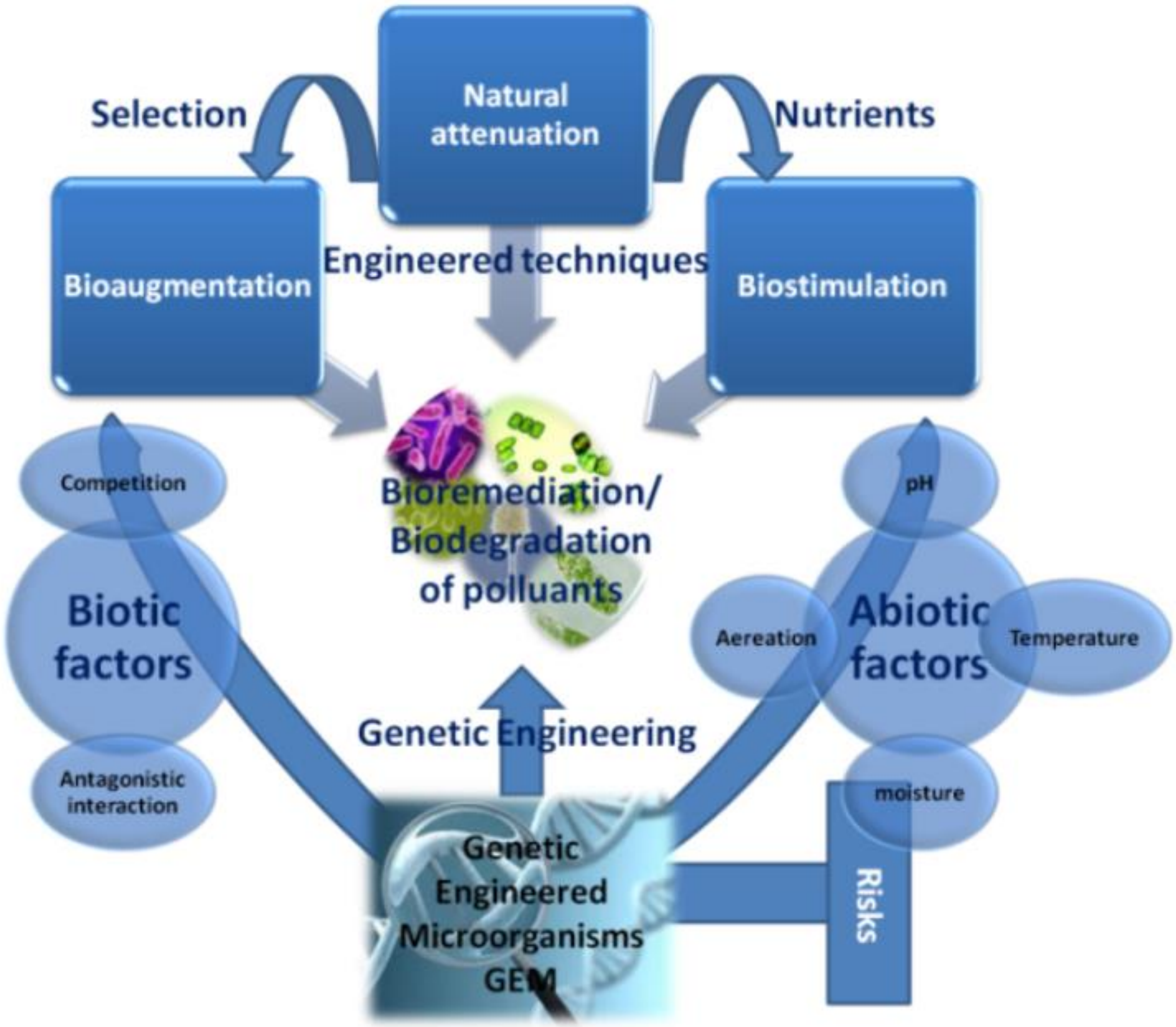



- Bioremediation of pollutants utilizing biodegradation abilities of microorganisms include the natural attenuation, although it may be enhanced by engineered techniques, either by addition of selected microorganisms (bioaugmentation) or by bio stimulation, where nutrients are added.
- Genetic engineering is also used to improve the biodegradation capabilities of microorganisms by GEM.
- Nevertheless, there are many factors affecting the efficiency of this process and risks associated to the use of GEM in the field.

**Degradation by genetically engineered microorganisms**

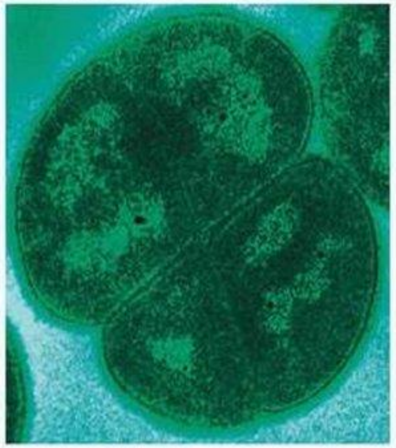
- A genetically engineered microorganism (GEM) or modified microorganism (GMM) is a microorganism whose genetic material has been altered using genetic engineering techniques inspired by natural genetic exchange between microorganisms
- Genetically engineered microorganisms (GEMs) have shown potential for bioremediation of soil, groundwater and activated sludge, exhibiting the enhanced degrading capabilities of a wide range of chemical contaminants.
- There are at least four principal approaches to GEM development for bioremediation application:
    1) Modification of enzyme specificity and affinity;
    2) Pathway construction and regulation;
    3) Bioprocess development, monitoring and control;
    4) Bio affinity bioreporter sensor applications for chemical sensing, toxicity reduction and end point analysis.

# *Engineering Deinocossus radiodurans*

## Introduction

*Deinococcus radiodurans* is an extremophilic bacterium, one of the most radiation-resistant organisms known. It can survive cold, dehydration, vacuum, acid and has been listed as the world's toughest bacterium in *The Guinness Book Of World Records*. Also, they are mesophiles.

**Deinococcus radiodurans**



A tetrad of *D. radiodurans*

*Gram-positive bacteria (* although its cell envelope is unusual and is reminiscent of the cell walls of Gram negative bacteria)
*Colonies- convex, smooth, pink to red in color (deinocrates - carotene)
*Size of cells- 1.5 to 3.5 µm.
*Do not form endospores, non-motile
*Obligate aerobic chemo-organo-heterotroph
* Habitat - rich in organic materials, such as soil, feces, meat, or sewage, but has also been isolated from dried foods, room dust, medical instruments and textiles

| Scientific classification | |
|---|---|
| Domain: | Bacteria |
| Kingdom: | Eubacteria |
| Phylum: | Deinococcus-Thermus |
| Class: | Deinococci |
| Order: | Deinococcales |
| Family: | Deinococcaceae |
| Genus: | *Deinococcus* |
| Species: | ***D. radiodurans*** |
| **Binomial name** | |
| ***Deinococcus radiodurans*** Brooks & Murray, 1981 | |

## Applications

- genetically engineered for use in bioremediation to consume and digest solvents and heavy metals, even in a highly radioactive site.
- For example, the bacterial mercuric reductase gene has been cloned from *Escherichia coli* into *Deinococcus* to detoxify the ionic mercury residue

## Mechanisms of Ionizing-radiation resistance

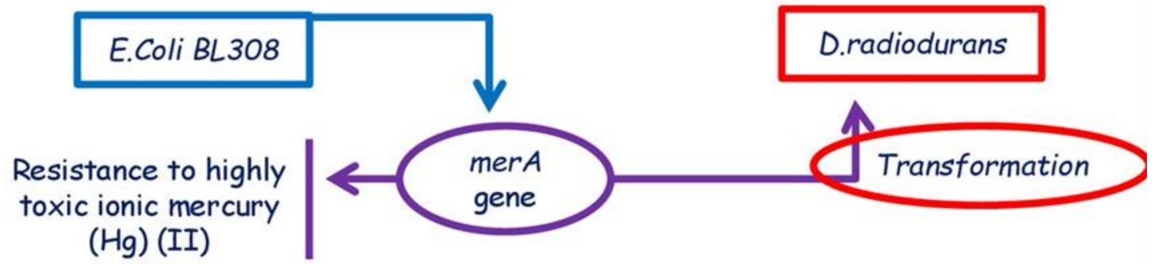
- resistance to radiation by having multiple copies of its genome and rapid DNA repair mechanisms.
- usually repairs in its chromosomes within 12–24 hours by a 2-step process.

