**Semester - I**

**I7MBP105A** **MARINE MICROBIOLOGY** **4H – 4C**

**Instruction Hours / week: L: 4 T: 0 P: 0** **Marks:** Internal: **40** External: **60** Total: **100**

**End Semester Exam: 3** Hours

**SCOPE**

This course has been intended to provide knowledge about the origin and maintenance of microbial diversity and its role in the structure and function of marine ecosystems.

**OBJECTIVE**

Students get an idea about isolation, Identification and preservation of the marine microbes and its application in various fields.

**UNIT – I**

Marine microorganisms: collection, preservation, enumeration (total and viable counts), isolation of culture and identification based on morphological, physiological and biochemical characteristics. International and national collection centres.

**UNIT – II**

Extremophiles: Thermopiles, basophiles, halophiles, psychrophiles, acid – alkalinophiles, oligotroph, toxitolerant, xerotolerant, endolith – Extremophiles and their environment, biodiversity. Genomics of extremophiles, phylogeny of extremophiles, 16S RNA classification in mitochondrial DNA genome, RAPD, RFLP studies.

**UNIT – III**

Microbiology of degradation of xenobiotic environment: Ecological considerations, decay behaviour, degradative plasmids, hydrocarbons, oil pollution, surfactants, pesticides, Bioremediation:- Factors affecting bioremediation – role of microbes in the marine nutrient cycles – diseases of marine organisms and its impact on marine biodiversity.

**UNIT – IV**

Brief account of photosynthetic and accessory pigments. Phytoplanktons and Zooplanktons, Red tides, Zones, Bioluminescence and Biopigment, Marine micro and macro organisms, Coral reefs, Mangrooves, Hydrothermal vents and water currents.

**UNIT – V**

Bar coding of marine organisms: Genome sequencing and physical mapping of genome. Marine exploration, Aquaculture-inland and freshwater, Isolation of marine bioactive compounds-separation, purification and identification techniques, cryopreservation.

**SUGGESTED READINGS**

**TEXT BOOKS**


1. Colin Munn. (2011). *Marine Microbiology: Ecology & Applications*. (2nd ed.). Black Well Publishers.
2. David Sigee. (2005). *Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment*. (1st ed.). Black well Publishers.
3. Se-Kwon Kim. (2013). *Bioactive compounds and biotechnological applications*. CLS Publishers


**REFERENCES**


1. Dube, H.C. (1994). *A text book of fungi, bacteria and viruses*. Vikas Publishing House, New Delhi.
2. Dale, J.W. (1994). *Molecular genetics of Bacteria*. John Wiley and Stones.
3. Pelczar, M., JR., Chan, E.C.S., and Noel, R. K., (2006). *Microbiology*. Tata McGraw, Hill. Co. (5th ed.). New Delhi.
4. Presscott, L.N., Harley, J.P. and Klein, D.A., (1999). *Microbiology*. W.C. Brown Publishers.
5. Stanier, R.Y., Ingharam, J.L., Wheelis, M.L., and Painter, P.R., (1986). *General Waste water engineering Treatment, Disposal and Reuse*. Metcaff and Eddy. Inc., Tata Mc Grew Hill, New Delhi.

# 2017

## I M. Sc Microbiology – Marine Microbiology

### Objective of the course

In addition to fulfilling the learning objectives provided by individual lecturers, the student should be able to do the following.

- Students get an idea about isolation, Identification and preservation of the marine microbes and its application in various fields.

**Prepared by : Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE**

# 2017

## M.Sc Microbiology - Marine microbiology

| LECTURE PLAN - UNIT -1 | | | |
|---|---|---|---|
| **S. no** | Lecture duration(Hr) | **Topics covered** | **Supporting materials** |
| 1 | 1 | Marine microbes – Collection, preservation | W1 |
| 2 | 1 | Enumeration TVC | T1 99-111 |
| 3 | 1 | Isolation and identification of marine microbes | T1 110-121 |
| 4 | 1 | Morphological identification | T2 39-79 |
| 5 | 1 | Physiological identification | T2 39-79 |
| 6 | 1 | International and internal collection cnetres | W1 |
| 7 | 1 | Unit I test | |
| **Textbooks :** | | T1-Microbiology- pelczar – McGraw Hill publishing<br>T2-Microbiology- presscott – McGraw hill publishing<br>T3-Microbial genetics – David friefelder- | |
| **Reference books:** | | | |
| **Website:** | | W1- www.microbiology.com<br>W2 – www.marinestudy.com | |
| **Journals:** | | | |

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# 2017

## Introduction of marine microbiology

### Microbial Life in Marine Environments

More than 70% of the Earth is covered by ocean. When we think of life in the ocean we often think of fishes and whales, life that is visible to the naked eye, but we may be astounded to learn that most life in the ocean is microbial life' new! Microbes account for more than 70% of ocean biomass and constitute a hidden majority of life that flourishes in the sea. What is even more surrisin is that much of this microbial life remains unknown because we cannot culture it in a test tube and it is difficult to observe in nature.

Sampling tools for the marine environment

Introduction

[1]All methods of physical capture are inherently selective. Small fish may pass through large-meshed nets; large fish may out-swim trawls; gill nets will catch fish mainly of a certain size range. Fish may react differently to fishing gear with respect to species, size, biological state, environmental conditions including ambient light and the acoustic noise field, among many other factors.

[2]This is why organisms are subdivided out of practical necessity, in that the sampling approach and sample size that are appropriate for one group are often inappropriate for another. The disparity in appropriate techniques for different sizes of groups of organisms has contributed greatly to the paucity of studies on more than one taxonomic grouping at a given locale.

Unfortunately, where conflicting conclusions have been drawn patterns in different groups of organisms, it is rarely possible to know whether the patterns truly vary among groups or merely reflect differences in sampling efforts. The choice of a suitable sampler is a compromise between a variety of factors.

Sampling tools for pelagic organisms

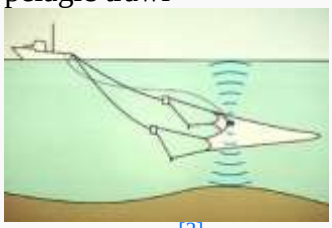
Midwater or pelagic trawl

[1]A midwater or pelagic trawl is a set of gear that is used to catch fish that are between the sea surface and bottom, generally staying clear of the bottom. Occasionally, midwater trawls are configured with floats to perform catching in the shallow-surface layer.

[3]A midwater trawl consists of a cone shaped body, normally made of four panels, ending in a codend with lateral wings extending forward from the opening.

[1]Midwater and bottom trawls (see further) have many parts in common, if differing in dimensions and shapes due to their different fishing objects and hydrodynamic regimes of operation. Midwater trawls are designed to catch fish in the midwater column, hence must be capable of rapid maneuvering while maintaining an open net mouth. This is reflected in differences in the body of the net, rigging, and even trawl doors.

pelagic trawl [1]



pelagic trawl [3]

Plankton nets

**Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE**

[4]Plankton nets are a modification on the standard trawl used to collect planktonic organisms, of nearly any size, intact. Towed by a research vessel, plankton nets have a long funnel shape that allows them to catch differently sized plankton simply by changing the mesh size of the net. At the end of the funnel is a collection cylinder called a cod-end.

**Ring net**

ring net[1]

[1]The ring net consists of a fine-meshed bag attached at its mouth, or opening, to a metallic ring. The net itself is terminated in a bottle or jar where the unfiltered plankton and other particulate matter are collected.

The net is usually deployed vertically for non-quantitative purposes from a platform, such as a vessel or pier. It may also be towed, although lacking in devices for controlling its passage through the water column, which is otherwise determined by hydrodynamic forces generated naturally during towing or hauling. Towing applications are mainly non-quantitative.

**Bongo nets**

bongo nets[5]

[1]Floating or suspended fish eggs and newly hatched larvae are often caught with Bongo nets. The mesh size is very fine, ranging from 20 µm up to 1000 µm (1 mm), thus allowing eggs and larvae with sizes of order 1-20 mm to be caught. The nets, mounted on a rigid yoke, can be towed from the surface to near the bottom for sampling throughout the water column.

In order to obtain quantitative samples of phytoplankton, zooplankton, other invertebrates, and large fish, it is critical to estimate the volume of water that is filtered during the sample. Most bongo and ring nets are deployed with mechanical or electronic flow meters positioned in the mouth of the net to quantify the volume of water filtered.

**MOCNESS**
**(Multiple Opening/Closing Nets and Environmental Sampling System)**

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MOCNESS[1]

[1]The Multiple Opening/Closing Nets and Environmental Sampling System, which is generally known by its acronym MOCNESS, is an operational, widely used system for capturing plankton at specific depths on the command of the operator. It also routinely carries a number of sensors for measuring environmental parameters as it is towed. These sensors measure, for example, conductivity, temperature, pressure, fluorescence, optical transmission, dissolved oxygen, and light levels.

