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Programme: M.Sc. Microbiology

COURSE TITLE: EXTREMOPHILES

Course Code: 24MICCBE2E

Unit – I: EXTREME MICROBIAL COMMUNITIES

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Microbes inhabiting Extreme Environments.

What are extremophiles?

The extremophiles are the organisms which grow under **extreme environmental conditions like**

temperature, salinity, pH,

anaerobic conditions (sensitive to O_2),

extreme atmospheric pressure,

water stress and others

under which other organisms will generally not grow or the conditions that may kill other organisms.

- The term ‘extremophile’ has generally not been used in the old literature as **it came up with the discovery of a *unique group of prokaryotes* from extreme environments**.
- It is from Latin (extremus = extreme, and Greek phila = love or friend of)….

The extremophiles include extreme

- **Psychrophilic** (cold loving)
- **Halophiles:** Salt loving microorgansims
- **Capnophile -** requires CO_2
- Extreme **thermophiles** (heat loving)
- **Alkalophiles** (bacteria that live at very high pH or alkaline conditions),
- **Acidophiles** (microorganisms) which show preference for growth at low pH, approximately 2.0),
- **Methanogens** (methane producing microorganisms)
- **Osmophiles** (the organisms which grow optimally in or on media of high osmotic pressure),
- **Radio tolerant: radiation reisistant microbes**
- **Piezophilic/Barophiles** (atmospheric pressure lovers)
- and **sulphur metabolizers;** *Archaeoglobus profundus* a sulphur reducer bears optimum growth **temperature of 82°C.**

First extremophile to have its genome sequence?

- The first extremophile to have its genome sequence was ***Methanococcus jannaschii*, an archaeobacterium** which lives near sea level where **temperature reaches boiling point of waters and pressure** enough to crush an ordinary submarine.
- These atmospheric pressure lovers are called **barophiles**.
- It exhibits high growth rate and high enzymatic activities at elevated temperatures.

Other than Archaeobacteria that come under extremophiles.

The Archaeobacteria alone do not come under extremophiles rather they include bacteria like ***Deinococcus radiodurans,***

Thermus aquaticus, Spirochaeta americana and the **animals Pompeii worm** (Deep sea polychaete worm, found only at hydrothermal vent)

Psychrophilic insects: Antarctic krill and water bear.

Cell wall-less Archaea.

Typical example of cell wall-less Archaea is the genus ***Thermoplasma***, in which the cells range from spheres to filaments.

The cytoplasmic membrane of cell of *Thermoplasma* are made of **diglycerol tetra ethers with 40-carbon isoprenoid hydrocarbons**.

The species of *Thermoplasma* are **thermopile and acidophilic growing optimally at about 60°C and pH 2**.

They like **sulphur-metabolizing archaea under anaerobic conditions require sulphur and reduce to H_2S**.

- However, 16S rRNA sequence homology points out that *Thermoplasma* is closely **related to methanogen/halophile branch of archaeal phylogenetic tree than to sulphur metabolizing thermophiles**.
- *Thermoplasma* **grow in nature in coal refuse piles, and Solfatanas** (natural volcanic steam vent) i.e., the **hot sulphur rich environments, volcanic areas or vents which yield sulphur vapours & steam**.

- **Survival time** of this cell wall less archaea has been estimated to be **15 years**.
- *Thermoplasma acidophilus* is **heterotrophic thermoacidophile found in acidic environments** created by chemolithotrophic (energy is obtained from the oxidation of inorganic compounds) sulphur oxidisers but does not produce acid itself.

Acetogenesis

- Some facultatively chemoautotrophic anaerobes are able to **reduce CO_2 with H_2 to acetate in place of methane,** ***Clostridium thermoaceticum*** **and** ***Acetobacterium woodii.***

- In the acetogenesis, the energy yield of the reaction is less favorable than the methanogenesis.

- Besides acetogenesis from **CO_2 and H_2 these organisms can also ferment formate & methanol to acetate** and have many metabolic features in common with methanogenic bacteria.

Psychrophile

- An organism which grows optimally at or below **15°C**, has an upper limit for growth at about **20°C** and which has lower limit for growth of **0°C** or below.

- Psychrophilic organism include certain **algae & fungi**, a number of **Gram negative bacteria** e,g. some species of ***Pseudomonas* and *Vibrio***, and a few **Gram positive** bacteria ***Clostridium*** spp.

- The term **psychrotroph** has been used for the organism which can grow at low temperature e.g. **0 – 5°C**) but which has an **optimum growth temperature >15°C** and an upper limit of growth **>20°C**.
- Psychrotrophs include certain **algae** and **fungi** and various **Gram negative and Gram positive bacteria.**

Psychrophiles

Acidophile

- An organism that grows optimally under acidic condition having an optimum **growth pH below 6** and sometimes **as low as pH 1** or below and **which typically grows poorly or not at all at or above pH 7**

  e.g. ***Sulpholobus, Thermoplasma* and *Thiobacillus***.

Microorganisms tolerate extreme pH

The microorganisms generally cannot tolerate extreme pH values.

Under **highly alkaline or acids conditions** some microbial **cell components may be hydrolyzed or their enzymes may get denatured**.

There are, however, some **acidophilic folerate** or need extreme pH conditions for growth.

Many fungi are **acidotolerant** but most bacteria are not.

Some acidotolerant bacteria like ***Thiobacillus*** and acidosis like ***Thiobacillus*** and ***Sulfolobus*** create their own low pH environment by producing acids.

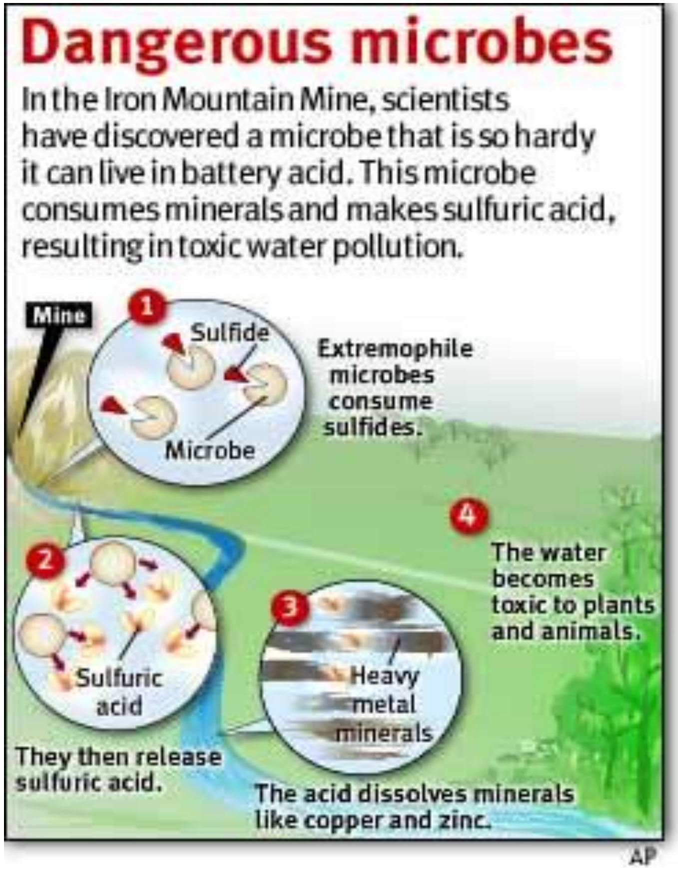
- *Lactobacillus* is a **mixed acid fermenter and Sulfolobus produces H_2SO_4** ***Bacillus* & *Acidophilus*** are environments created by chemolithotrophic sulphur oxidisers.
- ***Thermoplasma*** is an **archeon** without cell wall and live in **hot acid coal refuse piles**.
- ***Bacillus acidocaldarius*** is the native of acid hot spring.

Applications of Acidophiles:

- (i) *Lactobacillus* is used to **prepare silage (stored cured fodder) and fermented foods.**
- (ii) *Thiobacillus* and *Sulfolobus* are employed in **bioleaching of low-grade copper and uranium ores**.

Acidophiles

pH 0-1 of waters
at Iron Mountain



Alkaliphiles

- Many bacteria and fungi are known to bear alkaline pH **upto 9** but **prefer pH optimum near neutrality.**
- The examples of true alkaliphiles are ***Bacillus*** strains like ***B. alcalophilus*** and ***B. pasteuri***.
- Some cyanobacteria like ***Microcystis aeruginosa, Plectnonema nostocorum*** and some species of ***Spirulina*** are also alkalophilic.
- ***Halobacterium, Natronobacterium*** **and** ***Natronococcus*** are the category of alkalophiles found in **saline lakes with high pH**.