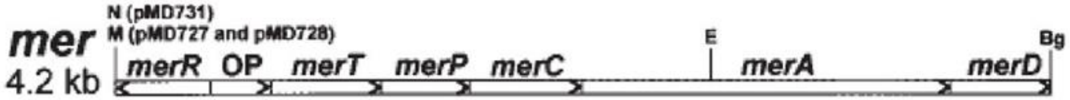
1.single-stranded annealing.
2. homologous recombination.

- tightly packed into toroids

# 1. Genetic methods: Transformation

Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments

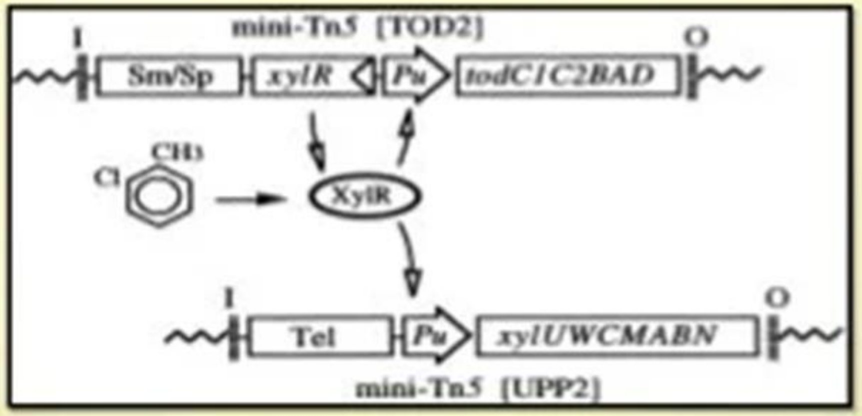



4.2-kb *mer* operon of pBD724 encodes six proteins: MerR, activation/repression of the *mer* operon; MerT, mercuric ion transport protein; MerP, periplasmic mercuric ion binding protein; MerC, transmembrane protein; MerA, mercuric reductase; and MerD, putative secondary regulatory protein. OP, operator/promoter sequence; M, *Mfe*I; N, *Nco*I; E, *Eco*RI; Bg, *Bgl*II.

## Conti.........

- ***Deinococcus radiodurans*****,** which naturally reduces Cr(VI) to the less mobile and less toxic **Cr(III),** was engineered for complete toluene degradation by cloned expression of ***tod*** and ***xyl*** genes of *Pseudomonas putida*.




- http://ars.els-cdn.com/content/image/1-s2.0-S0168165600003679-gr3.gif

## Drawback

- The major problem encountered in successful bioremediation technology pertains to hostile field conditions for the engineered microbes.
- Besides, the molecular applications are mainly confined to only few well characterized bacteria such as *E. coli*, *P. putida*, *B. subtilis*, etc.
- Other bacterial strains need to be tried for developing the engineered microbes.
- The main concern is to construct GE bacteria for field release in bioremediation with an adequate degree of environmental certainty.
- Efforts should be made to examine the performance of engineered bacteria in terms of their survival, potential of horizontal gene transfer, which may affect the indigenous microflora within a complex environmental situation.
- Often the novel scientific researches always give rise to still more fascinating questions pertaining to public concern.
- In the majority cases, the bacteria designed for bioremediation processes have been designed for specific purpose under the laboratory conditions, ignoring the field requirement and other complex situations.
- However, there is no evidence that the deliberate release of GE bacteria for bioremediation has caused a measurable adverse impact on the natural microbial community.

# Bioleaching

- Bioleaching (or biomining) is a process in mining and biohydrometallurgy (natural processes of interactions between microbes and minerals) that extracts valuable metals from a low-grade ore with the help of microorganisms such as bacteria or archaea.
- Metals can be extracted from large quantities of low grade ores This process has been used for centuries to recover copper form the drainage water of mines and the role of microbes in the bioleaching came into notice some 40 years ago.
- At present bioleaching is used essentially for the recovery of copper, uranium and gold, and the main techniques employed are **heap, dump and in situ leaching**. Tank leaching is practiced for the treatment of refractory gold ores.
- Bioleaching can involve numerous ferrous iron and sulfur oxidizing bacteria, including ***Acidithiobacillus ferrooxidans*** **(formerly known as** ***Thiobacillus ferrooxidans*****) and** ***Acidithiobacillus thiooxidans*** **(formerly known as** ***Thiobacillus thiooxidans*****)**.

- At the present time bioleaching processes are based more or less exclusively on the activity of *T. ferrooxidans*, *L. ferrooxidans* and *T. thiooxidans* which convert heavily soluble metal sulfides via biochemical oxidation reactions into water-soluble metal sulfates.

- A consortium of microorganisms namely *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Leptospirillum ferrooxidans, Sulpholobus spp*. and thermophilic bacteria including *Sulpholobus thermosulphidoxidans and Sulpholobus brierleyi a*re known to be involved in bioleaching. Anaerobes would also be found in leaching area.

- The most important player in the bioleaching process is *Acidithiobacillus ferrooxidans*.

- It is a chemoautotrophic acidophile, meaning that it obtains its energy from inorganic sources and fixes its own carbon while growing in an acidic medium.

- Its unique ability to oxidise ferrous to ferric, and sulphur and reduced sulphur compounds to sulphuric acid, leads to leaching of metals from their oxide and sulphide ores.

- These properties make it most attractive for commercial leaching operations. Due to its specific attributes, *Acidithiobacillus ferrooxidans* has been the most studied of these chemoautotrophic microorgansims.

# Bioleaching Process

## 1. The Inputs of Bioleaching:

- Metal ore or concentrate **to provide energy** for microbes.
- **Proper air** is supplied based on whether they are aerobic or anaerobic.
- $CO_2$, because bioleaching microbes need the macro-nutrient **C; N, P, Ka, Mg** nutrients needed for bioleaching microbes.
- **pH control** is needed, optimum: 2.3-2.5.
- Bioleaching microbes cultivation for inoculation.
- Temperature control mechanisms, optimum: $30^oC$- $50^oC$.
- Distribution system, stirring, sprinklers, airflow, tubes allowing for the circulation of microbes.
- Reaction catalysts if needed.

# General Leaching Process

Ores

↓

Bioleaching
(*T.ferrooxidans*)

↓

Extracted Metals
(Copper, Silver, gold,
uranium,silica,zinc,lead)

↓

Electroplating

↓

Pure Metals

# Mechanism involved in bioleaching

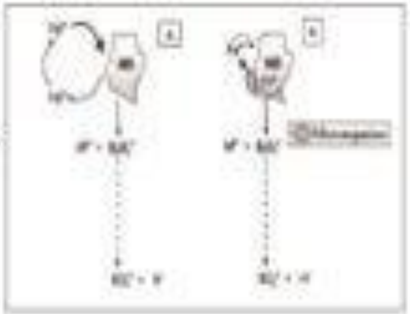
Two processes are used in bioleaching:

- Direct bioleaching

In direct bioleaching minerals which are susceptible to oxidation undergoes direct enzymatic attack by the microorganisms.

- Indirect bioleaching

In indirect method of bioleaching of minerals bacteria produce strong oxidizing agent which reacts with metals and extract them from the ores.



A: In direct mechanism
B: Direct mechanism

# MECHANISM OF BIOLEACHING

1. **DIRECT BIOLEACHING:**

   In this bioleaching, bacteria directly oxidize minerals and solubilize metals.