Neuston nets

neuston net[6]

[6]These types of nets are towed at the surface to sample neuston. Neuston are those organisms associated with the water surface, where they are supported by surface tension. Scientist can determine the number of organisms per unit volume of water filtered.

Drift nets

Whales and dolphins have been caught in drift nets[7]

[8] Drift nets are not set or fixed in any way, are in fact ‘mobile’, and they are allowed to drift with the prevailing currents. Drift nets are used on the high seas for the capture of a wide range of fish including tuna, squid and shark, and off north-east England for salmon An EU-wide ban on all drift nets was introduced from January 2002 but problems still exist.

Gill nets

**Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE**

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gill net[9]

[8]Gill nets are walls of netting which may be set at or below the surface, on the seabed, or at any depth in between. Gill netting is probably the oldest form of net fishing, having been in use for thousands of years. True gill nets catch fish that attempt to swim through the net, which are caught if they are of a size large enough to allow the head to pass through the meshes but not the rest of the body. The fish then becomes entangled by the gills as it attempts to back out of the net. The mesh size used depends upon the species and size range being targeted

Fyke nets


fyke net[10]

[3]Fish communities in shallow water are sampled using fyke nets. A fyke net is a fish trap. It consists of cylindrical or cone-shaped netting bags mounted on rings or other rigid structures. It has wings or leaders which guide the fish towards the entrance of the bags. The fyke nets are fixed on the bottom by anchors, ballast or stakes.

Sampling tools for benthic organisms

[11]The type of gear selected for sampling seabed substrata and the benthic macrofauna at aggregate dredging sites is primarily determined by the hardness/ compactness of the substrata. Whilst a wide variety of sampling methods are available, only a small proportion of these have the ability to effectively collect samples from areas of relatively coarse sediments which are characteristic of dredging sites. In certain situations, it may be necessary to use more than one technique in order to sample the full range of benthic organisms present in an area.

Bottom Grabs

grab

[11]Grab sampling is the simple process of bringing up surface sediments from the seafloor. Once it is launched, the jaws of the grab sampler open and it descends to the seafloor. A spring closes the jaws, and they trap sediments or loose substrate. The grab sampler is then brought up to the surface where its contents are studied in detail.

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**Hamon grab**

Hammon grab

[11]The Hamon grab is the recommended tool for sampling the benthic macro-infauna from coarse substrata. This grab consists of a rectangular frame forming a stable support for a sampling bucket attached to a pivoted arm. On reaching the seabed, tension in the wire is released which activates the grab. Tension in the wire during in hauling then moves the pivoted arm through a rotation of 90°, driving the sample bucket through the sediment. At the end of its movement, the bucket locates onto an inclined rubber-covered steel plate, sealing it completely. This results in the sediment rolling towards the bottom of the sample bucket, thereby reducing the risk of gravel becoming trapped between the leading edge of the bucket and the sample retaining plate, and thus preventing part of the sample being washed out. Weights are attached to the grab to minimize the lateral movement of the supporting frame during sample collection. A drawback of the Hamon grab is that the sediment sample is mixed during the process of collection and retrieval, thereby precluding the examination or sub-sampling of an undisturbed sediment surface.

**Van Veen grab**

Van Veen grab

[11]The van Veen grab in common with many other grabs, relies on the closure of two opposing jaws for the collection of a sediment sample. The van Veen grab has long arms attached to each bucket, thus giving better leverage during closure. This mode of action is not ideally suited for the collection of coarse sediments as large particles of gravel tend to become caught between the jaws, resulting in loss of the sample upon retrieval of the grab. Thus, whilst this type of grab has been used widely in benthic macrofauna studies, it is not recommended for use on coarser substrata.

Bottom Trawl

Bottom trawls are commonly used for remotely sampling the epifauna. They are designed to sample at and just above the surface of the seabed and, because of the relatively large area that can be covered in one deployment, they are appropriate for collecting the larger, rarer or more motile species.

[12]The design requirements of a bottom trawl are relatively simple, a mechanism for keeping the mouth of the net open in horizontal and vertical dimensions, a "body" of net which guides fish inwards, and a "cod-end" of a suitable mesh size, where the fish are collected. The size and design of net used is determined by the species being targeted, the engine power and design of the fishing vessel and locally enforced regulations.

**Beam Trawling**

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[12]The simplest method of bottom trawling, the mouth of the net is held open by a solid metal beam, attached to two "shoes", which are solid metal plates, welded to the ends of the beam, which slide over and disturb the seabed. This method is mainly used on smaller vessels, fishing for flatfish or prawns, relatively close inshore

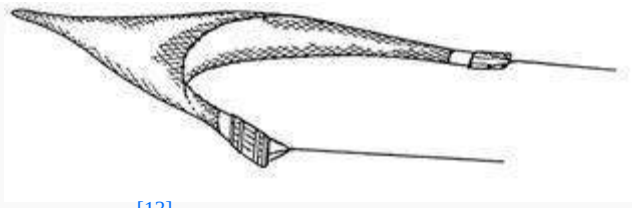
beam trawl

beam trawl [11]

**Otter trawling**

[12]Otter trawling derives its name from the "trawl doors" or "otters" which are used to keep the mouth of the net open. As these are towed along the seabed, hydrodynamic pressure pushes them outwards, preventing the mouth of the net closing. They also act like a plough, digging up to 15cm into the seabed, creating a turbid cloud, and scaring fish towards the trawl net mouth. The net is held open vertically on an otter trawl by floats and/or kites attached to the "headline" (the rope which runs along the upper mouth of the net), and weighted "bobbins" attached to the "foot rope" (the rope which runs along the lower mouth of the net). These bobbins vary in their design depending on the roughness of the sea bed which is being fished, varying from small rubber discs for very smooth, sandy ground, to large metal balls, up to 0.5 m in diameter for very rough ground. These bobbins can also be designed to lift the net off the seabed when they hit an obstacle. These trawls are commonly used to collect organisms from a sandy bottom.

otter trawl[1]



otter trawl[13]

**Shrimp trawling**

shrimp trawl[14]

[1]A special small-mesh bottom trawl is used to catch northern shrimp. This follows the basic design of the otter trawl, but with modified shape and dimensions. The groundrope and sweep are configured to optimize the capture of shrimp.

Dredges

[11]In general, the use of towed dredges for evaluation of epifaunal community structure should be avoided when other sampling tools (e.g. beam trawls) can be effectively employed. However, where the hard or uneven nature of the substrata precludes the use of a trawl it is often possible to obtain adequate samples using dredges, a variety of which are available

**Newhaven Scallop dredge**

Newhaven Scallop dredge[11]

[11]The Newhaven Scallop dredge is a commercially-used towed device that may be operated over very coarse terrain but would be likely to suffer damage if towed over bedrock or through large boulders. The dredge itself consists of a triangular steel frame supporting, on its underside, a spring-loaded plate to which a tooth bar, designed to dig into the sediment, is bolted. When the dredge encounters rock or large stones, the springs allow the tooth-bar to swing back thus avoiding snagging and reducing the quantity of stones caught. Also attached to each frame is a bag whose lower surface is made up of heavy-duty metal links with an upper surface of heavy gauge nylon mesh. The maximum diameter of particle likely to be retained within the dredge is approximately 20 mm. A number of these dredges may be attached to a robust metal beam which is fitted with large rubber rollers at each end.

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The dredges are deployed over the stern or side of a vessel and towed for a pre-determined time. Care must be taken to ensure that the dredge is deployed the right way up. The sampling efficiency of the dredge for each tow can be assessed on deck, normally by the quantity of material collected . The use of this device is recommended for the collection of qualitative samples as a last resort in areas of coarse, unconsolidated sediments which are too rough or uneven to permit the deployment of less robust gear. The Scallop dredge may be used to test the suitability of the ground prior to the deployment of less robust gear (e.g. beam trawl). This may be particularly useful if the ground is thought to be very coarse or uneven.

**Rallier-du-bathy dredge**

Rallier-du-bathy dredge[11]

[11]The Raillier-du-Baty dredge is designed to work in a range of substrata from sands to cobbles. It consists of a robust metal ring attached to a central towing arm. An open ended bag of the desired mesh size is attached to the ring, and the trailing end of the bag is tied to prevent loss of material during collection of the sample. This inner bag is protected by an outer, coarser bag which is, in turn, enclosed by a heavy duty apron of fishing net, in order to reduce chafing. The warp is attached to a fixing point on the metal ring, and a weak link is placed between this point and the central arm. This optimizes the digging capability of the edge of the ring and reduces the chances of the edge being lifted away from the seabed.

Corers

Corers work by boring a large tube into the benthos and then bringing up a column, or core, of sediment intact within the tube. Caps can automatically seal off the ends of the core after it has pulled up a sample, protecting the sample and keeping it intact. Different sizes and approaches work with different organisms and sediment types.