Application of alkaliphiles

- Some alkaliphilic *Bacillus* strains produce **proteases** & **lipases** - stable at high temperature at **alkaline pH in the presence of detergent.**
- Therefore, these are used in some **laundry detergents** to **clean fat & proteinaceous stains**.
- It is interesting to note that *Clostridium paradoxum* can withstand pH greater than **9.3 at 55°C and its optimal generation time is 13 min.**

Alkaliphile

e.g. Mono Lake
alkaline soda lake, pH 9
salinity 8%

Thermopiles

- The thermopiles like optimum growth temperature above 40°C.
- Bacterium ***Bacillus stearothermophilus*** grow at comparatively high temperature (**55°C to 70°C**).
- The maximum growth temperature for most thermophilic bacteria is about **99°C** and many thermopile bacteria have temperature ranging from **55° to 60°C.**

Optimal temperature for extreme thermopiles

- The **optimal temperature of extreme thermopiles** is above **80°C**.
- They grow in nature in **hot-springs and effluents from Laundromats** (washing machines and dryers for public use)**.**
- Many extreme thermopiles, however, can **remain live at freezing temperature and have been reported to have survived in Antarctic frozen soil**.

How thermopiles maintain their semipermeable properties at high temperature?

- They have comparatively high **molecular weight and branched fatty acids in their membranes** which permit them **to maintain their semipermeable properties at high temperatures**.

Unique habitats of thermopiles

- They grow in areas of **volcanic activity**; **steam vents** in such areas may have a **temperature of 500°C**.
- They also grow in **hot springs, like Yellow Stone National Park**, and in other parts of the world.

Thermal vent communities and colonies of hyper thermophiles

- The thermal vent communities occur at depth of **800 to 1000 m** where sea floor permits seawater to percolate deeply inside the crust (outside of the hard layer) and to react with the hot core material.
- The vent community of bacteria like ***Thermotoga, Begiatoa, Thiomicrospira*** and sulphide/sulphur oxidisers of various types.
- The archaeobacteria that may be growing are ***Thermococcus litoralis, Archaeoglobus pyrodictium*** **and** ***Pyrobaculum***.

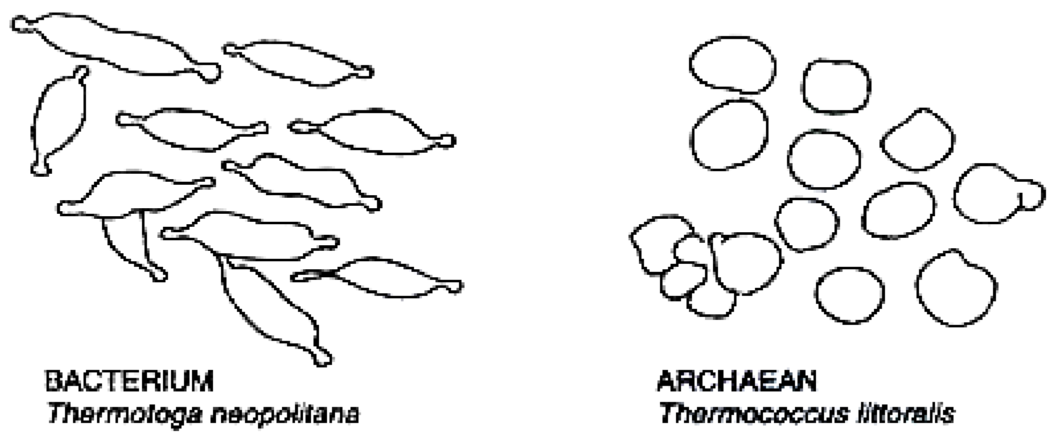
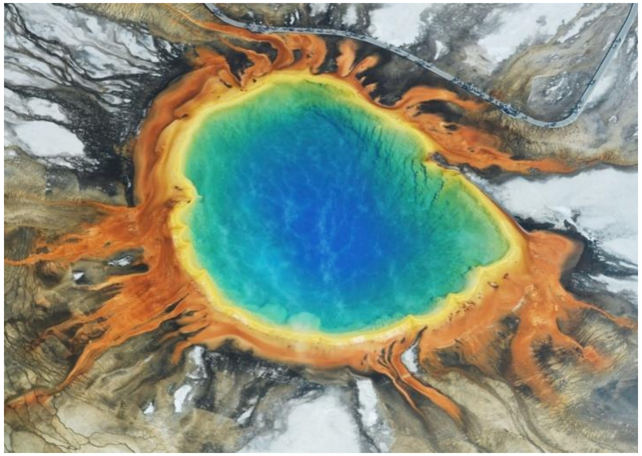


Fig. 6.5. Diagramatic scanning electron micrographs of hyperthermophilic bacterium and archaeobacterium.

Thermophiles

Obsidian Pool, Yellowstone National Park
Hydrothermal Vents



Life at High Temperatures, Thomas M. Brock


Barophiles

- The organisms which grow best or only under conditions of **high pressure** in the **depth of oceans** are called barophiles.

Barotolerants

- The organisms which can grow under conditions of **high pressure but do not show preference for growth under conditions of high pressure**.

Range of conditions that permitted growth of barophiles

- The barophiles have been isolated from the extreme depth of **10,500 m**.
- Some of the deep isolates were also **psychrophilic** and their optimum temperature and pressure influenced each other in a complex manner.
- An isolate from **3600 m deep waters was clearly barophilic at 4°C** recorded for that bacterium.

Significance of slow growing barophilic marine bacteria

- They make significant contributions to **biodegradation and marine food web**.

Can barophilic bacteria be cultured?

Yayanos *et al.* succeeded in isolating deep sea ***Spirillum* and grew that 15 times faster at pressure between 300 and 600 atm pressure than at 1 atm pressure**.

Osmophiles

- The microorganisms which can tolerate or prefer **high concentrations of organic solutes as sugars are called *osmotolerant or osmophiles***.
- Some of the noted habitats of osmophilic microorganisms are **honey, sap flows, nectar of flowers, molasses and sugary syrups**.
- Some yeasts like ***Debaromyces hansenii* and *Zygosaccharomyces rouxii*** are **good examples** of **osmophiles**.
- The moulds ***Aspergillus*** and ***Penicillium*** **also are osmotolerant.**

Halophiles

- Microorganisms which can tolerate or need **high concentration of salt** are called *halophiles* or *halotolerants*.

Halophiles

Solar salterns
Owens Lake,
Great Salt Lake
coastal splash zones
Dead Sea

“S” organisms

- It is the organism **syntrophic** with **methanogens**.
- ***Methanobacterium bryantii*** (***M. omelianskii***) was kept in culture collection for 26 years after its original description is 1941 before it was revealed to be syntrophic association of the methanogen proper and fermentative “S” organism.
- The “S” organism and **syntrophic fermenters** of similar type were later classified and named ***Syntrophomonas* and *Syntrophobacter.***
- Both these genera are H_2 process and need the presence of **methanogens as H_2 removers called mutualism based hydrogen transfer**

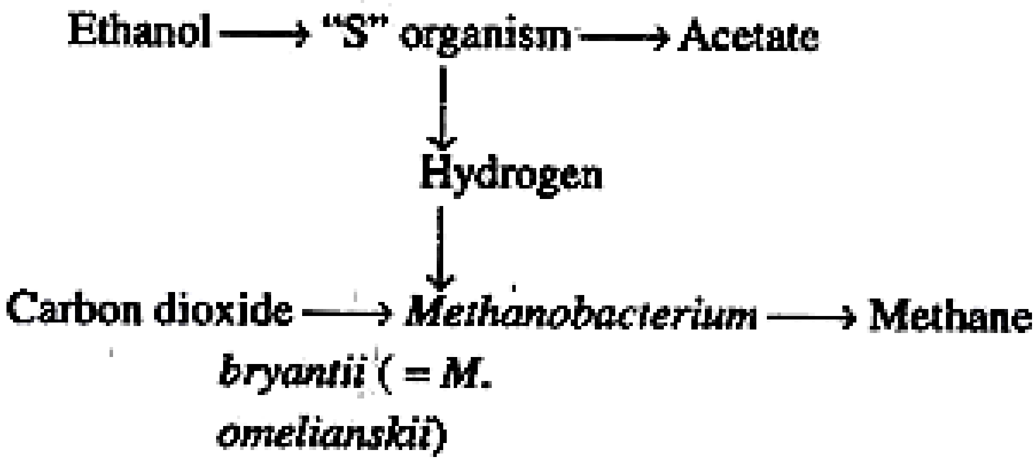


Fig. 6.8. Mutualism leading to hydrogen transfer in methanogens.

Arsenic loving extremophiles

- According to **NASA** scientist as reported in the journal Science, the first organism able to ' substitute one of the **six chemical elements** (C, H, O, N, S, P) **crucial to life has been found,** which bacterium is found in a California Mona lake (USA) that **uses** arsenic**, a poisonous element, in place of phosphorus**.

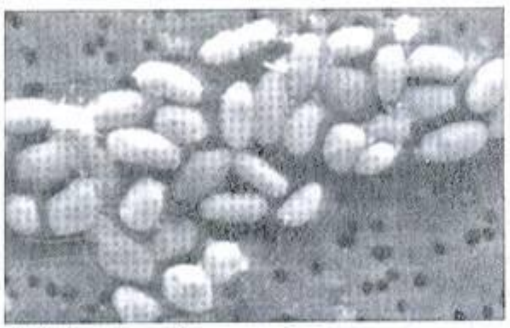


Fig. 6.11. Extremophilic bacteria growing on arsenic.

