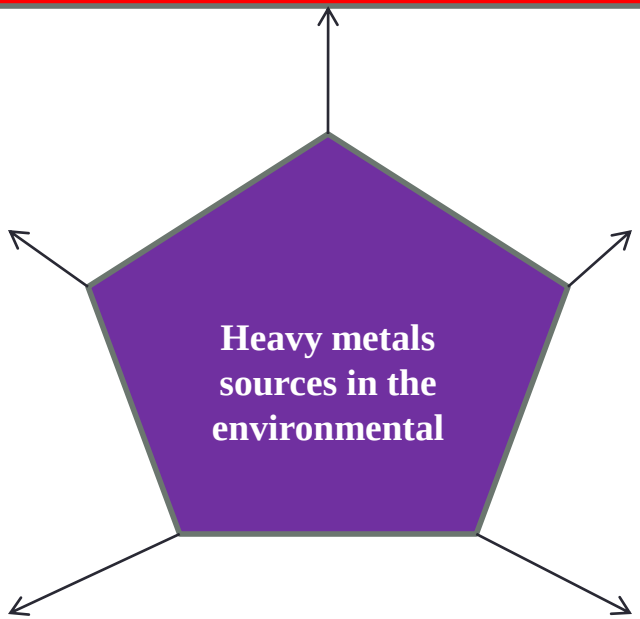
- Exopolysaccharide.
- Lipoproteins.

- H_2S producing bacteria
- Siderophores.
- Metal reduction.

SOURCES OF HEAVY METALS IN THE ENVIRONMET

Mining activities
smelting, river dredging,
mine spoils and tailings,
metal industries etc.

Industries
plastics, textiles,
microelectronics, wood
preservatives, refineries
etc.



Atmospheric deposition
urban refuse disposal,
hydrometallurgical
industries,
automobile exhausts,
fossil
fuel combustion etc.

Agrochemicals
Excessive use of fertilizers
and pesticides etc.

Waste disposal
sewage sludge leach ate
from landfill, fly ash disposal
etc.

BIOSURFACTANTS

- The biosurfactants are chemical compounds characterized by hydrophobic and hydrophilic (non-polar and polar) regions in one molecule (amphipathic molecules).
- Biosurfactants from bacteria, cyanobacteria, fungi and yeast are classified into:

1. Glycolipids.
2. Lipopeptides.
3. Phospholipids.
4. Glycoproteins.
5. Polymeric biosurfactants.

BIOSURFACTANTS PRODUCING GEM

- A genetically engineered *Pseudomonas aeruginosa*.
- This new strain can produce a **glycolipid emulsifier.**
- It can reduce the surface tension of an oil water interface. The reduced interfacial tension promotes biodegradation of oils.

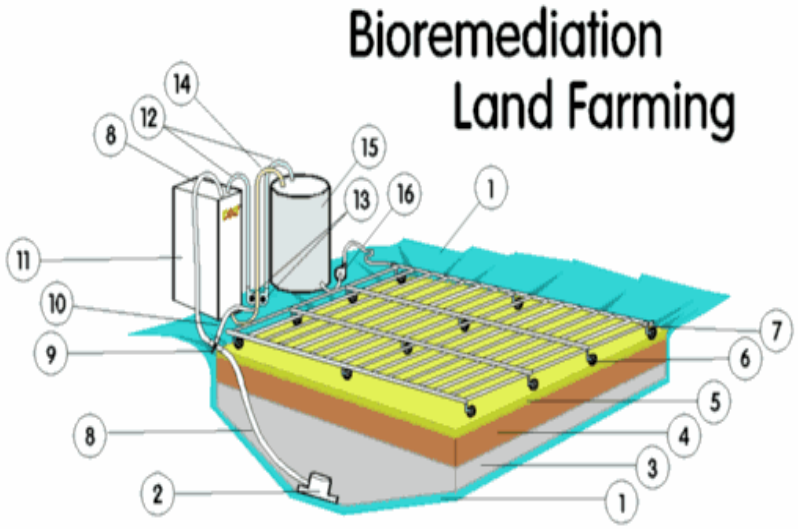
BIOREMEDIATION OF INDUSTRIAL WASTES

- A variety of pollutants are discharged in the environment from a large no of industries & mills.

1. Bioremediation of Dyes
2. Bioremediation in the paper and pulp industry

BIOREMEDIATION OF CONTAMINATED SOILS & WASTE LANDS

SLURRY-PHASE BIOREACTOR



LANDFARMING-BIOREMEDIATION

Table 4 Summary of bioremediation strategies.