**The gravity corer**

gravity corer[15]

Gravity corers are widely used for the collection of the smallest marine metazoans (meiofauna) from subtidal grounds

[16]The gravity corer is basically a weighted tube mounted within a frame that descends by gravity from the research vessel to the sea floor, where it penetrates the sediment to a given depth, filling the tube with sediment in the process. The hydraulically-damped gravity corer has a slow rate of penetration that is controlled by a water-filled piston. Disturbance of the water-sediment interface is minimal and maximum penetration in mud is about 70 cm. A core-catcher on the bottom of the tube moves into place when retrieval begins, trapping the sediment sample in the corer. A winch, onboard the ship, slowly brings the corer back to the surface.

**Multiple corer**

multiple corer

To test local faunal variations, it is necessary to recover several discrete samples from a single station. The samples could be obtained by multiple casts with a single core tube, but this approach requires much valuable ship time. A multiple corer incorporates the separate coring tubes into a single core body. It consists of a system to which a series of tubes measuring about 4 cm in diameter are attached. Above the system, a weight is mounted and this falls down onto the assembly system when the multicorer touches the sediment. The falling weight drives the tubes into the sea bed so that when they are raised again each of them contains a drilling core with sediment from the sea floor. When studying the benthic communities, scientists then take account of the depth at which these tiny creatures were found below the sea floor.

**Reineck boxcorer**

Reineck boxcorer

[17]The boxcorer takes relatively undisturbed samples. The equipment operates by a self releasing trigger system triggered by the frame touching the sea bed. The square box is pushed into the bottom by gravity force of the weight mounted on the top of the box retainer. A spade freed by the trigger-mechanism closes the sample box during the recovery of the unit preventing the sample being washed-out.

**Multi boxcorer**

multi boxcorer

A multi boxcorer incorporates the separate coring tubes of a boxcorer into a single core body.

Hyperbenthos sledge

[18]The benthic carriage drags a net over the sea floor. This net is divided into various compartments one above the other so that the benthic communities can be collected whether they are close to or further away from the sea floor.

hyperbenthos sledge

hyperbenthos sledge

Hyperbenthos net

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hyperbenthos net

Instruments and sensors to measure environmental parameters
Measurement of environmental parameters

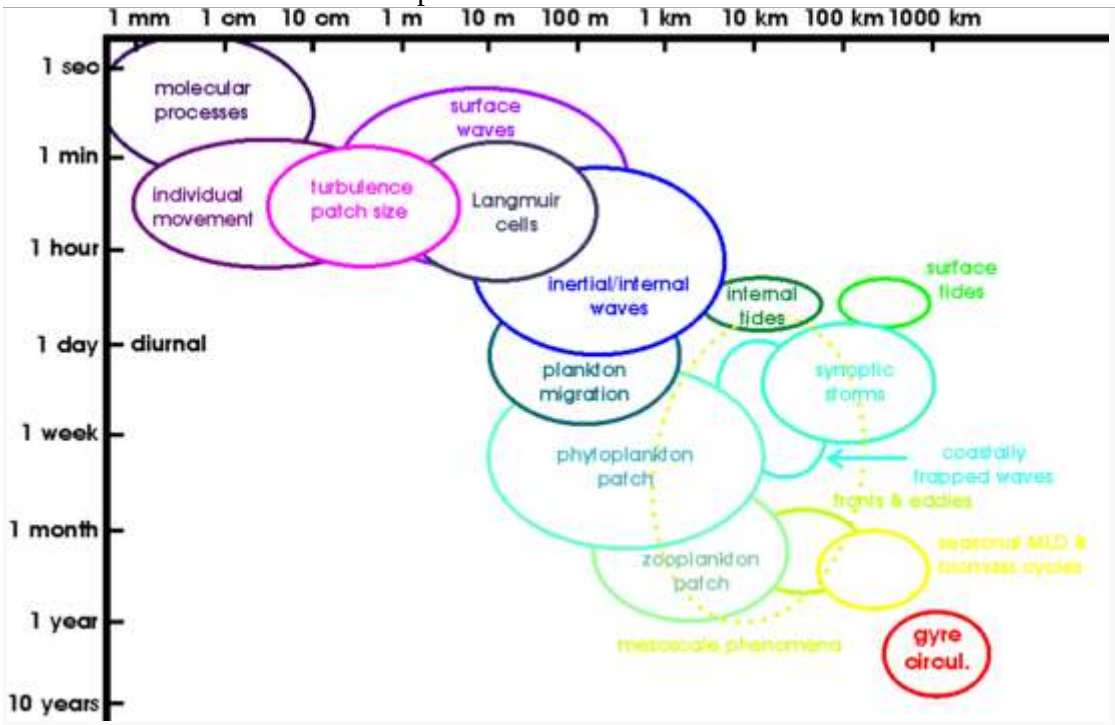


**Figure 1 Temporal and spatial scales of ocean processes**

The simplest way in which on can measure the environmental parameters of water, is to take samples and then analyze them after returning to the laboratory. It is a powerful approach since specialized laboratory equipment can be used to analyze a multitude of parameters. The main shortcomings of this approach are that only a limited number of measurements (samples) can be processed and the time between samples taken at the same location (to gain information about the temporal variation) usually spans from weeks to months. Processes that occur on time-scales shorter than weeks or episodic and transient events are therefore not captured. As a result, the importance of these processes and events for the distribution of parameters cannot be assessed.

In oceanography, there is a vast range of processes spanning many orders of time and space (see Figure 1). To allow for the investigation of these processes, a large volume of data must be gathered on the appropriate time and space scales. To achieve this task, instruments are needed that measure environmental parameters automatically in situ.

Oceanographic instruments

**Introduction**

**Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE**

# 2017

An oceanographic instrument generally consists of one or more sensors as well as a signal processing unit that converts the sensor signal and carries out scaling and conversion to engineering units and to the output data protocol. Figure 2 shows a schematization of an oceanographic instrument. The analyte (property to be measured) interacts with the detector (in some cases after a stimulus has been exerted by the instrument). The detector produces a signal, that is transformed into an electrical signal by the transducer. Detector and transducer together constitute the sensor. The electrical signal is fed to the signal processing (and conditioning) unit that creates the signal output of the instrument.

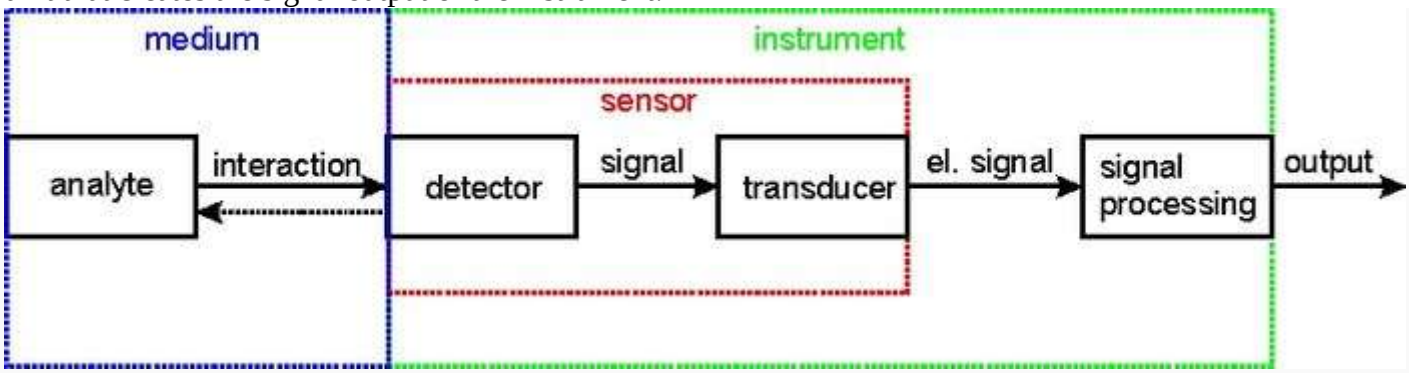


**Figure 2 Schematization of a generalised oceanographic instrument**

Oceanographic instruments can contain data loggers to store measurement data for readout after the deployment.

**Important properties**

- **Accuracy**: deviation of the measured value from the true value
- **Precision**: deviation of a measured value from another measured value of the same quantity (but at different environmental conditions (e.g. the two measurements taken at different temperatures))
- **Resolution**: smallest change in the measured quantity that can be detected by the instrument
- **Measurement rate**: number of measurements that can be carried out per unit time (e.g. measurements/hour)
- **Power consumption**: mean of electrical power uptake during deployment (usually measured in Watts [W])
- **Deployment time**: time period for which the instrument can be deployed (usually depends on environmental conditions, such as biofouling, or on stored energy and power consumption)

Sensors

**Introduction**

In an oceanographic instrument the stimulus can interact either directly with the detector (e.g. in a temperature, pressure or light sensor) or a stimulus can be exerted by the instrument. The stimulus is then modified by the property to be measured and then interacts with the detector, such as a fluorometer that sends out a light pulse (stimulus), which is transformed by chlorophyll fluorescence in the water (modification of stimulus). The transformed light (modified stimulus) then interacts with the detector.