Fig. 6.10. Mona lake, the ecological niche or natural home of salt loving extremophiles.

It is fascinating that bacterium breaks the golden rule of biochemistry. So far the idea has been that life on earth is composed of at least six elements : carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. But this bacterium is an exception to this rule and has broken the fundamental tenet of the biochemistry.

Life in extreme environments

- Extreme habitats - outside the range of conditions in which most of organisms live.
- Extreme includes **physical extremes**, e.g. temperature, radiation, pressure, and **geochemical extreme**, e.g. desiccation, salinity, pH, depletion of O_2/extreme redox potential.
- The discovery of inhabited extreme environments & extremophilic organisms made **higher possibility to find life outside of Earth, gave new pathways in biotech industry & biology.**

Extremophiles - **thrive beyond 'normal' environmental parametres**.

The future perspective on research of **extremophiles is promising because of their economic potential**, **their role in geochemical processes, threatening issues & searching life in the Universe**.

- Normal environments would mean those with **temperatures between 4 & 40°C**, with **pH values between 5 & 8.5, salinity between that of freshwater & seawater**.
- All **3 domains** of life (Archaea, Eubacteria & Eukaryotes) are found at extreme conditions.

Extremophiles……..

- Psychrophiles (thrive at low temperatures), thermophiles (high temperature),
- Acidophiles (low pH),
- Alkaliphiles (high pH),
- Piezophiles /barophiles (extremes of pressure),
- Xerophiles (desiccation),
- Halophiles (salinity)
- Methanogens (methane producing microorganisms)
- Osmophiles (the organisms which grow optimally in or on media of high osmotic pressure),
- Capnophile - requires CO_2
- Radio tolerant: radiation reisistant microbes

LIST OF EXTREME PARAMETRES AND LIFE IN THESE CONDITIONS

High temperature creates problems & chlorophyll degrades above 70°C, which excludes photosynthesis.

- Proteins & NAs are normally denaturated at 100°C and the fluidity of membranes to lethal level is then increased.
- Hyperthermophilic organisms have maximum growth >80°C & psychrophilic <15°C.
- The first and the most hyperthermophilic organism isolated by Brock in 1977 is ***Pyrolobus fumarii (Crenarchaeota) from*** **archaea** - optimum temperature for growth at 106°C & at 113°C it still continues to grow.

- Some phototrophs like cyanobacteria, purple & green bacteria (Seckbach & Oren, 2007) eubacteria (*Clostridium, Thiobacillus, Bacillus etc.)* and the archaea (*Pyrococcus, Thermococcus, Sulpholobus etc.) can* have temperature optimum up to 142°C (Rothschild & Mancinelli, 2001).

- The eukaryotes have the upper limit life at~60°C and this limit is still suitable for some protozoa, algae & fungi (Mangan, 2007).
- Mosses have the maximum temperature of 50°C, vascular plants 48°C & fish 40°C.
- At high temperatures **solubilzation of cellular lipids** can occur and **cells in this case loose their integrity.**

- In habitats with **temperatures below 0°C** we can find representatives from all major taxa.
- The lowest temperature for active microbes is -18°C.
- Between animals, an insect found in 1984 in Himalaya by Kohshima, living at **-16°C** in glacier, the most cold insect habitat ever recorded.

- Liquid water is not important only as a solvent but also as product/reactant in many metabolic processes.
- When temperature is low, water freezes, **ice crystals destroy CM.**
- In absence of liquid water biochemical reactions stop what results in **cell death.**
- A **nematode** *Panagrolaimus davidi* is only one discovered so far which can survive when **all body water freeze.**

- Organisms are not only capable to live at high/low temperature, but they can **modify the temperature of their habitat.**

Recent studies show that microbial activity can produce temperature increases in their habitat.

pH

- Biological processes in normal conditions considered by humans occur in middle of a pH spectrum (between 5 & 8.5).
- Organisms function best at pH values close to neutrality.
- Very high (acidic)/very low (alkaline) concentrations of H^+ ions will have a big effect on activity & ability of organisms to live in an environment.

- Due to **surface barriers of cells which are very impermeable for protons and selective mechanisms (active pumps) preventing H+ ions to enter in the cell, acidophiles use proton pumps as defence to maintain their intracellular pH at near neutral values**.

- There are few unicellular eukaryotes which live below pH 1 like red alga *Cyanidium caldarium* (described from natural environment at pH 0.5, however in culture it has growth optimum at pH 2-3), green alga *Dunaliela acidophila* *(it survives at* pH 0, and it has maximum growth rate at pH 1) (Rothschild & Mancinelli, 2001), fungi for example *Acontium velutium* & *Cephalosporium sp.* were found at pH 0.

- Diversity among acidophilic anoxygenic phototrops is limited to only 2 species purple nonsulphur bacteria (*Rhodoblastus aacidophilus* & *Rhodopila globiformis),* due to unsuitability of acidic habitats for anoxygenic phototrophs.
- Because of **acidic habitats have usually high level of metals which in high concentrations have negative effect** on these organisms.

- Two species of anaerobic heterotrophs, archaea, (*Picrophilus oshimae and Picropilus torridus)* were isolated from Japanese soils penetrated with solfataric gases, and show an optimal growth at pH 0.7 & 60°C.

PRESSURE

- Pressure causes volume changes of living organisms, **decreasing fluidity of cell membranes by compressing lipids & inhibiting the biochemical reactions in cells**. Sudden changes in pressure can have let**hal effect.**

Hydrostatic pressure is high in deep lakes and it is even more characteristic for all deep sea vent sites.

Hydrostatic pressure increases at a rate of 10.5 kPa/metre depth compared with 22.6 kPa/metre for lithostatic pressure.

The boiling point of water increases with pressure, and this enables that at the bottom of the sea water is liquid even at 400°C.

- Microorganisms **inhabiting the deep sea and subsurface of Earth** have the ability to survive at **hydro- and litho- static pressures** much > 1 atm.
- Studies of barotolerant (tolerance to high pressure) and barophilic (depending on high pressure) cultures of deep sea bacteria show that both types are present.

- Extreme **piezophiles**, live at high hydrostatic pressure associated with big depths, grow well in deep sea & even in deep subsurface sediments deep as 1,000 m
- Below seafloor under anaerobic conditions.
- The barophiles have been isolated from extreme depth of **10,500 m**.

- Under pressurized conditions at **155°C in slurry deep sediments**, prokaryotes produce **H_2, CH_4, organic acids & energy for themselves** & substrates for other prokaryotes and can catalyse some reactions which are considered to be thermogenic.

One component of pressure is gravity.

Until now it was believed that on Earth organisms lived at 1 *g.*

Space exploration included research in locations with different gravity regimes, for example launched space shuttles with variable *g, Moon which have* 0.17 *g, Mars etc.*

Gravity affects human health; it has effects on microbes & directs changes in biomass.

RADIATION

- Radiation is energy which can appear like particles (e.g. neutron, protons, electrons, alpha particles/heavy ions)/electromagnetic waves (gamma rays, X-rays, UV radiation, infrared, microwaves/radiowaves).

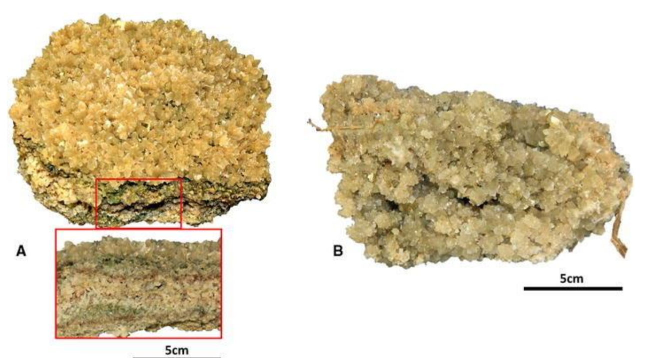
- Normally, extreme levels of radiation appear rarely on Earth.
- Man caused intense levels of irradiation are well studied due to their role in production of goods (preserving of food, fast drying of substrates, control of pests & microorganisms), in medical applications & space travel.
- When the wavelength is shorter, the potential of damage of an organism is higher.
- Due to depletion of O_3 layer in atmosphere, the exposure to UV has increased and its influence on organisms has received lately more attention.

- The most harmful UV radiation is UV-A: 320-400 nm, following by UV-B: 290-320 nm & UV-C: 200-290 nm.
- UV affects organisms by inhibiting photosynthesis & damaging the nucleic acids.

- Organisms protect themselves either actively/ passively, by moving away from lethal environments, by engaging mechanisms to repair damaged cell components, and
- By accumulation of protecting substances that protect against the harmful radiation (e.g. synthesis of **carotenoids** which absorb the visible spectrum above 400 nm).

- One of extreme cases of resistance to irradiation is, *Synechococcus* sp. isolated from an **intertidal evaporitic gypsum** (group of sedimentary rocks - originally thought to have formed from the evaporation of sea water) **crust from Mexico** which was exposed in outer space during two-week space flight in June 1994.
- In an experiment it was exposed to the high radiation (between 200 & 400 nm).
- Interestingly in such conditions only a small reduction in viability & activity was observed.