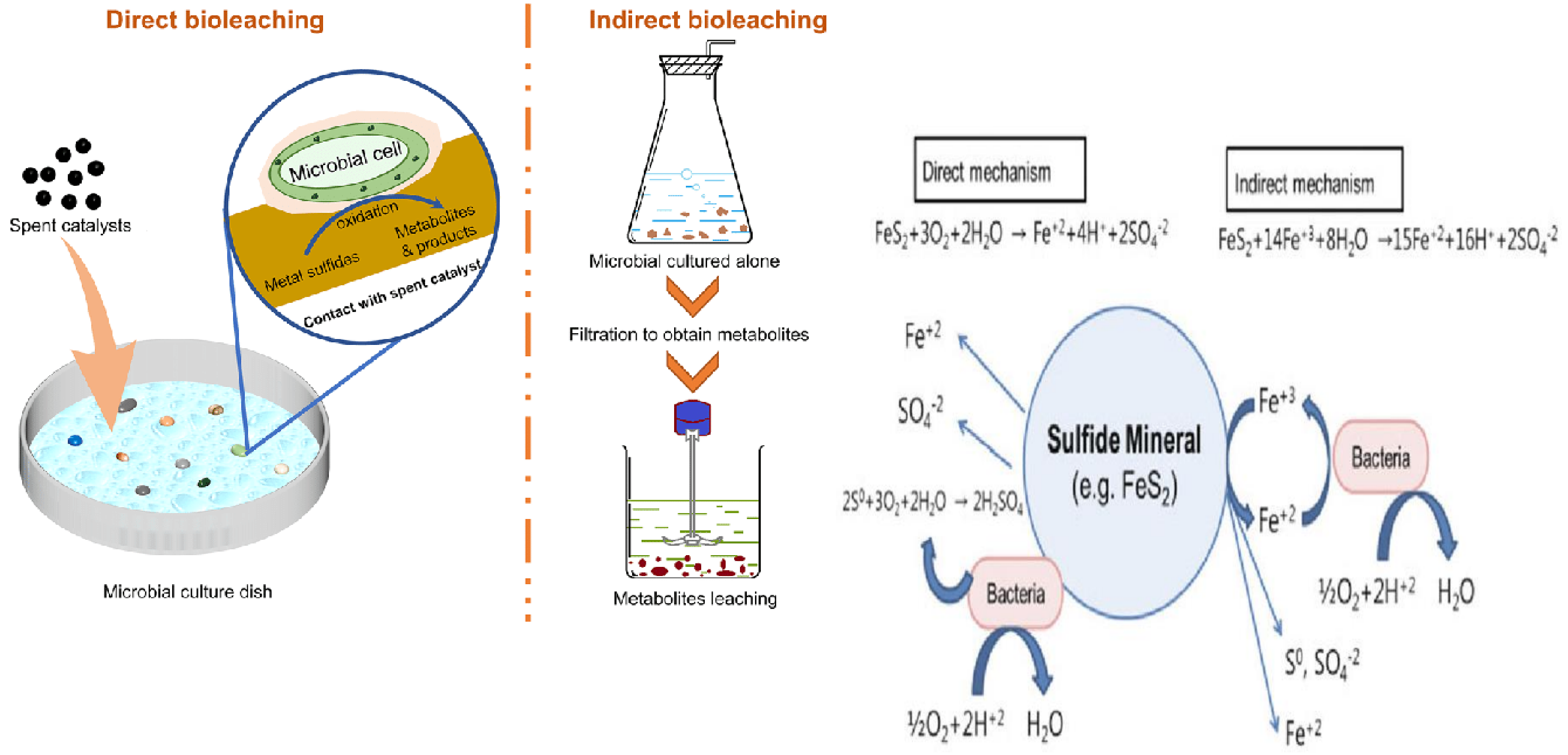
   In direct leaching , a physical contact exist between bacteria and ores and oxidation of minerals takes place through enzymatically catalysed steps.

   Example; pyrite is oxidised to ferric suphate

   $2FeS_2+7O_2+7H_2O \longrightarrow 2feSO_4+2H_2SO_4$

- Indirect bacterial leaching

  in this process the microbes are not in direct contact with minerals, but leaching agents are produced by these microbes which oxidize the ores.



Direct bioleaching
Spent catalysts
Microbial cell
oxidation
Metabolites & products
Metal sulfides
Contact with spent catalyst
Microbial culture dish
Indirect bioleaching
Microbial cultured alone
Filtration to obtain metabolites
Metabolites leaching
Direct mechanism
$FeS_2+3O_2+2H_2O \rightarrow Fe^{+2}+4H^{+}+2SO_4^{-2}$
Indirect mechanism
$FeS_2+14Fe^{+3}+8H_2O \rightarrow 15Fe^{+2}+16H^{+}+2SO_4^{-2}$
$Fe^{+2}$
$SO_4^{-2}$
$2S^0+3O_2+2H_2O \rightarrow 2H_2SO_4$
Sulfide Mineral
(e.g. $FeS_2$)
Bacteria
$½O_2+2H^{+2}$ $H_2O$
$Fe^{+3}$
$Fe^{+2}$
Bacteria
$½O_2+2H^{+2}$ $H_2O$
$S^0$, $SO_4^{-2}$
$Fe^{+2}$

**Table 1.** Factors that influence bioleaching

| Factors | Effects |
|---|---|
| **Nutrients** | Chemolithotrophic microbes are used for metal extraction from sulphide materials or sulphide-containing ores. These microorganisms require only inorganic materials for optimal growth. However, sulphur compounds, phosphate, iron compounds, magnesium salt and ammonium should be to achieve optimum microbial growth. The mineral nutrients required for microbial growth are usually sourced from the leaching environment and from the ore to be leached. |
| **Gases ($O_2$ and $CO_2$)** | Carbondioxide ($CO_2$) and oxygen ($O_2$) are both required for optimal growth of the bioleaching microbe. $CO_2$ is usually the only carbon source for the bioleaching bacteria; and $O_2$ which can be supplied via aeration, shaking or stirring at a laboratory scale is required for optimal growth of the organism. |
| **pH** | Correct pH is required for the optimal growth of the bioleaching bacteria. Bacterial oxidation of ferrous iron and sulphide ores occur at pH range of 2.0 –2.5. The growth of *Thiobacillus ferrooxidans* may be inhibited at a pH that is lower than 2.0 but the addition of acid to the medium or growth environment can as well support the growth of the organism. |
| **Temperature** | The extraction of metals from their ores occurs at optimal temperature levels (e.g. 28°C – 30°C) that are supportive to the growth of the bioleaching microbe. Thermophilic bacteria such as *Thiobacillus* species can also thrive at high temperature ranges of 50°C – 80°C. There is usually a decrease in metal extraction at lower temperature levels. However, the solubilization of some metals such as copper, zinc, nickel and cobalt can also occur at lower temperatures (e.g. 4°C). |
| **Mineral substrates** | The addition of some mineral substances such as carbonate and acids in the leaching environment encourage the optimal growth of the bioleaching bacteria. |
| **Heavy metals** | The presence of some heavy metals such as copper and nickel in the leaching environment favour the optimal growth of some bioleaching bacteria such as *Thiobacillus* species. It is not all bioleaching microbes that are tolerable to the presence of heavy metals in the leaching environment. Thus, increasing the tolerance of microbes to heavy metals in leaching environments through genetic manipulations is critical to the success rate of bioleaching activities. |
| **Organic extractants and surfactants** | The organic extractants and surfactants used in solvent extraction have growth inhibitory effect on the bioleaching bacteria. And this is usually due to the reduction in mass transfer of $O_2$ molecules as well as decrease in the surface tension of the leaching environment or medium. |

# Types

There are three commercial process use bioleaching;