If the detector signal is of a property (such as color) it can be converted to an electrical signal by a not an electrical signal (e.g. an optical signal or the change transducer). The sensor is made up of both the detector and the transducer.

**Types of sensors**

There are numerous sensors in oceanographic work:

*Some of the most commonly used are*

- Temperature sensors (under construction)
- Salinity sensors (under construction)
- Turbidity sensors such as

Secchi disk

Optical backscatter point sensor (OBS)

Optical transmissiometers (Theme 9 wanted page)

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- Oxygen sensors
- Fluorescence sensors
- Multi-probe sensors (Theme 9 wanted page)

*Less common are*

- pH sensors
- Optical Laser diffraction instruments (LISST)
- Flow cytometers
- pCO2 sensors
- Acoustic point sensors (ASTM, UHCM, ADV)
- Acoustic backscatter profiling sensors (ABS)

*Examples of specialized sensor systems are*

- Nutrient analyzers
- Trace metal analyzers (Theme 9 wanted page)
- Measuring instruments for fluid velocity, pressure and wave height
- Measuring instruments for sediment transport
- Instruments for bed level detection
- Waverider buoys (under construction)
- Underwater video systems

**Important properties**

- **Sensitivity**: The smallest change in the property being measured that leads to a measurable change in the detector signal.
- **Selectivity**: How those properties, other than the one being measured, lead to changes in the detector signal. High selectivity sensors exhibit little change in the detector signal from properties other than the one being measured.
- **Range**: The span between the extremes of the property being measured, at which no further change in the detector signal occurs.
- **Linearity**: A measure of how far equal amounts of change in the property being measured, lead to equal amounts of change in the detector signal.

**Life in Extreme Environments**

**Dry environments**

Imagine a desert and a feeling of dehydration follows. In the absence of water, lipids (fats) , proteins and nucleic acids (DNA, RNA) suffer structural damage. The Atacama desert located on the high northern Andean plains of Chile is one of the oldest, driest hot deserts on the Earth, while the Antarctic dry valleys are the coldest, driest places on Earth. In both cases, despite environmental extremes, life exists in the form of microbes: cyanobacteria, algae, lichens, and fungi.

Anhydrobiosis is a strategy organisms use to survive dry spells. During anhydrobiosis their cells come to contain only minimal amounts of water. No metabolic activity is performed. A variety of organisms can become anhydrobiotic, including bacteria, yeast, fungi, plants, insects, the aforementioned tardigrades, mycophagous (fungi-eating) nematodes, and the brine shrimp *Artemia salina* (also known as "Sea Monkeys" when marketed to school age children). During the drying out process (desiccation), less available water forces substances to increase in their concentration. Such increases lead to stressful responses within a cell that are similar to those of a cell experiences when exposed to high salt environments.

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The ultimate dry environment is the "desert" of space. Adaptations to desiccation are critical for organisms to survive in interplanetary space. One organism in particular (described below) is a natural born space traveler.

**Salinity**

As airplanes descend into the San Francisco area, red patches on the eastern shore of the South Bay are conspicuous. These are evaporation ponds of Cargill Salt Company. The cause of the red color is halophilic (salt-loving) microbes that produce red pigments called carotenoids. The microbes involved are either members of the Archaea, a major group of microbes superficially similar to bacteria, or the green alga Dunaliella salina. At a bit lower (25-33%) salinity, bacteria, cyanobacteria, other green algae, diatoms and protozoa are found. Some Archaea, cyanobacteria, and *Dunaliella salina* can even survive periods in saturated sodium chloride - about as salty an environment as one can imagine.

Salt water can evaporate leaving deposits ("evaporite deposits") consisting of salts such as sodium chloride (halite) and calcium sulfate (gypsum). Within evaporates are fluid inclusions - small trapped pockets of water - which can provide a refuge for microbes for at least six months. Our research group showed that cyanobacteria trapped within dry evaporite crusts can continue to have low levels of metabolic function such as photosynthesis. These deposits also form nice fossils of the organisms trapped within. Although highly controversial, others claim that bacteria might survive for millions of years in the fluid inclusions of salt deposits including evaporates. Tantalizingly, such deposits have been found on Mars.

So how do cells adapt to this potentially deadly environment? To prevent an exodus of water from the cell, halophiles offset the high salt in the environment by accumulating such compounds as potassium and glycine-betaine. This allows a balance of salts inside and outside of the cell preventing water from flowing outward as would be the case if lower salt levels existed within the cells.

**Acidity and Alkalinity**

Yellowstone National Park has bubbling acid hotsprings that would make a witch's cauldron seem benign. They also teem with life. Once again we have been astounded that such environments harbor life.

Acidity and alkalinity are measures of the concentration of protons, the units used are pH units. The lower the number (down to zero), the higher the acidity. The higher (up to 14), the more alkaline. A neutral pH near 7 is optimal for many biological processes, although some - such as the light reactions of photosynthesis - depend on pH gradients. In nature, pH can be high, such as in soda lakes or drying ponds, or as low as 0 and below. Organisms that live at either extreme do this by maintaining the near-neutral pH of their cytoplasm (i.e.) the liquid and materials within their cells.

Low pH is the realm of acidophiles - "acid lovers". If you are looking for champion acid lovers, forget fish and cyanobacteria which have not been found below pH 4, or even plants and insects which don't survive below pH 2 to 3. The extreme acidophiles are microbes. Several algae, such as the unicellular red alga *Cyanidium caldarium* and the green alga *Dunaliella acidophila*, are exceptional acidophiles both of which can live below pH 1. Three fungi, *Acontium cylatium, Cephalosporium sp.*, and *Trichosporon cerebriae*, grow near pH 0. Another species, *Ferroplasma acidarmanus*, has been found growing at pH 0 in acid mine drainage in Iron Mountain in California. These polyextremophiles (tolerant to multiple environmental extremes) thrive in a brew of sulfuric acid and high levels of copper, arsenic, cadmium, and zinc with only a cell membrane and no cell wall.

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**High Temperature**


Octopus Spring, an alkaline (pH 8.8Ð8.3) hotspring in Yellowstone National Park, USA, is situated several miles north of Old Faithful geyser. The water flows from the source at 95°C to an outflow channel, where it cools to a low of 83°C. About every 4Ð5 minutes a pulse of water surges from the source raising the temperature as high as 88°C. In this environment the pink filamentous *Thermocrinis ruber* thrives.

Temperature is a critical parameter because it determines whether liquid water is present. If temperature is too low, enzymatic activity slows, membrane fluidity decreases. Below freezing ice crystals form that slice through cell membranes. High temperatures can irreversibly alter the structure of biomolecules such as proteins, and increase membrane fluidity. The solubility of gasses in water is correlated with temperature, creating problems at high temperature for aquatic organisms requiring oxygen or carbon dioxide.

As it happens, organisms can outwit theory. Geysers, hotsprings, fumaroles and hydrothermal vents all house organisms living at or above the boiling point of water. The most hyperthermophilic (VERY hot loving) organisms are Archaea, with *Pyrolobus fumarii* (of the Crenarchaeota), a nitrate-reducing chemolithotroph (an organism that derives energy from minerals), capable of growing at up to 113°C, is the current champion. As such, these hyperthermophiles are able to prevent the denaturation and chemical modification (breakdown) of DNA which normally occurs at or around a comparatively cool 70°C. The stability of nucleic acids is enhanced by the presence of salts which protect the DNA from being destroyed.

Thermophily (living in hot places) is more common than living in scalding, ultra hot locales, and includes phototrophic bacteria (i.e., cyanobacteria, and purple and green bacteria who derive energy from photosynthesis), eubacteria (i.e., *Bacillus, Clostridium, Thiobacillus, Desulfotomaculum, Thermus,* lactic acid bacteria, *actinomycetes, spirochetes,* and numerous other genera), and the Archaea (i.e., *Pyrococcus, Thermococcus, Thermoplasma, Sulfolobus,* and the methanogens). In contrast, the upper limit for eukaryotes is ~ 60°C, a temperature suitable for some protozoa, algae, and fungi. The maximum temperature for mosses is another 10° lower, vascular plants (house plants, trees) about 48°C, and fish 40°C.

**Low Temperature**

Representatives of all major forms of life inhabit temperatures just below 0°C. Think winter, think polar waters. While sperm banks and bacterial culture collections rely on the preservation of live samples in liquid nitrogen at -196°C, the lowest recorded temperature for active microbial communities and animals is substantially higher at -18°C.