The most common marine evaporites are calcite, gypsum & anhydrite, halite, sylvite, carnallite, langbeinite, polyhalite & kainite.



The **Atacama Desert** (Spanish: *Desierto de Atacama*) is a desert plateau located on the Pacific coast of South America, in north of Chile. Stretching over a 1,600 km (990 mi) strip of land west of the Andes Mountains, it covers an area of 41,000 sq mi, which increases to 49,000 sq mi if the barren lower slopes of Andes are included.

Rare rainfall events cause the flowering desert phenomenon in the southern Atacama Desert.

Vegetation in Pan de Azúcar National Park on the coast of the Atacama Desert

A **lizard** native to the southern reaches of the Atacama Desert

Flamingos in Atacama

Flora: Over 500 species have been gathered within the border of this desert, herbs & flowers such as thyme, llareta, saltgrass & trees.

The llareta is one of the highest-growing wood species in the world. It is found at altitudes between 3,000 & 5,000 m, 3 to 4 m (9.8 to 13.1 ft) thick.

Fauna: Sand-colored **grasshoppers, beetles** and their larvae provide a valuable food source in the *lomas* (hills). Desert **wasps, butterflies, Red scorpions** also live in the desert.

Surprisingly few reptile species, fewer amphibian species. lava lizards, salt flat lizards.

Birds: Humboldt penguins, Andean flamingos, while Chilean flamingos, etc.

Microbial Life at dessert eco-niches

- Members of the genus *Streptomyces such as Streptomyces deserti* from the hyper-arid **Atacama** Desert are also reported from arid habitats (Harwani, 2013; Santhanam et al., 2013), *Streptomyces bullii* from the hyper-arid Atacama Desert (Santhanam et al., 2013).
- *Micromonospora* from Atacama Desert (Carro *et al.,* 2018)

- Two new *Streptomyces* species from Atacama Desert soils (Santhanam et al., 2011, 2013) were shown to produce new ansamycin and 22-membered macrolactones with antibacterial and antitumor activity (Rateb et al., 2011).
- Another *Streptomyces* strain isolated from the Chilean highland soil of the Atacama Desert produces novel aminobenzoquinones which show inhibitory activity against bacteria and dermatophytic fungi (Schulz et al., 2011).

Mojave Desert

The Mojave Desert, the driest & smallest of the North American deserts, occupies only 7 percent of the total arid lands of North America, yet offers a wide diversity of habitats, from salt pans to mesa tops, high sand dunes to alkaline springs. It is home to over 2,500 species of plants and animals, more than 100 of which are considered to be in some degree of peril.

Temperature: above 100°F (38°C)
Annual average precipitation of 2 to 6 inches (51 to 152 mm)

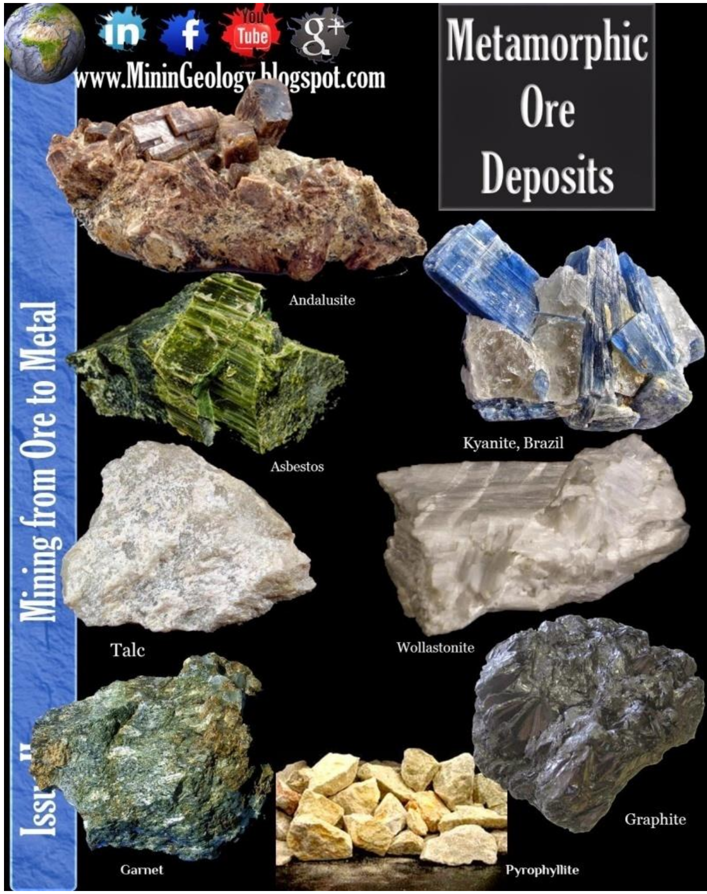
A desert tortoise, of the Mojave Desert

Life at The Mojave Desert is dominated by low, widely spaced shrubs, few trees, several parasitic plants and a vast variety of wildflowers.

- 25 percent of the plant species in the Mojave are endemics--found nowhere else in the world.
- The fauna of the Mojave Desert is composed of both wide-ranging species, such as the desert bighorn sheep (Ovis canadensis) and jackrabbit, and highly specialized species that live only in small relict habitats, including the Inyo Mountain's slender salamander (*Batrachoseps campi*) and several species of pupfish (*Cyprinodon* sp.).

Life at ore deposits

- An ore deposit consists of one/more ore bodies.
- An ore body is a mass of rock that contain ore & from which a commodity of value will be extracted.
- Not all ore within an ore body will be extracted.
- Ex.: Talc is a naturally occurring mineral, mined from earth, composed of Mg, Si, O & H. Chemically, talc is a hydrous magnesium silicate with a chemical formula of $Mg_3Si_4O_{10}(OH)_2$. Talc has many uses in cosmetics & other personal care products.
- Absorbs moisture well & helps cut down on friction, making it useful for keeping skin dry & helping prevent rashes.



Mining areas

Mineral deposits are accumulations of valuable minerals that are of economic interest to humans.

These deposits can be found in a variety of geological settings, including igneous, sedimentary, & metamorphic rocks.

They are formed through a range of geological processes.

The minerals in these deposits may be metals, such as Cu, Ag, Zn, nonmetals, such as salt/S.

- **Biomining:** Extraction of specific metals from their ores through biological means, usually microorganisms.
- Biomining began with the discovery of the bacterium *Thiobacillus ferrooxidans.*

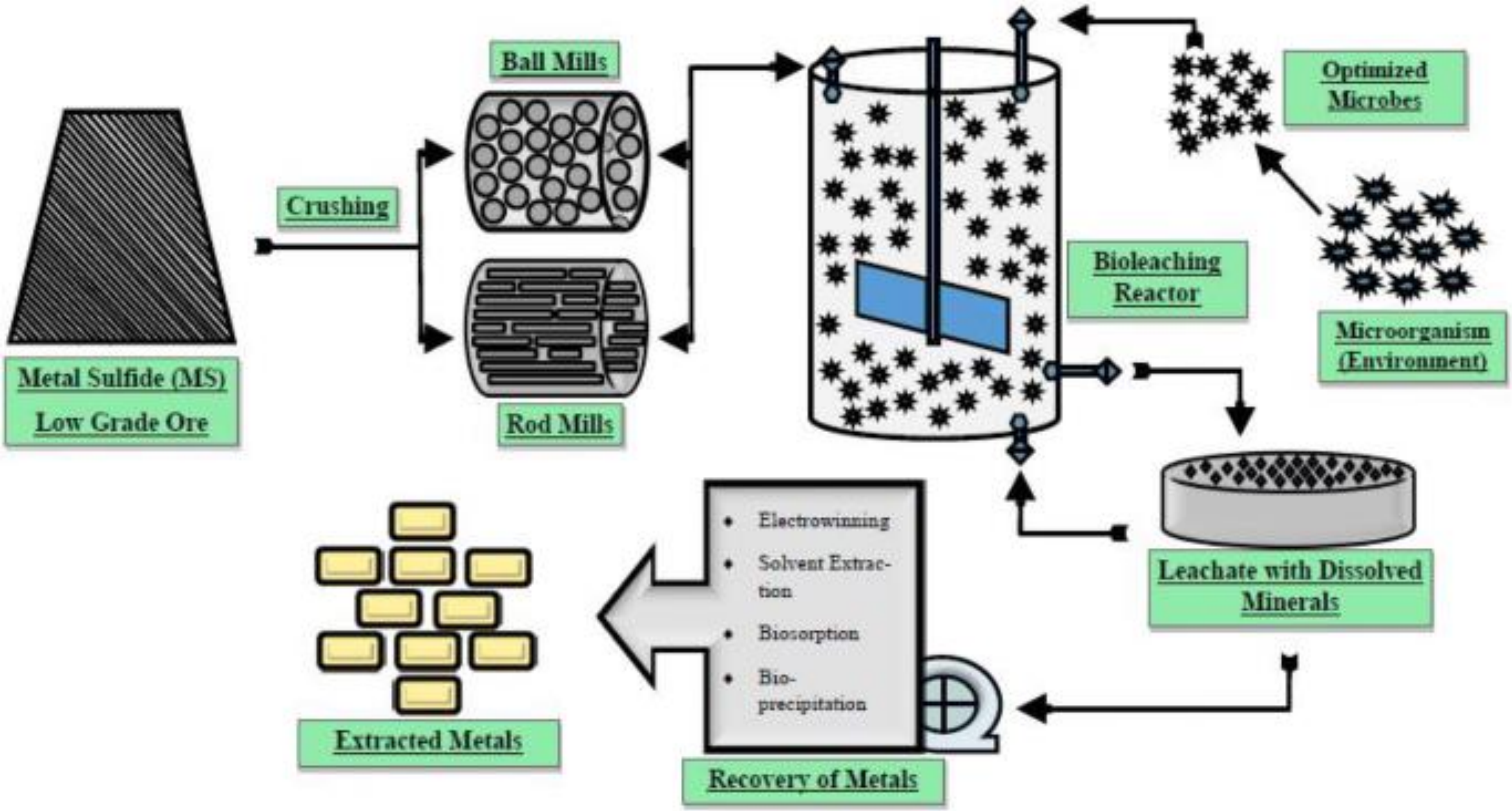
Microscopic image of *Thiobacillus ferrooxidans*