a. Slope leaching
b. Heap leaching
c. In situ leaching

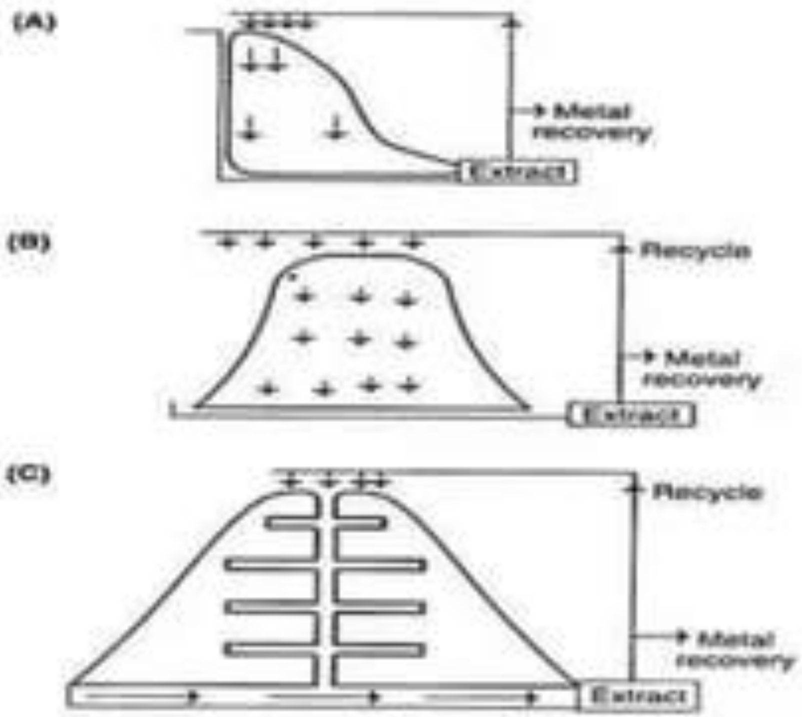
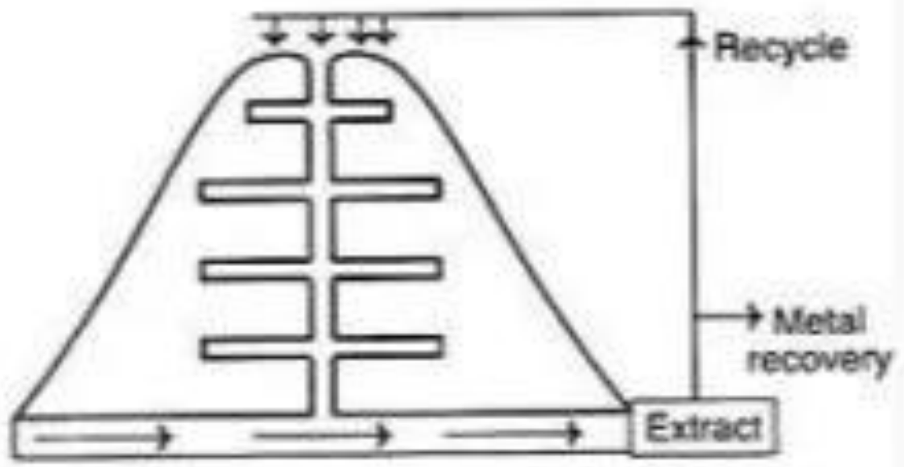



Fig. 32.2 : Commercial bioleaching processes (A) Slope leaching (B) Heap leaching (C) In situ leaching.

# In situ bioleaching

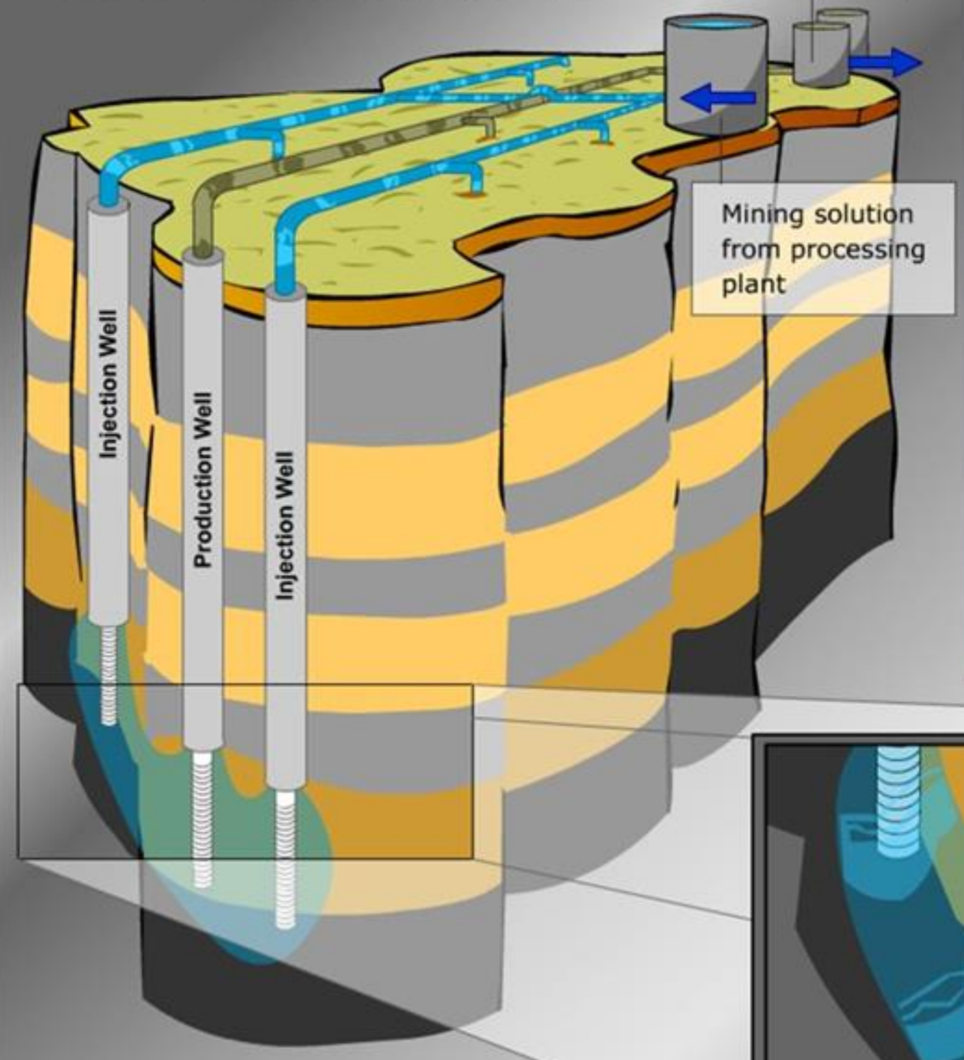
- In this process the ore remains in its original position in earth.
- Surface blasting of earth is done to increase the permeability of water.
- Water containing thiobacillus is pumped through drilled passages to the ores
- Acidic water seeps through the rock and collects at bottom

# In situ leaching




In situ leaching (ISL), also known as solution mining, or in situ recovery (ISR), involves leaving the ore where it is in the ground, and recovering the minerals from it by dissolving them and pumping the pregnant solution to the surface where the minerals can be recovered.

# IN SITU LEACHING



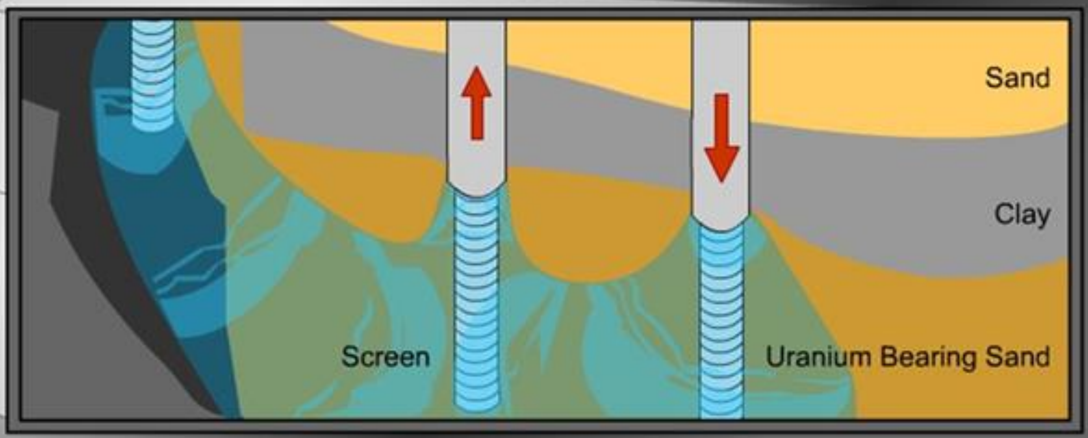

Bore holes are drilled from the surface into the orebody.

A leach solution of weak sulphuric acid and oxygen is pumped into the into orebody.

The leach solution dissolves the uranium in the orebody.

The uranium-bearing solution is pumped back to the surface for treatment.

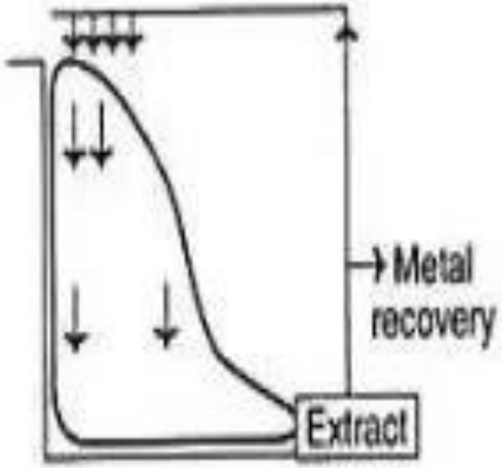
At the processing plant the uranium is separated from the leach solution and the solution is recycled.