Freezing of water located within a cell is almost invariably lethal. The only exception to this rule known from nature is the nematode *Panagrolaimus davidi* which can withstand freezing of all of its body water. In contrast, freezing of extracellular water - water outside of cells - is a survival strategy used by a small number of frogs, turtles and one snake to protect their cells during the winter. Survival of freezing must include mechanisms to survive thawing, such as the production of special proteins or "cryoprotectants" (additives that protect against the cold) called "antifreeze" proteins. The other method to survive freezing temperatures is to avoid freezing in the first place. Again "antifreeze" molecules are produced which can lower the freezing point of water 9 to 18°C. Fish in Antarctic seas manage to employ these mechanisms to their advantage.

Other changes with low temperature include changes in the structure of a cell's proteins - most notably their enzymes - so as to allow them to function at lower temperatures. The fluidity of cell membranes decreases with

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temperature. In response, organisms that are able to adapt to cold environments simply increase the ratio of unsaturated to saturated fatty acids thus retaining the required flexibility of membranes.

**Radiation**

Radiation is a hazard even on a comfortable planet like Earth. Sunlight can cause major damage unless mechanisms are in place to repair - or at least limit - the damage. Humans lacking the capacity to repair ultraviolet (UV) damage have xeroderma pigmentosa. This disease is so serious that suffers cannot leave their house during the day unless completely covered, and must even shade the windows in their homes.

Once you leave the protected surface of Earth, things can get more hostile. One of the major problems that organisms might face during interplanetary transfer (inside a rock blasted off of a planet by a large impact event for example), living on Mars, or even at high altitudes on Earth is the high levels of UV (ultraviolet) radiation.

"A "tetrad" of *Deinococcus radiodurans* cells.

In space there is cosmic and galactic radiation to contend with as well. The dangers of UV and ionizing radiation range from inhibition of photosynthesis up to damage to nucleic acids. Direct damage to DNA or indirect damage through the production of reactive oxygen molecules creates can alter the sequence or even break DNA strands.

Several bacteria including two Rubrobacter species and the green alga *Dunaliella bardawil*, can endure high levels of radiation. *Deinococcus radiodurans*, on the other hand is a champ and can withstand up to 20 kGy of gamma radiation up to 1,000 joules per s02. meter of UV radiation. Indeed, *D. radiodurans* can be exposed to levels of radiation that blow its genome into pieces only to have the organism repair its genome and be back to normal operations in a day.

This extraordinary tolerance is accomplished through a unique repair mechanism which involves reassembling damaged (fragmented) DNA. Scientists at the Department of Energy are looking to augment *D. radiodurans* genome such that it can be used to clean up mixed toxic and radioactive spills. So eager are biotechnologist to understand just how *D. radiodurans* does what it does that its genome was among the first organisms to be fully sequenced.

**Gravity**

Gravity is a constant force in our lives; who has not imagined what it would be like to be an astronaut escaping gravity even temporarily? The universe offers a variety of gravitational experiences, from the near absence of gravity's effects in space (more accurately referred to as microgravity) to the oppressive gravitational regimes of planets substantially larger than ours.

Gravitational effects are more pronounced as the mass of an organism increases. That being said, flight experiments have revealed that even individual cells respond to changes in gravity. Cell cultures carried aboard various spacecraft including kidney cells and white blood cells showed marked alterations in their behavior, some of which is directly due to the absence of the effects of a strong gravity field. Indeed, recent work conducted aboard Space Shuttle missions has shown that there is a genetic component (as yet understood) to kidney cell responses to microgravity exposure.

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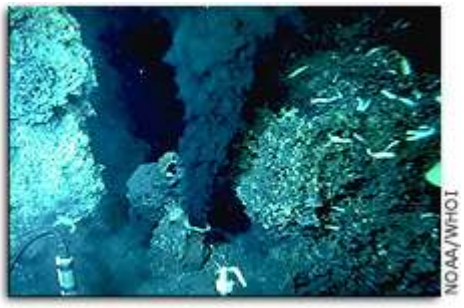
**Pressure**



A deep ocean hydrothermal vent belching sulfide-rich hot water. The black "smoke" is created as sulfide minerals form in the mixing process between vent water and colder ocean water. These minerals settle and can accumulate to great thicknesses.

Pressure increases with depth, be it in a water column or in rock. Hydrostatic (water) pressure increases at a rate of about one-tenth of an atmosphere per meter depth, whereas lithostatic (rock) pressure increases at about twice that rate. Pressure decreases with altitude, so that by 10 km above sea level atmospheric pressure is almost a quarter of that at sea level.

The boiling point of water increases with pressure, so water at the bottom of the ocean remains liquid at 400¡C. Because liquid water normally does not occur above ~100°C, increased pressure should increase the optimal temperature for microbial growth, but surprisingly pressure only extends temperature range by a few degrees suggesting that it is temperature itself that is the limiting factor.

The Marianas trench is the world's deepest sea floor at 10,898 m, yet it harbors organisms that can grow at temperature and pressure we experience everyday. It has also yielded obligately piezophilic species (i.e. organisms that are pressure loving and can only grow under high pressure) that can only grow at the immense pressures found at the ocean's greatest depths.

**Other extreme conditions**

A bit of creative thinking suggests other physical and chemical extremes not considered here, including unusual atmospheric compositions, redox potential, toxic or xenobiotic (manmade) compounds, and heavy metal concentration. There are even organisms such as *Geobacter metallireducens* that can survive immersion in high levels of organic solvents such as those found in toxic waste dumps. Others thrive inside the cooling water within nuclear reactors. While these organisms have received relatively little attention from the extremophile community, the search for life elsewhere may well rely on a better understanding of these extremes.

**Extremophiles and Astrobiology**

The study of extremophiles holds far more than Guinness Book of World Records-like fascination. Seemingly bizarre organisms are central to our understanding of where life may exist and where our own terrestrial life may one day travel. Did life on Earth originate in a hydrothermal vent? Will extremophiles be the pioneers that make Mars habitable for our own more parochial species?

Happily, extremophile research has lucrative side. Industrial processes and laboratory experiments may be far more efficient at extremes of temperature, salinity and pH, and so on. Natural products made in response to high levels of radiation or salt have been sold commercially. Glory too goes to those working with extremophiles. At least one Nobel Prize, that for the invention of the polymerase chain reaction (PCR), would not have been possible without an enzyme from a thermophile. As the world of molecular biology has become increasingly reliant on products from extremophiles, they will continue be the silent partner in future awards.

Current work on extremophiles in space focuses on four major environments: manned-flight vehicles, interplanetary space (because of the potential for panspermia), Mars and Europa because of the possibility of liquid water - and thus life.

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This Mars Global Surveyor spacecraft photo covers an area approximately 3 kilometers (1.9 miles) wide by 6.7 km (4.1 mi) high. The image shows gullies eroded into the wall of a meteor impact crater in Noachis Terra. Channels and associated aprons of debris that are thought to have been formed by groundwater seepage, surface runoff, and debris flow.

**Mars: Habitable?**

Mars is, at first blush, inhospitable. Temperatures are, for the most part, frigid, exposure to ultraviolet radiation is high, and the surface is highly oxidizing, precluding the presence of organic compounds on the surface. The atmospheric pressure is very low (similar to that of Earth's uppermost atmosphere) so liquid water is unstable on the surface. Yet hydrogeological evidence from Mars Global Surveyor hints that liquid water may even flow today under the surface. Previous evidence seems to show that it once flowed much more freely on the surface in ancient times.

Could Mars harbor subsurface life, similar to the subsurface or hydrothermal communities found on Earth? If so, it would be protected from surface radiation, damaging oxidants, and have access to liquid water. Mars is rich in carbon dioxide, the raw material used by plants to produce organic carbon. Life has been found at the depths of Earth's oceans and several kilometers below the surface inside of rocks. If it did arise during a warmer, wetter period in Mars' history, perhaps it managed to migrate into warmer, more clement regions of the planet's interior before the surface became uninhabitable.

**The Large Moons of Jupiter: Underground Oceans**

With evidence mounting that one or more of the large moons of Jupiter (Europa, Ganymede, Callisto) have ice-covered lakes, the possibility of life on these moons becomes a subject of scientific discourse. One of these, Europa, has an ice layer too thick to allow enough light to get through to allow photosynthesis, the process that drives much of terrestrial life including those under the perennially ice-covered lakes of Antarctica. However, Chris Chyba from the SETI Institute has suggested that chemistry in the ocean's ice cover, driven by charged particles accelerated in Jupiter's magnetosphere, could produce sufficient organic and oxidant molecules for a Europan biosphere to be sustained. The Galileo spacecraft has detected a weak magnetic field on Callisto, suggesting that salt water may lie beneath an ice-covered surface. Supportive evidence exists as well for an ocean with Ganymede. Several of Saturn's moons and other outer solar system bodies may also hold the potential for having a subsurface ocean.