- **Bioleaching**: Bioleaching is the process by which metals are dissolved from ore bearing rocks using microorganisms.
- Bioleaching is also called microbial leaching.
- The most commonly used microorganisms in bioleaching are:
- *Thiobacillus thiooxidans*
- *Thiobacillus ferrooxidans*

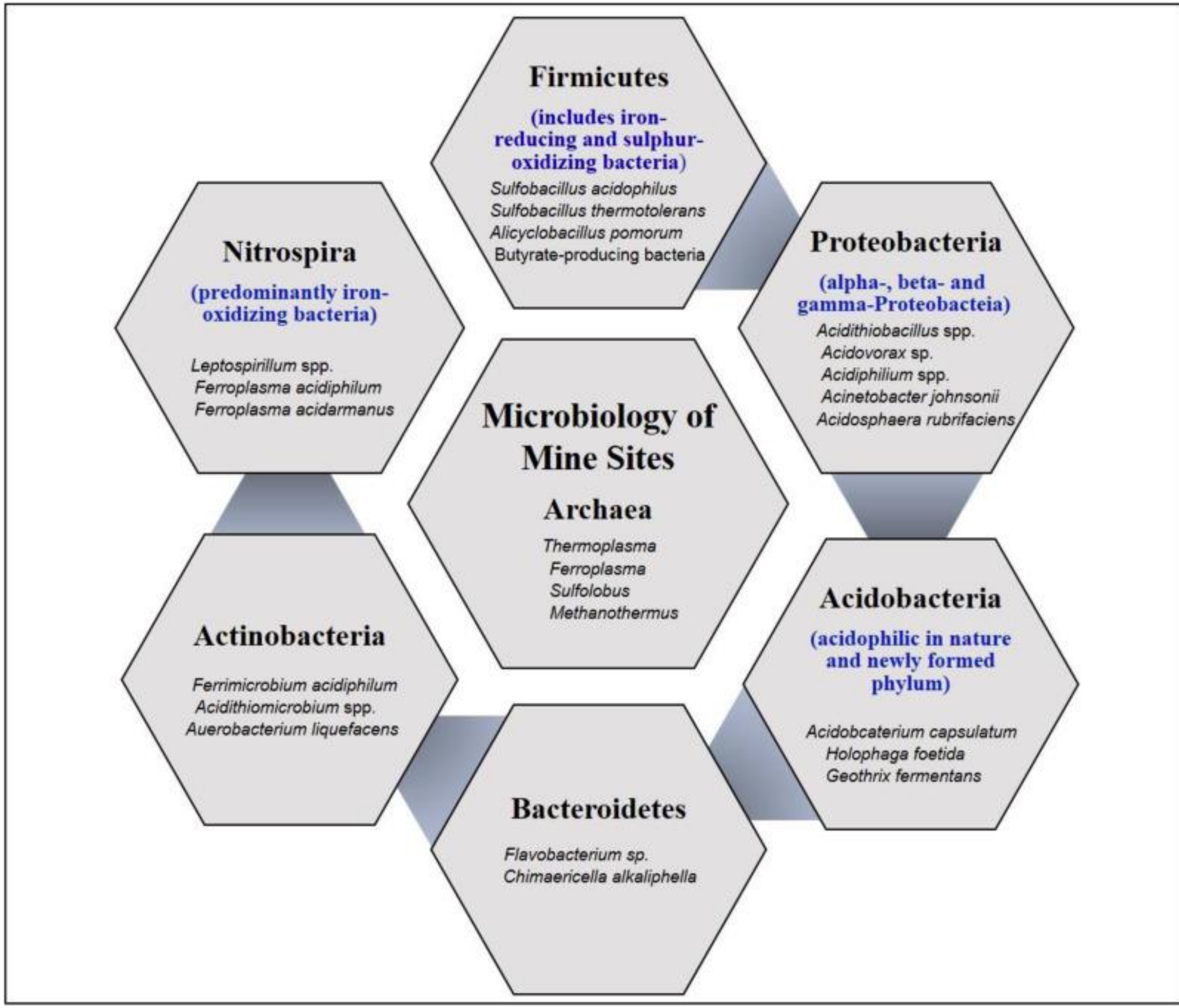
Other microorganism:

- *Sulfolobus* – Ca, As
- *Saccharomyces cerevisiae* – Cu, Pb, Sn
- *Penicillium simplicissium* - Cr

- The use of **acidiphilic, chemolithotrophic iron- and sulfur-oxidizing microbes** in processes to recover metals from certain types of copper, uranium, and gold-bearing minerals or mineral concentrates is now well established.
- During these processes insoluble metal sulfides are oxidized to soluble metal sulfates.


Ball Mills
Crushing
Metal Sulfide (MS)
Low Grade Ore
Rod Mills
Bioleaching
Reactor
Optimized
Microbes
Microorganism
(Environment)
Leachate with Dissolved
Minerals
Electrowinning
Solvent Extrac-
tion
Biosorption
Bio-
precipitation
Recovery of Metals
Extracted Metals

Biomining is a simple & effective technology for metal extraction and concentration. For this purpose, commonly used microorganisms are **Mesophiles, Moderately thermophilic bacteria, extremophiles**. For copper & gold, this method is already fruitful. Using the biomining technique, 10 to 15% of gold mining done.



Extremophiles from Animals

Methanogenic archaea found in the **rumen** acts as a symbiont to anaerobic ciliates.

These anaerobes are useful to break down **cellulose** in rumen, making it bio-available when otherwise **indigestible** by animals.

Termites - anaerobic bacteria to fix & recapture N.

Anaerobic bacteria differ from aerobic bacteria **in their O_2 requirement**.

O_2 is **toxic** to anaerobes, which can be explained by the **absence of enzymes** in the anaerobes of **catalase/ superoxide dismutase/peroxidase** enzymes.

- Anaerobic microbes **grow & survive in extreme environments and they can do so with the help of their genomes**.
- They can adapt to such harsh conditions because of their unique genomic composition.
- For instance, anaerobes have certain enzymes that scavenge O_2 & protect them from O_2.

Capnophiles: An organism with optimal growth conditions in high concentrations of CO_2.

- Example: *Mannheimia succiniciproducens*, a bacterium that inhabits a ruminant animal's digestive system.
- Employed for the efficient production of succinic acid.

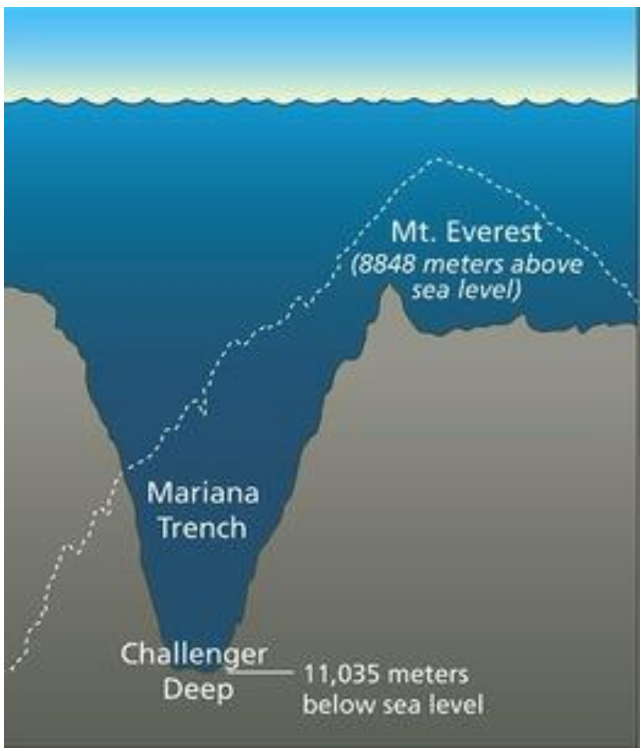
- *M. succiniciproducens* MBEL55E, a capnophilic & facultatively anaerobic gram-negative bacterium, was isolated from the rumen of a Korean cow.
- Efficiently fixes CO_2 & produces SA as a major fermentative product.
- *M. succiniciproducens* can metabolize a broad range of C sources such as glucose, sucrose, maltose, lactose, mannitol, xylose & glycerol. Especially, *M. succiniciproducens* can efficiently produce SA using cheap C sources such as whey/wood hydrolysate-based medium.