## Slope leaching

- Here the ores are first ground to get fine pieces and then dumped into large leaching dump
- Water containing inoculum of thiobacillus is continuously sprinkled over the ore
- Water is collected from the bottom and used to extract metals and generate bacteria in an oxidation pond

## Slope leaching

- 

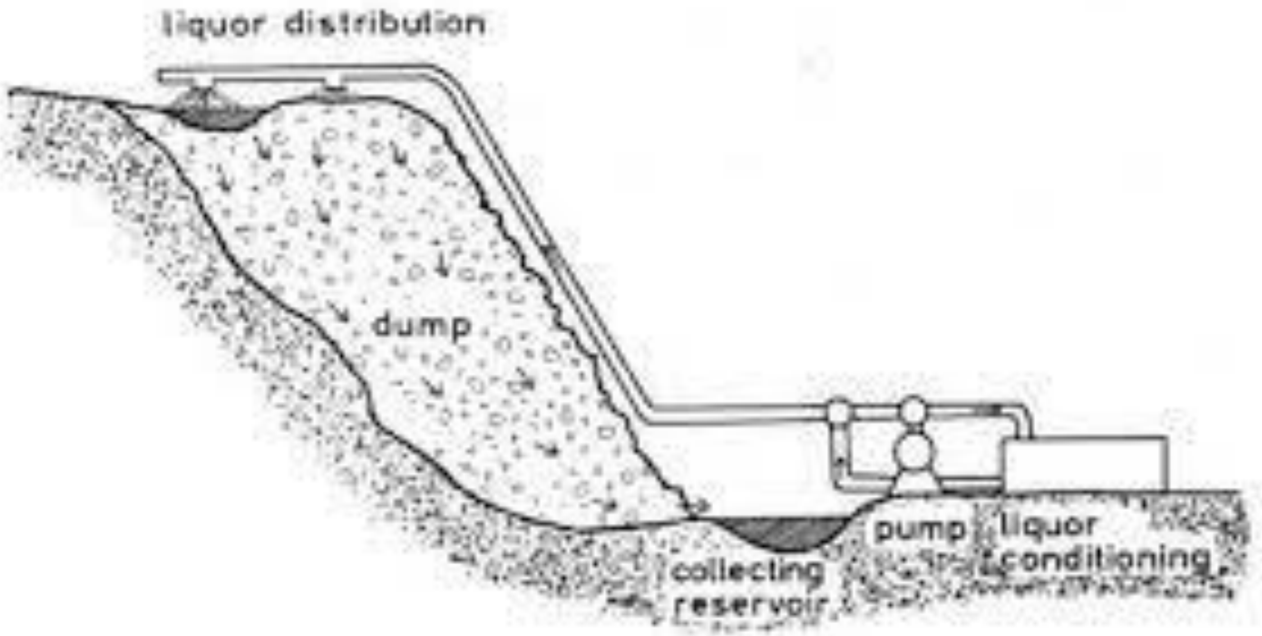
## •2.3.6.2 Heap and Dump Leaching

### A. Dump Leaching

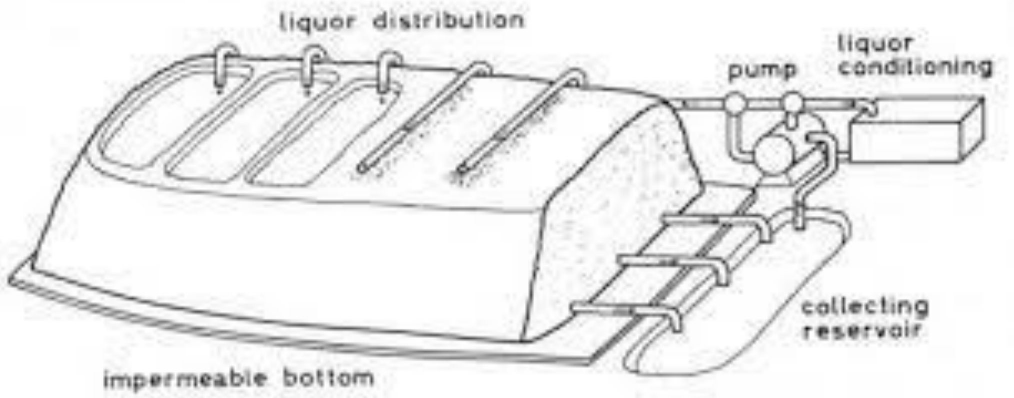
- Dump leaching is similar to heap leaching, however in the case of dump leaching ore is taken directly from the mine and stacked on the leach pad without crushing.

- In the case of gold and silver, the dump is irrigated with a dilute cyanide solution that percolates through the ore to dissolve gold and silver.

- The solution containing gold and silver exits the base of the dump, is collected and precious metals extracted. The resultant barren solution is recharged with additional cyanide and returned to the dump.




dump ore leaching on a slope




dump ore leaching

## B.Heap Leaching

- Heap leaching is, in contrast to many dump leaching operations, a pre-planned process where arrangements are made to optimize conditions for leaching.
- In brief, the mineral ore or concentrate is piled in a heap and lixiviate fluid is distributed over the surface to leach metal from the heap.
- Heap leaching is used to leach low-grade ores.
- Low-grade ores are broken and piled into small heaps on impervious ground/asphalted surface/concrete.

# Steps in Heap Leaching

1. The soil on a slightly sloping ground is first compacted and then covered with an impermeable pad like an asphalt layer or a flexible plastic sheet.
2. Crushed ore is stacked in big heaps on the pad. Fine particles are agglomerated to increase permeability.
3. The heap is sprayed with leaching reagent.
4. As the reagent percolates through the heap the wanted metals are solubilized.
5. The leachate (metal containing solution) drained from the heap is collected in a pond and the solution is subsequently sent for metal recovery.

## Summary operation of heap leaching

1. The construction of leaching pads
2. The formation of the heaps
3. The distribution of the lixiviants
4. The collection of the leach liquor in the solution pond
5. The recirculation of the barren solution to the heaps after the recovery of the metal values.