**Naked in Space: The Ultimate Exposure**

Panspermia ("seeds spread far"), the idea that life can travel through space from one hospitable location to another is no longer wild speculation. Space is extremely cold, subject to unfiltered solar radiation, solar wind, galactic radiation, space vacuum, and to negligible gravity. But this treacherous realm can be crossed by life. [Table II]

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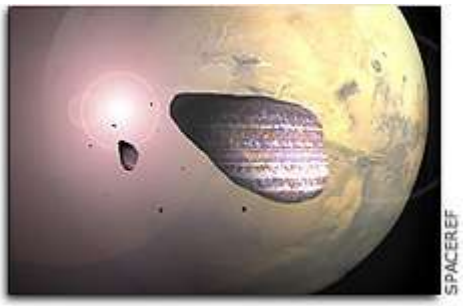
**TABLE II. Physical conditions prevailing in the interplanetary space environment**

| Parameter | Interplanetary Space |
|---|---|
| Pressure (Pa) | $10^{-14}$ |
| Solar electromagnetic radiation range | all |
| Cosmic ionizing radiation Gy/yr) | $\leq$ 0.1 |
| Gravity | < 10-6* |
| Temperature (K) | 4* |




Over the history of the solar large impact events may have served as a steady means of transporting rocks (which arrived as meteorites) between one world or another. Whether or not any of these rocks ever actually contained viable life forms is not known. However recent studies suggest that this is possible.

We know from Mars meteorites such as the (now) famous ALH84001 sample that a natural vehicle exists for interplanetary transport. These meteorites contain organic compounds from Mars, showing that such compounds can survive the journey. Moreover, studies have shown that given a rock of sufficient size, conditions within a rock thrown off of Mars - and then later entering Earth's atmosphere - can remain cool enough such that not just organic material - but also microbes contained within - could (theoretically) survive the trip.

The criticism that life cannot endure extended periods in space is now being tested experimentally in space simulation facilities in the U.S. and Germany, and through unmanned flight experiments. NASA's Long Duration Exposure Facility and the European Space Agency's BioPan space experiments showed that microbes can survive direct exposure to the raw conditions of space. Survivors to date include spores of *Bacillus subtilis* and halophiles in the active (vegetative) state. Hopes for further experiments of this nature rest on both unmanned flights and the ESA Exposed Facility planned for the International Space Station.

**Summary**

Earth provides us with a wondrous array of life's adaptations. Indeed, by studying the extremophiles here on Earth, we may get the first clear indication of what ET could be like - or at least the range of things they might eat and breathe.

**Life in Extreme Environments: The Universe May Be More Habitable Than We Thought**

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Artist's concept of an astronaut examining a rock sample on Mars. Life has been found living inside rocks in the various extreme environments on Earth. Clues derived from finding life in such terrestrial locations will serve as a guide to understanding where we might find life on other worlds.

Clearly there are physical and chemical extremes that should make life based on organic carbon difficult if not impossible. Yet, within the last few decades we have found organisms that have punctured these seemingly insurmountable limits and have come to called "extremophiles" from the Latin "extremus" (being on the outside) and the Greek "philos" for love. Organisms that can live in more than one extreme, for example *Sulfalobus acidocaldarius* (a member of the Archea - an ancient branch off the family tree of life) lives at pH 3 and 80°C, are called polyextremophiles.

**Who are the extremophiles?**

The word "Extremophile" often invokes images of microbes, and so-called "simple" ones at that, yet the taxonomic range spans all three domains. (Note that life itself is so complex that the human creation of life has remained elusive. Thus, it is unjustifiably arrogant of us to call any form of life "simple".) While all organisms that live at extremely high temperatures are Archaea or Bacteria, eukaryotes (organisms whose cells have nuclei) are common among organisms that thrive at low temperature, extremes of pH (high acidity or alkalinity) pressure, water, and salt levels. Extremophiles include multicellular organisms, cold-lovers include vertebrates such as penguins and polar bears.

To qualify as an extremophile, does an organism have to be an extremophile during all life stages? Under all conditions? Not at all. Spores, seeds, and sometimes eggs or larval stages are all far more resistant to environmental extremes than adult forms. Yet some adult organisms - trees, frogs, insects, and fish - can endure remarkably low temperatures during the winter as a result of seasonal shifts in physiology such as hibernation.

One of the most resilient organisms known are tardigrades ("water bears"). Tardigrades can go into a hibernation mode - called the tun state - one that is more akin to "suspended animation" whereby it can survive temperatures from -253°C to 151°C, as well as exposure to x-rays, and vacuum conditions. When you place tardigrades in perfluorocarbon fluid (again while hibernating), at a pressure of 600 MPa, (that's almost 6,000 times atmospheric pressure at sea level) they emerge from the experience just fine . Even the bacterium *Deinococcus radiodurans*, the most radiation resistant organism known, only achieves this resistance under some conditions such as fast growth and in nutrient-rich medium.


A Scanning Electron Micrograph of a Tartigrade

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**Classification and examples of extremophiles**

| Environmental parameter | type | Definition | examples |
|---|---|---|---|
| **temperature** | hyperthermophile<br>thermophile<br>mesophile<br>psychrophile | growth >80°C<br>growth 60-80°C<br>15-60°C<br><15°C | *Pyrolobus fumarii*, 113°C<br>*Synechococcus lividis*<br>*Homo sapiens*<br>*Psychrobacter*, some insects |
| **radiation** | | | *Deinococcus radiodurans* |
| **pressure** | barophile<br>piezophile | Weight loving<br>Pressure loving | unknown<br>For microbe, 130 MPa |
| **vacuum** | | tolerates vacuum (space devoid of matter) | tardigrades, insects, microbes, seeds. |
| **desiccation** | xerophiles | Anhydrobiotic | *Artemia salina*; nematodes, microbes, fungi, lichens |
| **salinity** | halophile | Salt loving | *Halobacteriacea, Dunaliella salina* |
| **pH** | alkaliphile<br><br>acidophile | pH >9<br><br>low pH loving | *Natronobacterium, Bacillus firmus* OF4, *Spirulina spp.* (all pH 10.5)<br>*Cyanidium caldarium, Ferroplasma sp.* (both pH 0) |
| **oxygen tension** | anaerobe<br>microaerophil<br>aerobe | cannot tolerate $O_2$<br>tolerates some $O_2$<br>requires $O_2$ | *Methanococcus jannaschii*<br>*Clostridium*<br>*Homo sapiens* |
| **chemical extremes** | gases<br>metals | Can tolerate high concentrations of metal (metalotolerant) | *Cyanidium caldarium* (pure $CO_2$)<br>*Ferroplasma acidarmanus*(Cu, As, Cd, Zn); *Ralstonia* sp. CH34 (Zn, Co, Cd, Hg, Pb) |

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17MBP105A
I MSC MICROBIOLOGY
MARINE MICROBIOLOGY

| Unit I Ques | option 1 | option 2 | option 3 | option 4 | answer |
|---|---|---|---|---|---|
| Attachment of small particles or molecules to a larger particle by electric charge is called as | Adsorption | absorption | fixation | attachment | absorption |
| _____ _____ is derived from an environment other than that in which it is found. | autothonous | Allochthonous | hetrothonous | xenothonous | autothonous |
| _____ ___are organism which grows at high pressure rather than at atmospheric pressure. | Barophile | halophile | thermophile | neutrophil | Barophile |

| | | | | | | |
|---|---|---|---|---|---|---|
| _____ ____ is Mass of living matter present | biogroup | Biomass | biodiverse | bioaccumulation | | Biomass |
| Particulate (organic) material which is only partly disintegrated is called as_____ __ | demeris | divergent | detritus | debris | | detritus |
| An organism which grows preferentially in high salinities. | Halophile | Barophile | Chemophile | Divergephile | | Halophile |
| Living together of two organisms with mutual advantage and without losing their identity is called as | Antagonism | Commensalism | Symbiosis | Mutualism | | Commensalism |

| | | | | | | |
|---|---|---|---|---|---|---|
| Which were the investigators lived at the same time? | Koch and Pasteur | Darwin and Woese | Van Leeuenhoek and Ricketts | Berg and Hooke | | Koch and Pasteur |
| The unifying | habitats which are | absence of a | presence of a cell | cytoplasmic | | habitats which are |
| Organisms can synthesize ATP by oxidative phosphorylation when they | ferment | oxidize glucose to pyruvate | pass electrons from the oxidation of chlorophyll through an electron transport system | pass electrons to oxygen through an electron transport system containing cytochromes | | oxidize glucose to pyruvate |
| How many molecules of carbon dioxide will be given off during ten turns of the Krebs cycle? | 10 | 20 | 30 | 40 | | 10 |
| In cellular metabolism, O2 is used | to provide electrons for photophosphorylation | in glycolysis | as a terminal electron acceptor | in the Krebs cycle | | to provide electrons for photophosphorylation |