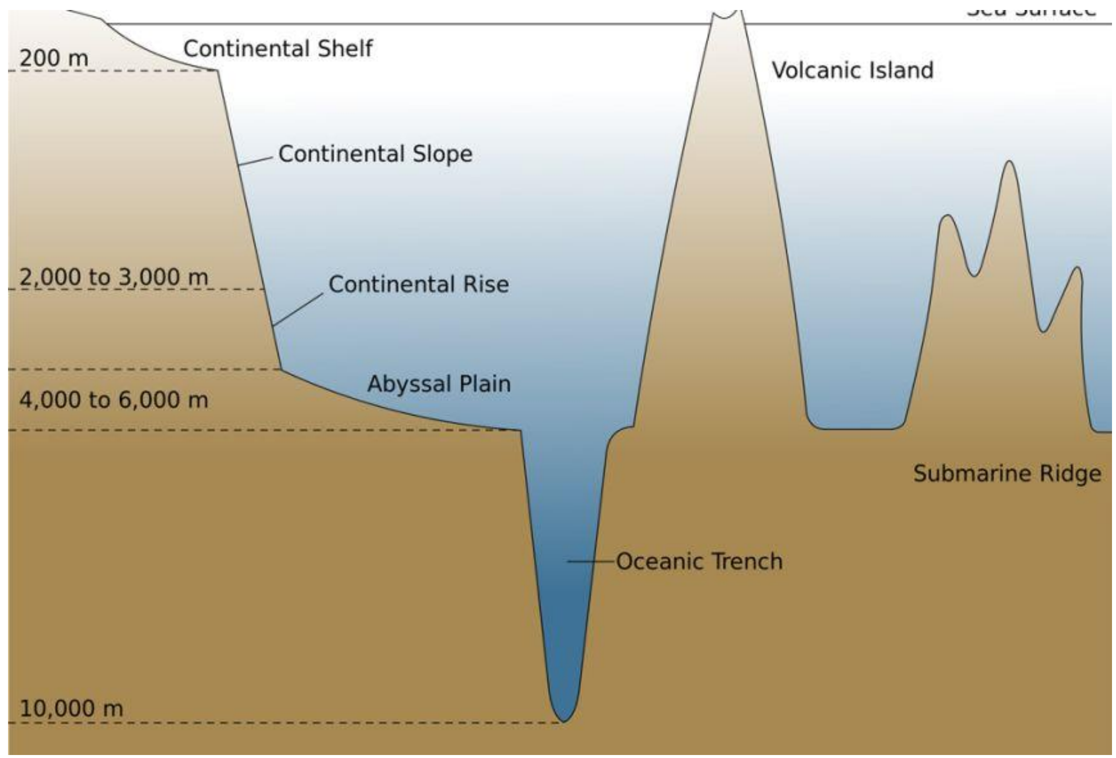
Extremophiles at deep biosphere (Marine)

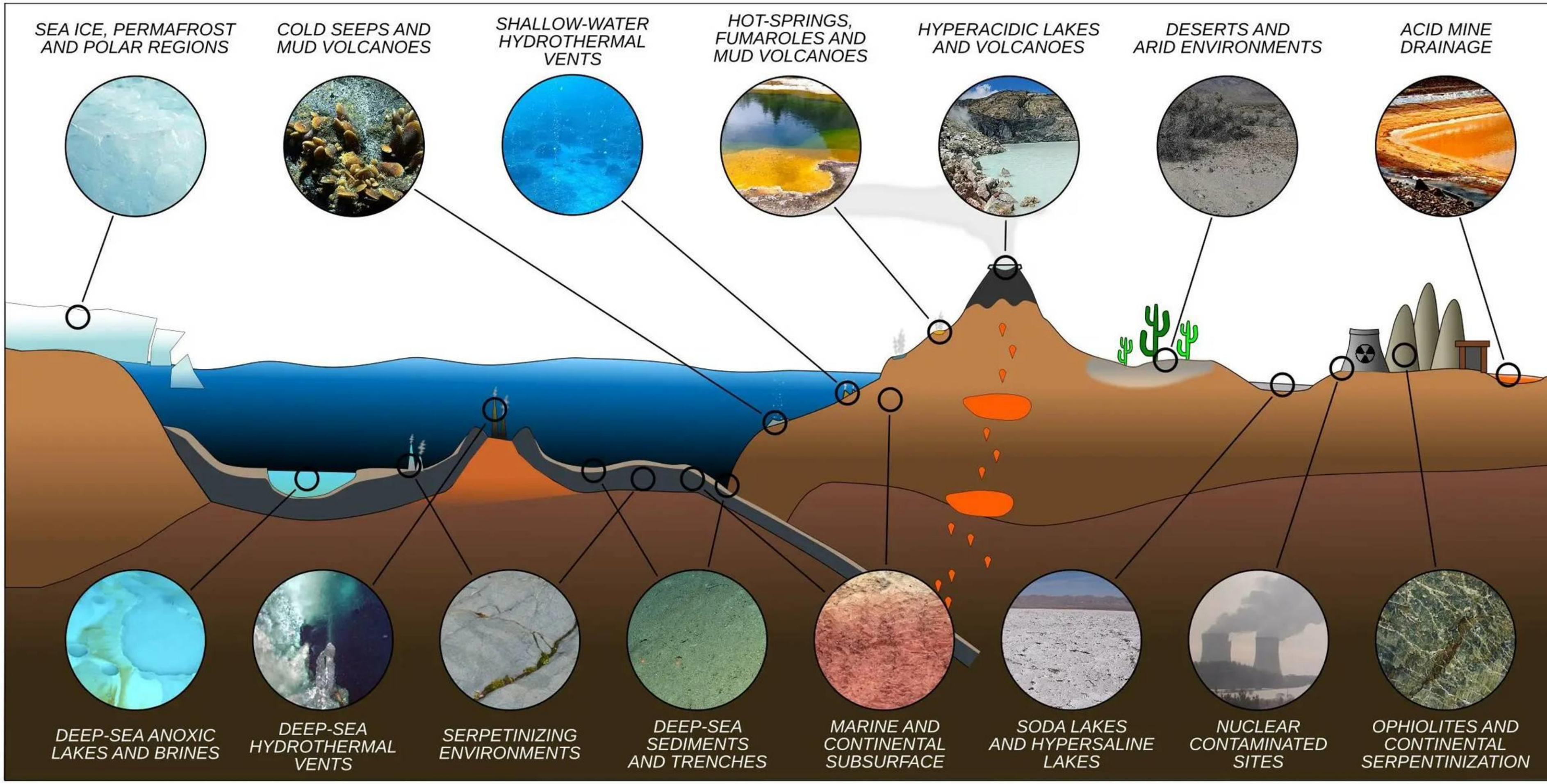
Environmental conditions of Deep-sea floor & trenches

Temperature (℃)	:	1.9–13.8
pH	:	7.3–8.1
Pressure (MPa)	:	2.1–112
Salinity (% NaCl)	:	3.4–3.9

The vast marine deep biosphere consists of microbial habitats within sediment, pore waters, upper basaltic crust & the fluids that circulate throughout it.







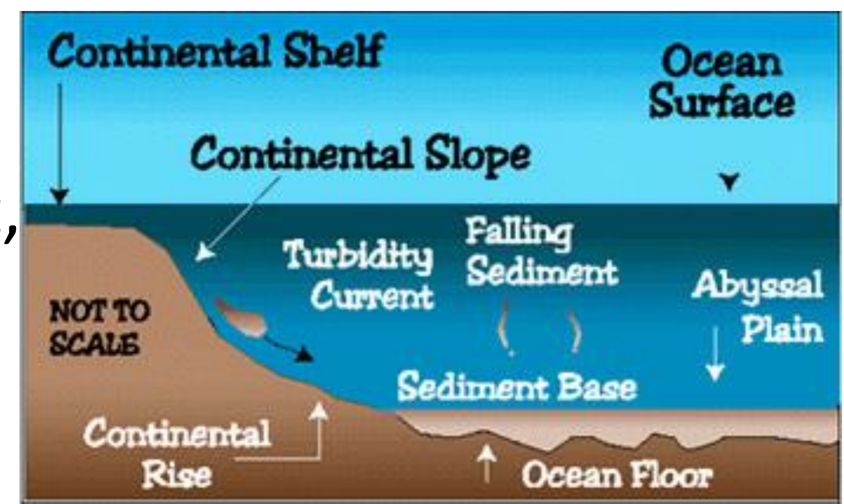
Serpentinization: Hydration & metamorphic transformation of ferromagnesian minerals.