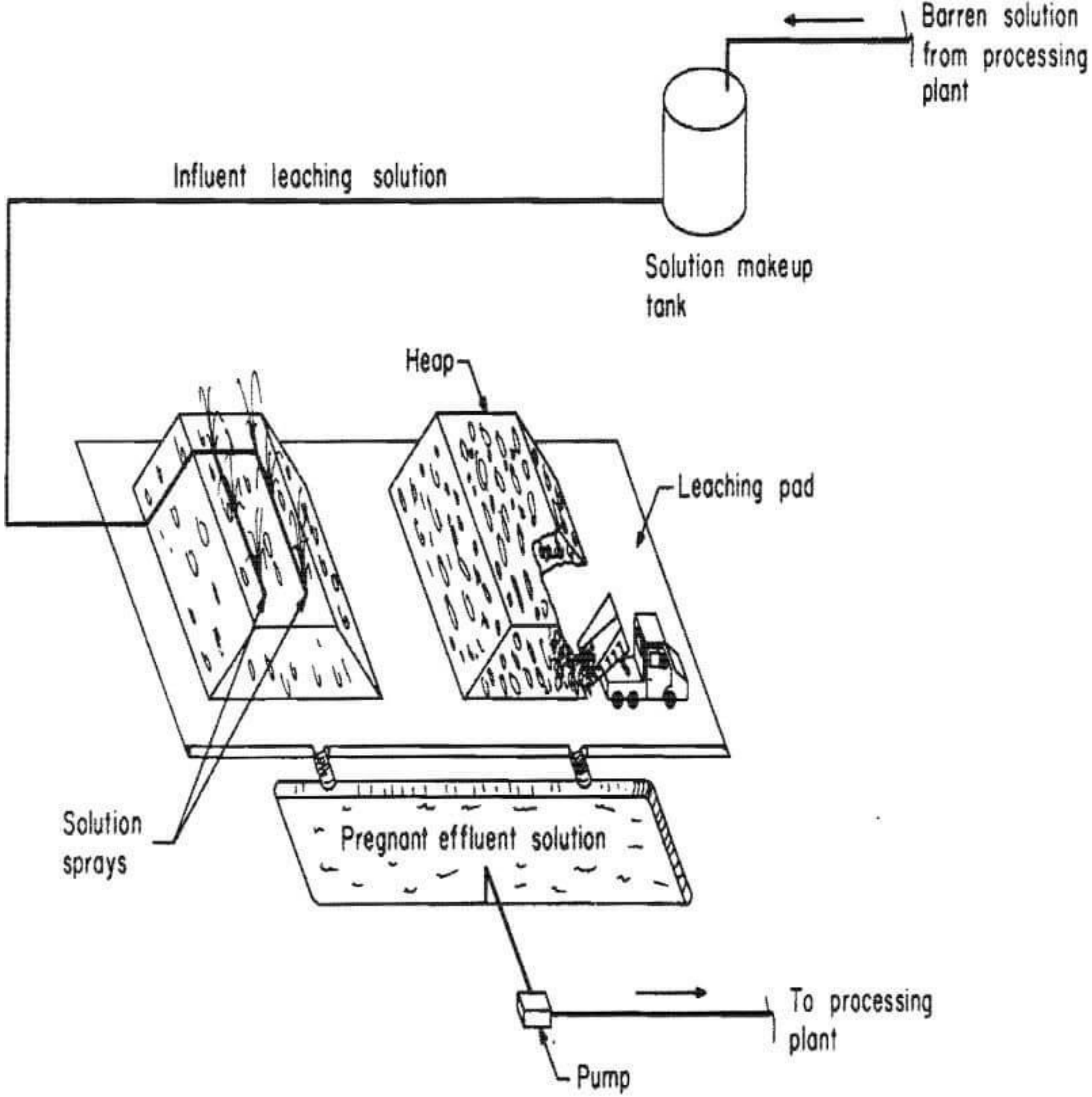
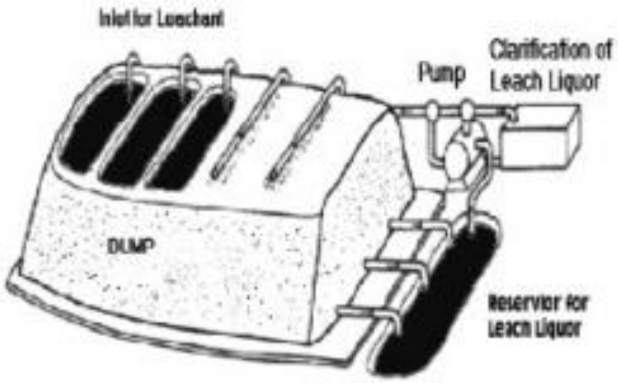
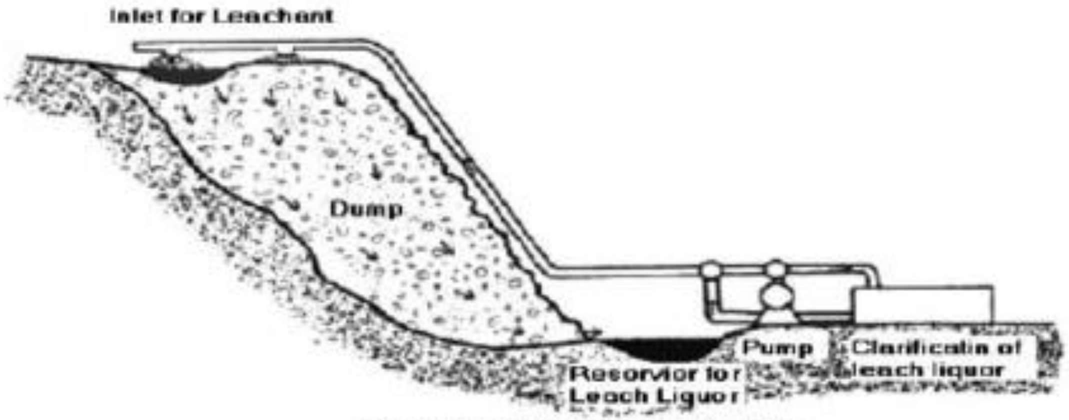


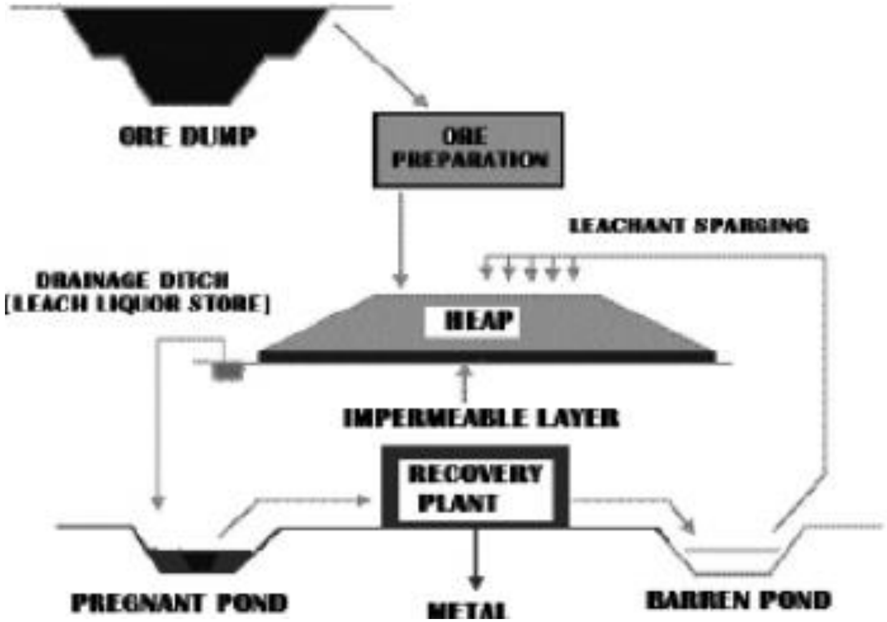

FIGURE 1. - Heap leaching system.