| | | | | | |
|---|---|---|---|---|---|
| In glycolysis, ATP is created by | photophosphorylation | the chemiosmotic mechanism | substrate level phosphorylation | the pentose phosphate pathway | substrate level phosphorylation |
| The concept of putting microbes to help clean up the environment is called | pasteurization | bioremediation | fermentation | biolistics | bioremediation |
| If a canning procedure is not properly followed, which type of microbe is most likely to grow in the canned food? | Obligate Aerobe | Acidophile | Mesophile | Obligate Anaerobe | Obligate Aerobe |

| | | | | | |
|---|---|---|---|---|---|
| Some cyanobacteria produce potent neurotoxins that, if ingested, will kill humans. These cyanobacteria are most likely to contaminate | water rich in organic carbon wastes but poor in phosphate | water that are anoxic | water rich in phosphate wastes but poor in organic carbon | none of the above | water rich in organic carbon wastes but poor in phosphate |
| A musty or muddy | the growth of | the mud at the | the growth of | none of the above | the growth of |
| The predominant kind of bacteria causing spoilage in fish at chilling temperature is | species of *Pseudomonas* | *Micrococcus* | *Bacillus* | E.coli | species of *Pseudomonas* |
| Preservation of foods by using salts and sugars works by | raising pH | lowering osmotic pressure | creating a hypertonic environment | creating a hypotonic environment | raising Ph |

| | | | | | | |
|---|---|---|---|---|---|---|
| Which of the following bacteria lack a cell wall and are therefore resistant to penicillin? | Cyanobacteria | Mycoplasmas | Bdellovibrios | Spirochetes | | Mycoplasmas |
| Chemotaxis is a phenomenon of | swimming away of bacteria | swimming towards bacteria | swimming away or towards of bacteria in presence of chemical compound | Swimming in media | | swimming away or towards of bacteria in presence of chemical compound |
| The structure responsible for motility of bacteria is | pilli | flagella | sheath | capsules | | flagella |
| _____ group of bacteria grows in high pressure. | Halophiles | thermophiles | Basophiles | psychrophiles | | Basophiles |

| | | | | | |
|---|---|---|---|---|---|
| The group of gram positive bacteria having high G+C contents are called as | cyanobacteria | Nanobacteria | Firmicutes | Actinobacteria | Actinobacteria |
| _____ group of bacteria grows in high temperature | Halophiles | Basophiles | thermophiles | psychrophiles | thermophiles |
| The group of gram positive bacteria having low G+C contents are called as | cyanobacteria | Nanobacteria | Firmicutes | Actinobacteria | Firmicutes |
| BGA expanded as | Blue Green Algae | Blue Grown Algae | Blue non Grown Algae | Brown Green Algae | Blue Green Algae |
| Bacteria are | Obligate | single celled | multicellular | seen by naked | single celled |
| Who is father of Marine Microbiology? | Leewenhoek | Zobell | Edward Jenner | Louis Pasteur | Zobell |
| Strain means_____ | dye | Agent | Bacteria | organisms | organisms |

| | | | | | | |
|---|---|---|---|---|---|---|
| Prokayotic ribosomes are made up of _____ _ subunits | Two | Three | Five | ten | | Two |
| The time required to kill 90% of the microorganisms in a sample at a specific temperature is the | decimal reduction time | thermal death point | F value | D value | | decimal reduction time |
| Cyanobacteria have | a gram-positive cell wall | a gram-negative cell wall | no cytoplasm | No cell wall | | a gram-positive cell wall |
| The unifying feature of the archaea that distinguishes them from the bacteria is | habitats which are extreme environments with regard to acidity | absence of a nuclear membrane temperature | presence of a cell wall containing a characteristic outer membrane | cytoplasmic ribosomes that are 70S | | habitats which are extreme environments with regard to acidity |

| | | | | | | |
|---|---|---|---|---|---|---|
| Suppose a eukaryotic cell had a mutation that prevented the production of cytochrome c. As a result of this mutation, which of the following processes would not occur? | Cellular respiration | Photosynthesis | Mitosis | Cell wall synthesis | | Photosynthesis |
| In cellular metabolism, O2 is used | to provide electrons for photophosphorylation | in glycolysis | as a terminal electron acceptor | in the Krebs cycle | | to provide electrons for photophosphorylation |
| The bacteria most often involved in the spoilage of fish are | Sarcina | Micrococcus or Bacillus species | Molds or yeasts | virus | | Molds or yeasts |

| | | | | | | |
|---|---|---|---|---|---|---|
| The red or pink color of the fish is generally caused from the growth of | part of the natural flora of the external slime of fishes and their intestinal contents | part of the natural flora of the internal slime of fishes only | no chage in structure | coliorms | | part of the natural flora of the external slime of fishes and their intestinal contents |
| The Archaea include all of the following except | methanogens | halophiles | thermoacidophiles | cyanobacteria | | cyanobacteria |
| The ocean contains _____ ___ bacteria per milliliter (mL) of water. | $10^5$ - $10^7$ | $10^7 – 10^9$ | $10^6$ - $10^7$ | $10^5 – 10^8$ | | $10^7 – 10^9$ |
| A major cause of waterborne disease is the bacterium *Vibrio cholerae* , which causes___ _____ | Cholera | dysentry | diarhoea | vomiting | | Cholera |

| | | | | | | |
|---|---|---|---|---|---|---|
| _____ __ causes diarrhea, urinary tract infections, bacteremia, and meningitis. | *Vibrio cholerae* | *E. coli* | *Salmonella typhi* | *Serratia* | | *Salmonella typhi* |
| When the _____ _ is ingested by drinking, the mature adult spreads in the human host where it reproduces just below the skin. | copepod | coliforms | plankton | gastropod | | copepod |
| Which of the following is a characteristic unique to the ciliates? | use flagella | Presence of both a macronucleus and several micronuclei | no cilia no flagella | Possess a light-detecting eye spot | | Presence of both a macronucleus and several micronuclei |

| | | | | | | |
|---|---|---|---|---|---|---|
| Suppose a eukaryotic cell had a mutation that prevented the production of cytochrome c. As a result of this mutation, which of the following processes would not occur? | Cellular respiration | Photosynthesis | Mitosis | Cell wall synthesis | | Photosynthesis |
| Some cyanobacteria produce potent neurotoxins that, if ingested, will kill humans. These cyanobacteria are most likely to contaminate | water rich in organic carbon wastes but poor in phosphate | water that is anoxic | water rich in phosphate wastes but poor in organic carbon | water rich in organic matters | | water rich in organic carbon wastes but poor in phosphate |

| | | | | | | |
|---|---|---|---|---|---|---|
| The cell walls of many gram positive bacteria can be easily destroyed by the enzyme known as | lipase | lysozyme | pectinase | peroxidase | | lysozyme |
| Bacteria reproduce by _____ mechanism | fission | own | fusion | Direct | | fission |
| Bacteria are sensitive to _____ | Interleukins | Interferons | Antibiotics | Antitumours | | Antibiotics |
| _____ __media is used for cultivation of bacteria | Nutrient agar | MacConkey agar | EMB agar | MHA | | Nutrient agar |
| Single bacteria will form a _____ __ colony | Multiple | Single | No | infinite | | Single |
| Which instrument is used for sterilization above 100° C | Flame | Autoclave | Filters | Desiccators | | Autoclave |

| | | | | | | |
|---|---|---|---|---|---|---|
| _____ is the first phase in growth curve | Log | Lag | stationary | death | | Lag |
| The last step in synthesis of peptidoglycan is | attachment of a peptide to muramic acid | attaching two amino acids to form a cross-link | attachment of a portion of peptidoglycan to a membrane lipid | binding of penicillin to a membrane protein | | attaching two amino acids to form a cross-link |
| Cytoplasmic inclusions exclude | ribosomes | mesosomes | fat globules | flagella | | flagella |
| The cocci which forms a bunch and irregular pattern are | Staphylococci | diplococcic | Tetracocci | Streptococci | | Staphylococci |
| Chemotaxis is a phenomenon of | swimming away of bacteria | swimming towards bacteria | swimming away or towards of bacteria in presence of chemical compound | Swimming in broth. | | swimming away or towards of bacteria in presence of chemical compound |

| | | | | | | |
|---|---|---|---|---|---|---|
| .The structure responsible for motility of bacteria is | pilli | flagella | sheath | capsules | | flagella |
| _____ ____ is Mass of living matter present. | biogroup | Biomass | biodiverse | bioaccumulation | | Biomass |