MARINE DEEP BIOSPHERE

- A wide range of temperature, pressure, pH, and electron donor & accept/conditions exists all of which can combine to affect carbon & nutrient cycling.
- Diverse and mostly uncharacterized microorganisms live in these habitats, and potentially play a role in mediating global scale biogeochemical processes.

MARINE DEEP BIOSPHERE

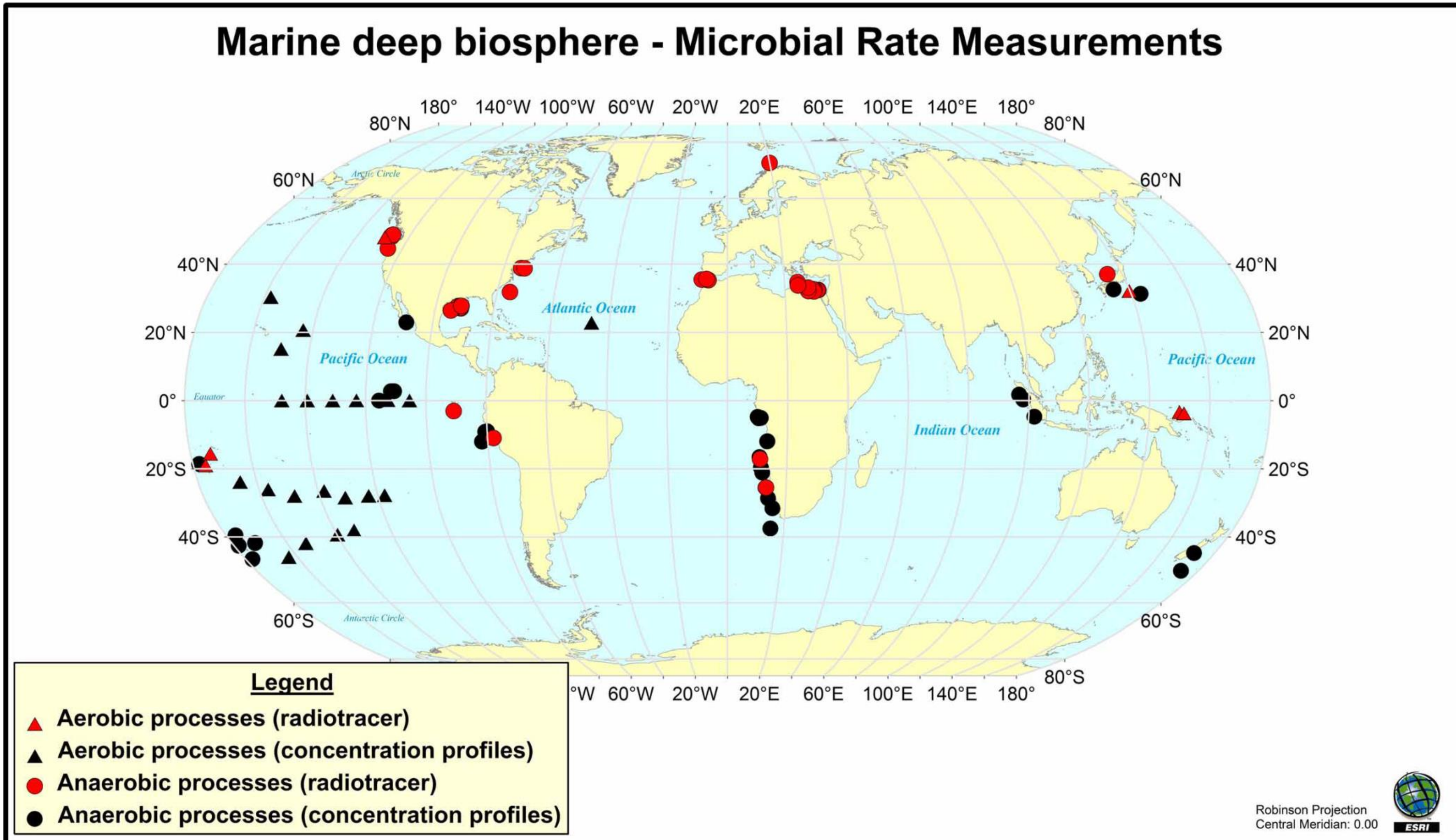
- The marine deep biosphere is often defined as "life existing deeper than one meter below seafloor (Jørgensen & Boetius, 2007), spanning from continental margins to abyssal plains".
- In 1992, Thomas Gold presented the thought: "If there exists this deep, hot biosphere, it will become a central item in the discussion of many/indeed most, branches of the Earth sciences.



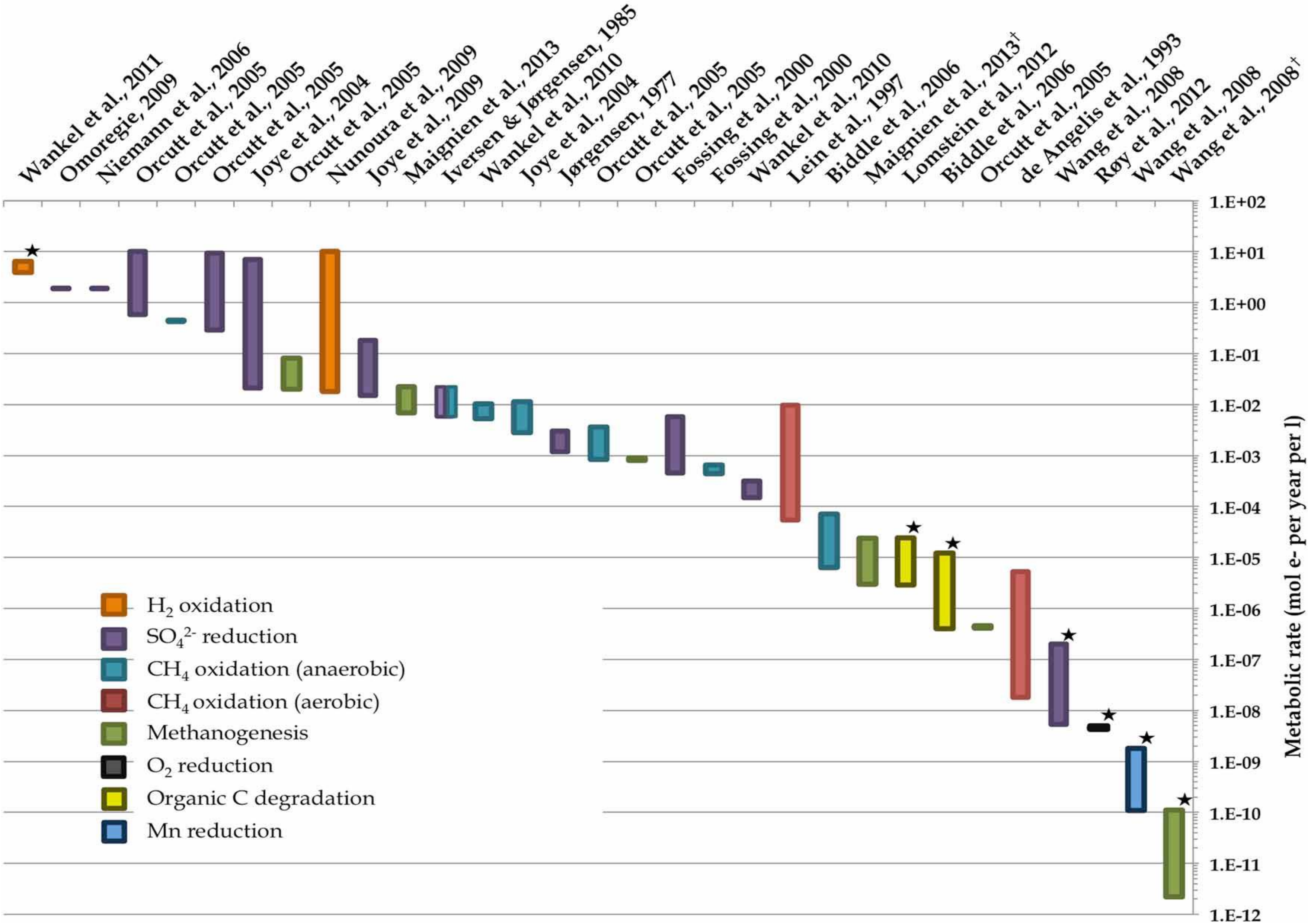
- Environments in the dark reaches of ocean depths, such as hydrothermal vent systems and newly formed oceanic crust (Orcutt et al., 2011a; Biddle et al., 2012) are also part of the marine deep biosphere, though more often viewed as “**windows**” to subsurface ecosystems.

- While recent estimates for the number of microorganisms living in the sedimentary deep biosphere have considerably decreased (Kallmeyer et al., 2012), the number of microbes in the crustal environment is still largely unconstrained (Edwards et al., 2012a), and the vastness of this ecosystem means that it is a **major reservoir for harboring microbial life on this plane**t.