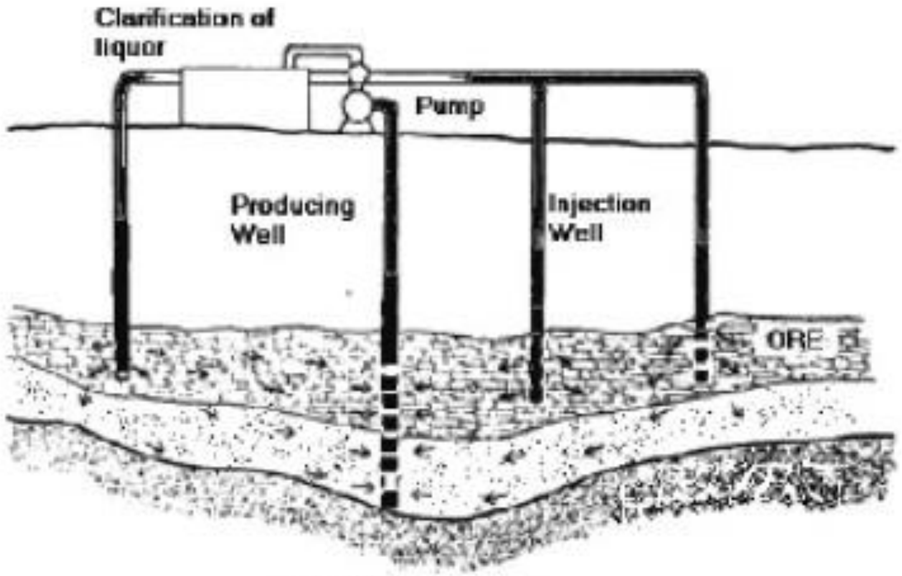



Dump Leaching- on ore bed




Dump Leaching- on slope

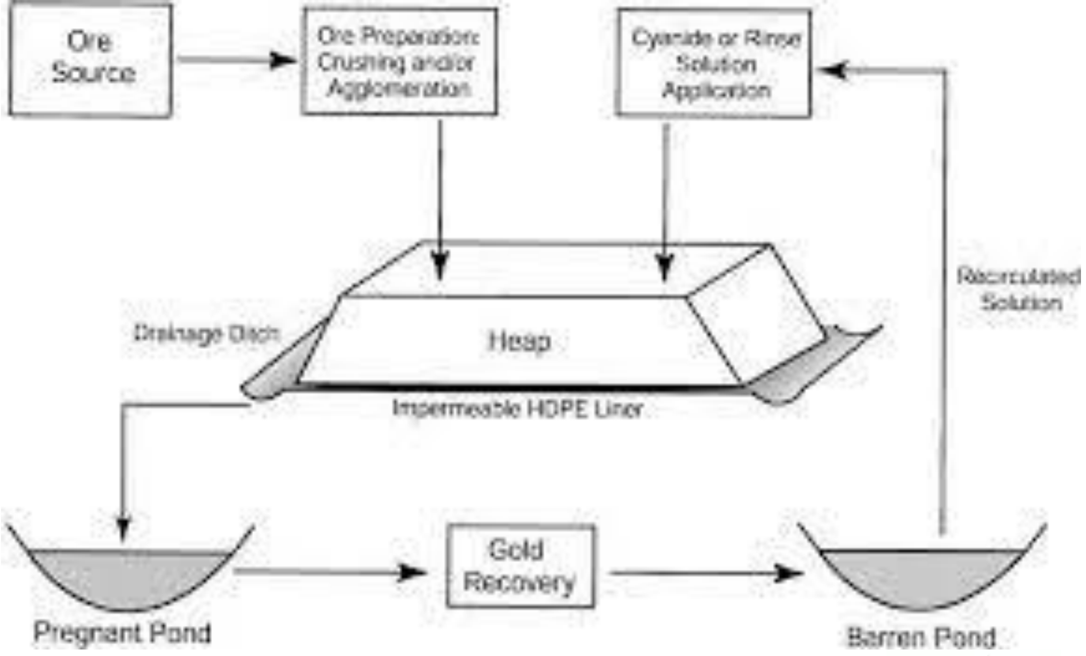


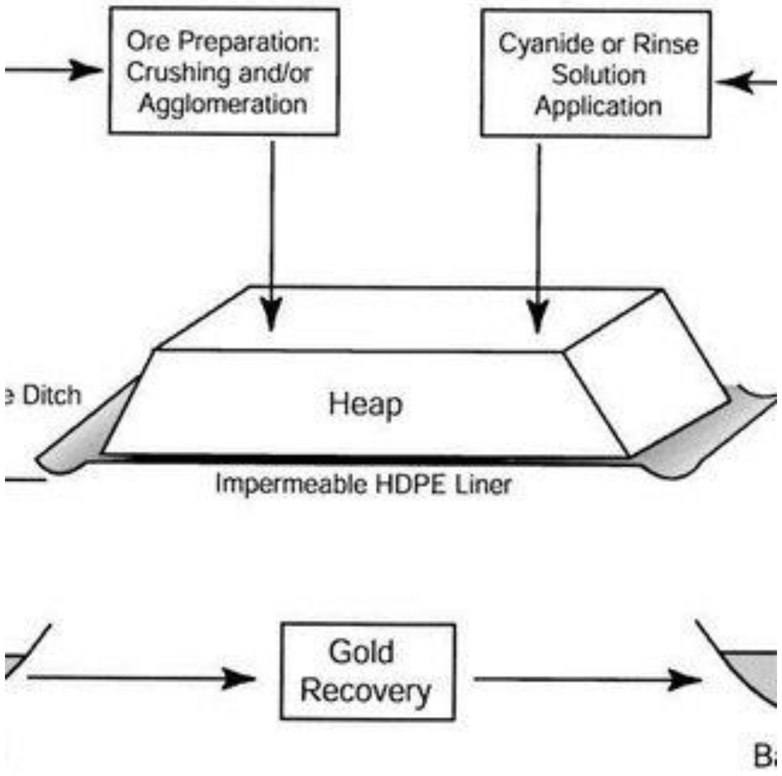




In-situ Leaching

**What is leaching of gold and silver?**

- Basically gold and silver leaching involves spraying a cyanide solution on the ore to dissolve the metal values, collecting the solution containing the dissolved metals, and recovering the metal from the solution. By eliminating milling, leaching reduces capital cost and startup time for new operations.
- Sodium cyanide solution is commonly used to leach gold from ore. There are two types of leaching: Heap leaching: In the open, cyanide solution is sprayed over huge heaps of crushed ore spread atop giant collection pads. The cyanide dissolves the gold from the ore into the solution as it trickles through the heap.
- **Thiourea leaching of precious metals such as gold and silver from ores has several advantages when compared with conventional cyanidation process**.
- Iron and sulfur oxidizing acidophilic bacteria are able to oxidize certain sulphidic ores containing encapsulated particles of elemental gold.




Leaching is done by cyanida process.

1. Ag ore is leached with NaCN (or KCN) solution in presence of air when silver and its salt are converted to soluble complex.

$$Ag_2S + 4CN^- \xrightleftharpoons{O_2} 2[Ag(CN)_2]^- + S^{2-}$$

Argento cyanide (soluble)

$O_2$ (air) is pumped to force the reaction in forward direction.

$$4S^{2-} + 5O_2 + 2H_2O \longrightarrow 2SO_4^{2-} + 2S\downarrow + 4OH^-$$

Zinc dust is then added to precipitate Ag.

$$[Ag(CN)_2]^- + Zn \longrightarrow Ag\downarrow + [Zn(CN)_4]^{2-}$$

(Pure)

# Bioleaching of Copper

- Chalcopyrite, Covellite and Chalcocite are ores of copper used for extraction of copper.
- Copper leaching is carried out by heap leaching and Insitu leaching process
- The ore is dumped as large piles down a mountain side.
- Water containing *T. ferrooxidans* is sprinkled upon the ore.
- *T. ferro oxidans* oxidizes insoluble chalcopyrite ($CuFeS_2$) to soluble copper sulphate ($CuSO_4$).
- Sulphuric acid is the byproduct of this reaction maintains necessary acidic environment for the extraction.

## Bioleaching of Copper

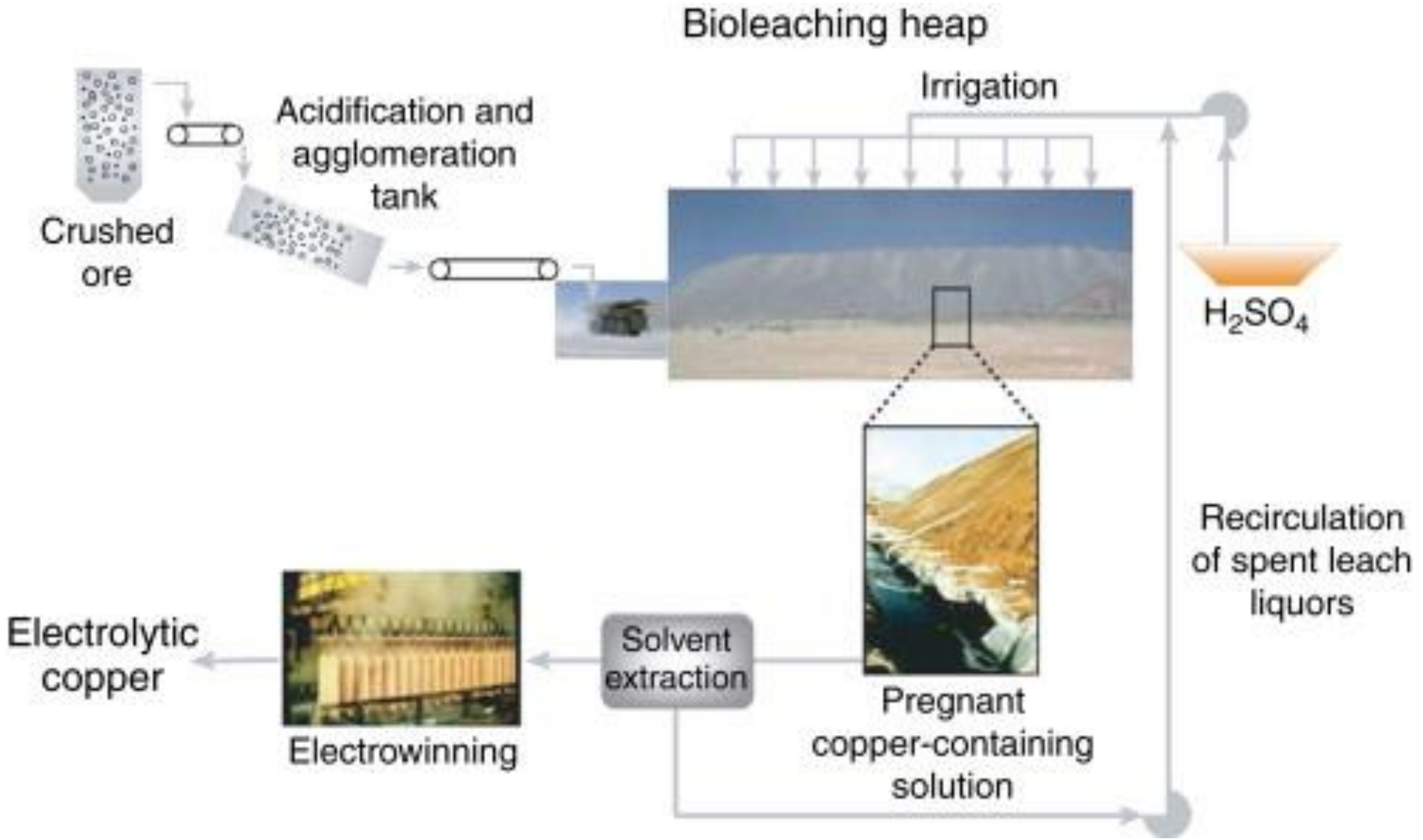
- Copper Ores: Calcopyrite ($CuFeS_2$), Chalcocite $Cu_2S$ , Covellite CuS
- Copper ore is a low grade ore.
- In bioleaching of Copper, the action of *Acidithiobacillus* involves the oxidation of $CuFeS_2$ via generation of ferric ions.