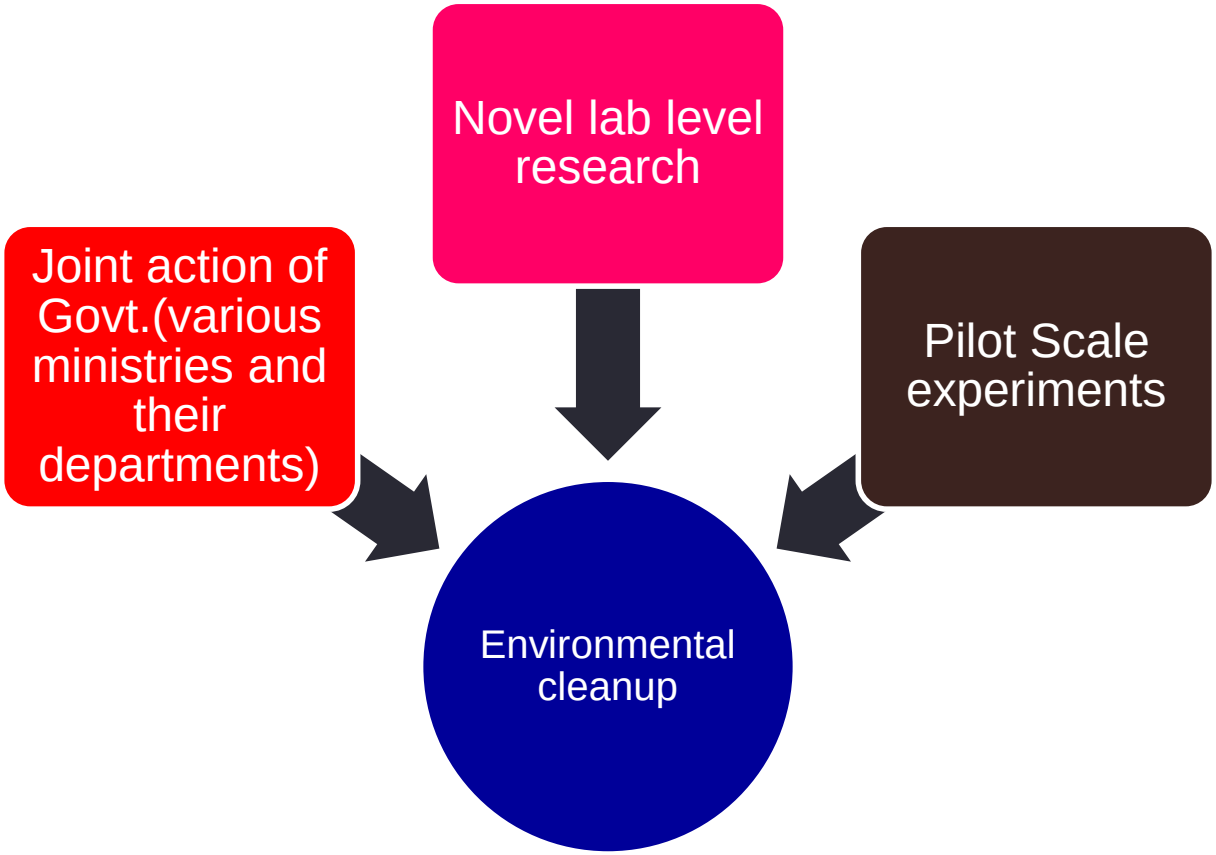
Technology	Examples	Benefits	Limitations	Factors to consider
In situ	*In situ* bioremediation Biosparging Bioventing Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation processes Treats soil and water	Environmental constraints Extended treatment time Monitoring difficulties	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

PREVENTION AND CONTROL OF POLLUTION

- These activities are supported by a set of legislative and regulatory promotional measures such as Policy Statement on Abatement of Pollution, 1992; and the National Environment Policy, 2006.