- Importantly, the marine deep biosphere is alive: it is not just a reservoir for buried, non-functioning microbial cells.



Map of the locations where rates of microbial activity in deep sediment have been measured (through radio-isotope tracer techniques) or inferred from modeling of vertical geochemical parameters during drilling program expeditions.



Representative ranges of microbial activity in the marine deep biosphere based on literature values of measured & modeled volumetric rates. Starred rate measurements derive from measurements of *in situ conditions; all others derive from ex situ incubation experiments.*

- The deep sea is one of the enigmatic sites that stayed unnoticed till the challenger deep sea mission was carried out using the Marina Trench, which is below 10,916 meters from the surface.
- The diversity analysis carried out using advanced high throughput sequence analysis revealed the presence of Chloroflexi (SAR202 & other lineages), Planctomycetes sp., Bacteroidetes sp., "Ca. Marinimicrobia" (Marine group A and SAR406), Thaumarchaeota, "Ca. Woesearchaeota" (Deep-sea Hydrothermal Vent Euryarchaeotic Group 6), & Gemmatimonadetes (archaeal α subgroup).
- Similarly, the deep sediment analysis performed from the south Colombian Caribbean Sea at a depth of 1681–2409 meters results highlighted the presence of 36 phyla, 89 classes, 93 orders, 104 families, 90 genera, and 53 species, among them Proteobacteria sp., Bacteroidetes sp., Firmicutes sp., Actinobacteria sp. & Chloroflexi sp., were the top 5 phyla identified from the selected deep-sea sediments.

- Marine deep biosphere research has greatly benefited from investigations by the scientific drilling community through the Integrated Ocean Drilling Program (IODP) and Ocean Drilling Program (ODP), and it has influenced the future scope of the international scientific drilling program (IODP, 2011).

Microbes of Hydrothermal vents

- Hydrothermal vents are located where the tectonic plates are moving apart & spreading.
- This allows water from the ocean to enter into the crust of the earth where it is heated by the magma.
- There are generally three kinds of vents that occur and are all characterized by its temperature and chemical composition.
- 1. **Diffuse vents** release clear water typically up to 30°C.
- 2. **White smoker vents** emit a milky-coloured water between 200-330°C, Chimneys formed from deposits of Ba, Ca & Si and
- 3. **Black smoker vents** generally release water hotter than the other vents between 300-400°C. Deposits of iron sulfide,
- The waters from black smokers are darkened by the precipitates of sulfide (S^{2-}) that are accumulated.

Black smokers

White smoker

Underwater volcanoes at spreading ridges & convergent plate boundaries produce **hot springs** known as hydrothermal vents.

- The **hydrothermal vent microbial community** includes all unicellular organisms that live & reproduce in a chemically distinct area around hydrothermal vents.
- These include organisms in the microbial mat, free floating cells/bacteria in an endosymbiotic relationship with animals.

- Chemolithoautotrophic bacteria derive nutrients & energy from the geological activity at Hydrothermal vents to fix C into organic forms.
- Viruses are also a part of the hydrothermal vent microbial community and their influence on the microbial ecology in these ecosystems is a burgeoning field of research.

- Due to the absence of sunlight at these ocean depths, energy is provided by chemosynthesis where symbiotic bacteria & archaea form the bottom of food chain and are able to support a variety of organisms such as *Riftia pachyptila* (giant tube worm - marine invertebrate) & *Alvinella pompejana* (the Pompeii worm, is a species of deep-sea polychaete worm - extremophile found only at hydrothermal vents in the Pacific Ocean).
- These organisms use symbiotic relationship in order to use & obtain chemical energy that is released at these hydrothermal vent areas.

- Large variation in temperatures at the surface of the water with the changing depths of thermocline seasonally.
- Waters immediately surrounding the hydrothermal vents which can get as high as 407°C.
- The approximate rate of pressure increase in the ocean is 10Mega-pascals (MPa) for every km that is travelled towards the seafloor. This means that hydrostatic pressure can reach up to 110MPa at the depths of the trenches.

- Salinity stay relatively constant within the deep seas communities around the world at 35 ppt.
- Although there is very little light in the hydrothermal vent environment, photosynthetic organisms have been found.
- Microbes that live here are known to be hyperthermophiles, microorganisms that grow at above 90°C.

- The organisms are found where the fluids from the vents are expelled & mixed with the surrounding water.
- These hyperthermophilic microbes are thought to contain proteins that have extended stability at higher temperatures due to intra-molecular interactions but the exact mechanisms are not clear yet.

- The stabilization mechanisms for DNA are not as unknown and the **denaturation of DNA are thought to be minimized through high salt concentrations, more specifically Mg, K, PO_4** which are highly concentrated in hyperthermophiles.
- Along with this, many of the microbes have proteins similar to histones that are bound to the DNA and can offer **protection** against the high temperatures.

- Microbes are also found to be in symbiotic relationships with other organisms in the hydrothermal vent environment due to their **ability to have a detoxification mechanism which allows them to metabolize the sulfide-rich waters** which would otherwise be toxic to the organisms & the microbes.

Bacterial Diversity

Sulfur-oxidizing

- These bacteria use various forms of available sulfur (S^{-2}, S^0, $S_2O_3^{-2}$) in the presence of O_2.
- They are the predominant in the majority of hydrothermal vents because their source of energy is widely available, and chemosynthesis rates increase in aerobic conditions.
- The bacteria at hydrothermal vents are similar to the types of sulfur bacteria found in other H_2S-rich environments - except *Thiomicrospira* has replaced *Thiobacillus.*
- Other common species are *Thiothrix* and *Beggiatoa,* which is of particular importance because of its ability to fix N_2.

Methane-oxidizing

- Methane is a substantial source of energy in certain hydrothermal vents, but not others: methane is more abundant in warm vents (25°C) than H.
- Many types of methanotrophic bacteria exist, which require O_2 & fix CH_4, $CH_{3,}$ NH_2, and other C_1 compounds, including CO_2 and CO, if present in vent water.
- These type of bacteria are also found in *Riftia* trophosome, indicating a symbiotic relationship.
- Here, methane-oxidizing bacteria refers to methanotrophs, which are not the same as methanogens: *Methanococcus* and *Methanocaldococcus jannaschii* are examples methanogens, which are found in hydrothermal vents; whereas *Methylocystaceae* are methanotrophs, which have been discovered in hydrothermal vent communities as well.

Hydrogen-oxidizing

- Little is known about microbes that use H as a source of energy, however, studies have shown that they are aerobic, and also symbiotic with *Riftia* (see below).
- These bacteria are important in the primary production of OC because the geothermally-produced H_2 is taken up for this process.
- Hydrogen-oxidizing and denitrifying bacteria may be abundant in vents where NO_3^--containing bottom seawater mixes with hydrothermal fluid.
- *Desulfonauticus submarinus* is a hydrogenotroph that reduces S-compounds in warm vents and has been found in tube worms *R. pachyptila* & *Alvinella pompejana.*

Iron- and manganese-oxidizing

- These bacteria are commonly found in Fe & Mn deposits on surfaces exposed intermittently to plumes of hydrothermal and bottom seawater.
- However, due to the rapid oxidation of Fe^{2+} in neutral and alkaline waters (i.e. freshwater & seawater), bacteria responsible for the oxidative deposition of Fe would be more commonly found in acidic waters.
- Manganese-oxidizing bacteria would be more abundant in freshwater & seawater compared to iron-oxidizing bacteria due to the higher concentration of available metal.

The list below is a cumulative representation of bacterial phyla and genera, in alphabetical order. As shown, proteobacteria appears to be the most dominant phyla present in deep-sea vents.

- Actinomycetota
- Aquificota
  - *Hydrogenobacter* and *Aquifex*
- Chloroflexota
- Chlorobiota
  - *Chlorobium*
- Deferribacterota
- Gemmatimonadota
- Nitrospirota
- *Nitrospinota*
- *Leptospirillum ferriphilum*

- Bacillota
  - Acetogen: *Clostridium*
- Pseudomonadota
  - Acidithiobacillia
  - Alphaproteobacteria
    - *Paracoccus*
  - Betaproteobacteria
    - *Thiobacillus*
    - *Sideroxydans lithotrophicus*
  - Gammaproteobacteria - major symbionts
    - *Allochromatium*
    - *Thiomicrospira*
    - *Thioalkalivibrio*
    - *Methylococcaceae*
    - *Beggiatoa*
    - *Thioploca*
  - Zetaproteobacteria
    - Mariprofundus ferrooxydans
- Campylobacterota[11][15][16]
  - *Sulfurovum lithotrophicum*
  - *Sulfurimonas paralvinellae*
  - *Nitratifactor salsuginis*
  - *Hydrogenimonas thermophila*
  - *Thiovulum*
- Thermodesulfobacteriota - sulfate-reducing, make up more than 25% of the bacterial community
  - *Desulfovibrio*
  - *Desulfobulbus*
  - *Desulfuromonas*