$$CuFeS_2 + 4\,Fe^{3+} \longrightarrow Cu^{2+} + 5\,Fe^{2+} + 2\,S_0 \quad \text{(Spontaneous)}$$
$$4\,Fe^{2+} + O_2 + 4\,H^+ \longrightarrow 4\,Fe^{3+} + 2\,H_2O \quad \text{(Iron oxidizers)}$$
$$2\,S^0 + 3\,O_2 + 2\,H_2O \longrightarrow 2\,SO_4^{2-} + 4\,H^+ \quad \text{(Sulfur oxidizers)}$$

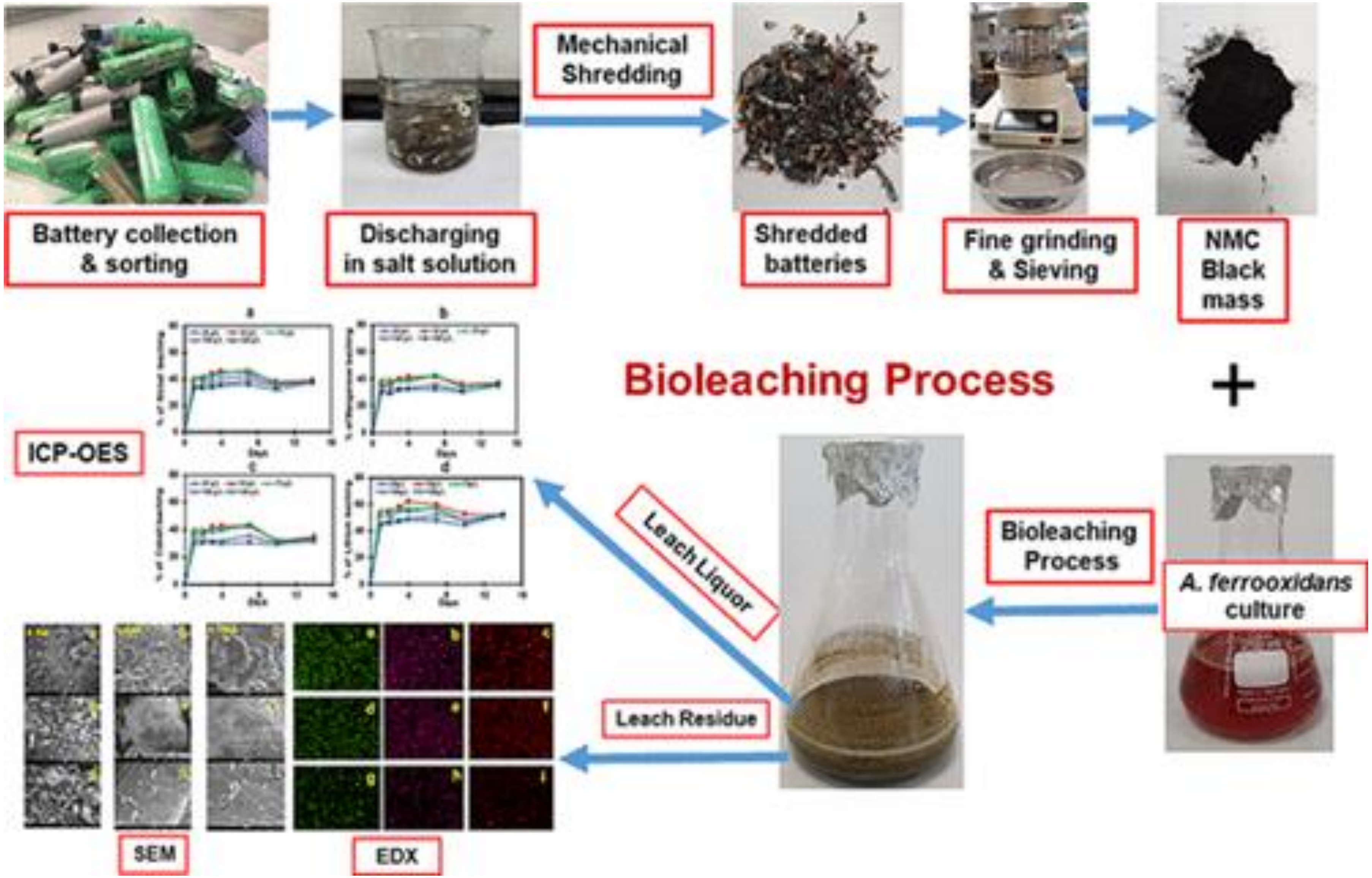
**Net Reaction:**

$$CuFeS_2 + 4\,O_2 \longrightarrow Cu^{2+} + Fe^{2+} + 2\,SO_4^{2-}$$

- Copper leaching is operated as simple heap leaching and *in situ* leaching process
- Dilute sulphuric acid is percolated down through the pile
- Liquid coming out of bottom of pile reach in mineral
- Liquid is collected and transported to precipitation plant
- Metal is precipitated an purified



Bioleaching heap
Irrigation
Acidification and agglomeration tank
Crushed ore
$H_2SO_4$
Recirculation of spent leach liquors
Electrolytic copper
Solvent extraction
Electrowinning
Pregnant copper-containing solution




**Bioleaching as an Eco-Friendly Approach for Metal Recovery from Spent NMC-Based Lithium-Ion Batteries at a High Pulp Density**

Joseph Jegan Roy, Madhavi Srinivasan*, and Bin Cao*

Cite this: ACS Sustainable Chem. Eng. **2021**, 9, 8, 3060–3069
Publication Date:February 18, 2021
https://doi.org/10.1021/acssuschemeng.0c06573

# Advantages of Bioleaching

- Bioleaching is **simpler, cheaper** to operate and maintain.
- The process is more **environmentally friendly** than traditional extraction methods.
- Bioleaching if used for all processing could drastically **reduce the amount of greenhouse gases** in our atmosphere.
- Bioleaching can be used extract metals from ores that are **too poor for other technologies**.

# ADVANTAGES OF BIOLEACHING

- Simple.
- Inexpensive.
- Recovery of metals from low grade ore.
- To extract refines and expensive metals which is not possible by other chemical processes.
- Employed for collecting metals from waste and drainages.
- No poisonous sulfur dioxide emission as in smelters.
- No need for high pressure or temperature.
- Ideal for low-grade sulfide ores.
- Environment friendly process.
- It is ideally suited for developing the countries.

# Disadvantages of bioleaching

- The bacterial leaching process is **very slow .**
- that the heat created from the dissolving process can **kill the bacteria.**
- **Toxic chemicals** are sometimes produced in the process.
- Unlike other methods, once started, bioleaching **cannot be quickly stopped.**