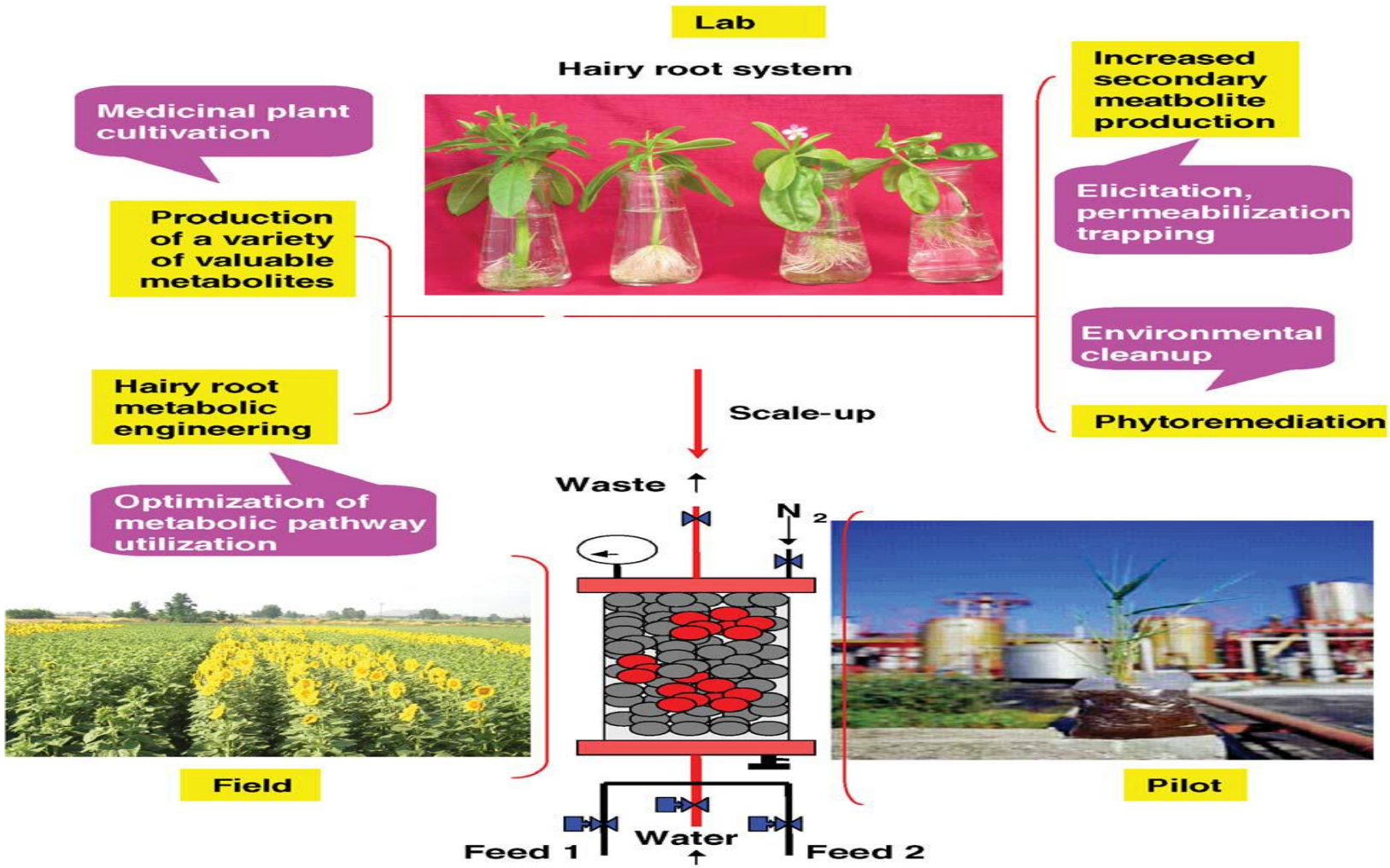
- The major actions on Abatement of Pollution and Environmental Cleanup are as follows: Clean Technology (CT), Control of Pollution (CP), Environment Education (EE), Environmental Impact Assessment (IA), Environmental Information (EI), Environmental Information System (ENVIS), Environment Research (RE), Forest Protection (FPR), Hazardous Substances Management (HSM) , Climate Change(CC) , Clean Development Mechanism (CDM).

- There is an immediate necessity for initiating an “All India Coordinated project on Bioremediation of the Contaminated Sites” involving premier institutions in this field for conducting large scale demonstration projects.
- Further, various departmental agencies and related ministries in India may plan a joint action plan to launch bioremediation from Lab-Pilot –Field scale projects at specific sites



Possible All India coordinated bioremediation demonstration projects at specifi c sites

BIOREMEDIATION FROM LAB-PILOT –FIELD SCALE



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.
- 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

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.
- Biological processes are often highly specific. microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- It is difficult to extrapolate (deduce) from bench and pilot-scale studies to fullscale field operations.
- Bioremediation often takes longer time than other treatment options.

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