# 2017

## I M.Sc Microbiology – Marine microbiology

| LECTURE PLAN - UNIT -11 | | | |
|---|---|---|---|
| **S. no** | Lecture duration(Hr) | **Topics covered** | **Supporting materials** |
| 1 | 1 | Extremophiles | T2 643-646 |
| 2 | 1 | Halophiles, Psychrophiles | T2 647-648 |
| 3 | 1 | Acid – alkalinophiles, oligotroph | T2 648-649 |
| 4 | 1 | Toxitolerant, Xerotolerant | T2 649-651 |
| 5 | 1 | Endolth – extremophile | T2 652 |
| 6 | 1 | Biodiversity | T1 543 |
| 7 | 1 | Genomics of extremophiles | T2 673-675 |
| 8 | 1 | 16s r RNA classification and phylogenetic tree | W1 |
| 9 | 1 | RAPD | T3 640-645 |
| 10 | 1 | RFLP | T3 646-650 |
| 11 | 1 | UNIT II Discussion | |
| 12 | 1 | UNIT I Test | |
| **Textbooks :** | | T1-Microbiology- pelczar – McGraw Hill publishing<br>T2-Microbiology- presscott – McGraw hill publishing<br>T3-Microbial genetics – David friefelder- | |
| **Reference books:** | | | |
| **Website:** | | W1- www.microbiology.com<br>W2 – www.marinestudy.com | |
| **Journals:** | | | |

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# 2017

**Life in Extreme Environments**

**Dry environments**

Imagine a desert and a feeling of dehydration follows. In the absence of water, lipids (fats) , proteins and nucleic acids (DNA, RNA) suffer structural damage. The Atacama desert located on the high northern Andean plains of Chile is one of the oldest, driest hot deserts on the Earth, while the Antarctic dry valleys are the coldest, driest places on Earth. In both cases, despite environmental extremes, life exists in the form of microbes: cyanobacteria, algae, lichens, and fungi.

Anhydrobiosis is a strategy organisms use to survive dry spells. During anhydrobiosis their cells come to contain only minimal amounts of water. No metabolic activity is performed. A variety of organisms can become anhydrobiotic, including bacteria, yeast, fungi, plants, insects, the aforementioned tardigrades, mycophagous (fungi-eating) nematodes, and the brine shrimp *Artemia salina* (also known as "Sea Monkeys" when marketed to school age children). During the drying out process (desiccation), less available water forces substances to increase in their concentration. Such increases lead to stressful responses within a cell that are similar to those of a cell experiences when exposed to high salt environments.

The ultimate dry environment is the "desert" of space. Adaptations to desiccation are critical for organisms to survive in interplanetary space. One organism in particular (described below) is a natural born space traveler.

**Salinity**

As airplanes descend into the San Francisco area, red patches on the eastern shore of the South Bay are conspicuous. These are evaporation ponds of Cargill Salt Company. The cause of the red color is halophilic (salt-loving) microbes that produce red pigments called carotenoids. The microbes involved are either members of the Archaea, a major group of microbes superficially similar to bacteria, or the green alga Dunaliella salina. At a bit lower (25-33%) salinity, bacteria, cyanobacteria, other green algae, diatoms and protozoa are found. Some Archaea, cyanobacteria, and *Dunaliella salina* can even survive periods in saturated sodium chloride - about as salty an environment as one can imagine.

Salt water can evaporate leaving deposits ("evaporite deposits") consisting of salts such as sodium chloride (halite) and calcium sulfate (gypsum). Within evaporates are fluid inclusions - small trapped pockets of water - which can provide a refuge for microbes for at least six months. Our research group showed that cyanobacteria trapped within dry evaporite crusts can continue to have low levels of metabolic function such as photosynthesis. These deposits also form nice fossils of the organisms trapped within. Although highly controversial, others claim that bacteria might survive for millions of years in the fluid inclusions of salt deposits including evaporates. Tantalizingly, such deposits have been found on Mars.

So how do cells adapt to this potentially deadly environment? To prevent an exodus of water from the cell, halophiles offset the high salt in the environment by accumulating such compounds as potassium and glycine-betaine. This allows a balance of salts inside and outside of the cell preventing water from flowing outward as would be the case if lower salt levels existed within the cells.

**Acidity and Alkalinity**

Yellowstone National Park has bubbling acid hotsprings that would make a witch's cauldron seem benign. They also teem with life. Once again we have been astounded that such environments harbor life.

Acidity and alkalinity are measures of the concentration of protons, the units used are pH units. The lower the number (down to zero), the higher the acidity. The higher (up to 14), the more alkaline. A neutral pH near 7 is optimal for many biological processes, although some - such as the light reactions of photosynthesis - depend on pH gradients. In nature, pH can be high, such as in soda lakes or drying ponds, or as low as 0 and below. Organisms that live at either extreme do this by maintaining the near-neutral pH of their cytoplasm (i.e.) the liquid and materials within their cells.

Low pH is the realm of acidophiles - "acid lovers". If you are looking for champion acid lovers, forget fish and cyanobacteria which have not been found below pH 4, or even plants and insects which don't survive below pH 2 to 3. The extreme acidophiles are microbes. Several algae, such as the unicellular red alga *Cyanidium caldarium* and the green alga *Dunaliella acidophila*, are exceptional acidophiles both of which can live below

pH 1. Three fungi, *Acontium cylatium, Cephalosporium sp.,* and *Trichosporon cerebriae,* grow near pH 0. Another species, *Ferroplasma acidarmanus,* has been found growing at pH 0 in acid mine drainage in Iron Mountain in California. These polyextremophiles (tolerant to multiple environmental extremes) thrive in a brew of sulfuric acid and high levels of copper, arsenic, cadmium, and zinc with only a cell membrane and no cell wall.

**High Temperature**

Temperature is a critical parameter because it determines whether liquid water is present. If temperature is too low, enzymatic activity slows, membrane fluidity decreases. Below freezing ice crystals form that slice through cell membranes. High temperatures can irreversibly alter the structure of biomolecules such as proteins, and increase membrane fluidity. The solubility of gasses in water is correlated with temperature, creating problems at high temperature for aquatic organisms requiring oxygen or carbon dioxide.


Octopus Spring, an alkaline (pH 8.8Ð8.3) hotspring in Yellowstone National Park, USA, is situated several miles north of Old Faithful geyser. The water flows from the source at 95°C to an outflow channel, where it cools to a low of 83°C. About every 4Ð5 minutes a pulse of water surges from the source raising the temperature as high as 88°C. In this environment the pink filamentous *Thermocrinis ruber* thrives.

As it happens, organisms can outwit theory. Geysers, hotsprings, fumaroles and hydrothermal vents all house organisms living at or above the boiling point of water. The most hyperthermophilic (VERY hot loving) organisms are Archaea, with *Pyrolobus fumarii* (of the Crenarchaeota), a nitrate-reducing chemolithotroph (an organism that derives energy from minerals), capable of growing at up to 113°C, is the current champion. As such, these hyperthermophiles are able to prevent the denaturation and chemical modification (breakdown) of DNA which normally occurs at or around a comparatively cool 70°C. The stability of nucleic acids is enhanced by the presence of salts which protect the DNA from being destroyed.

Thermophily (living in hot places) is more common than living in scalding, ultra hot locales, and includes phototrophic bacteria (i.e., cyanobacteria, and purple and green bacteria who derive energy from photosynthesis), eubacteria (i.e., *Bacillus, Clostridium, Thiobacillus, Desulfotomaculum, Thermus,* lactic acid bacteria, *actinomycetes, spirochetes,* and numerous other genera), and the Archaea (i.e., *Pyrococcus, Thermococcus, Thermoplasma, Sulfolobus,* and the methanogens). In contrast, the upper limit for eukaryotes is ~ 60°C, a temperature suitable for some protozoa, algae, and fungi. The maximum temperature for mosses is another 10° lower, vascular plants (house plants, trees) about 48°C, and fish 40°C.

**Low Temperature**

Representatives of all major forms of life inhabit temperatures just below 0°C. Think winter, think polar waters. While sperm banks and bacterial culture collections rely on the preservation of live samples in liquid nitrogen at -196°C, the lowest recorded temperature for active microbial communities and animals is substantially higher at -18°C.

Freezing of water located within a cell is almost invariably lethal. The only exception to this rule known from nature is the nematode *Panagrolaimus davidi* which can withstand freezing of all of its body water. In contrast, freezing of extracellular water - water outside of cells - is a survival strategy used by a small number of frogs, turtles and one snake to protect their cells during the winter. Survival of freezing must include mechanisms to survive thawing, such as the production of special proteins or "cryoprotectants" (additives that protect against the cold) called "antifreeze" proteins. The other method to survive freezing temperatures is to avoid freezing in the first place. Again "antifreeze" molecules are produced which can lower the freezing point of water 9 to 18°C. Fish in Antarctic seas manage to employ these mechanisms to their advantage.

Other changes with low temperature include changes in the structure of a cell's proteins - most notably their enzymes - so as to allow them to function at lower temperatures. The fluidity of cell membranes decreases with temperature. In response, organisms that are able to adapt to cold environments simply increase the ratio of unsaturated to saturated fatty acids thus retaining the required flexibility of membranes.

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# 2017

**Radiation**

Radiation is a hazard even on a comfortable planet like Earth. Sunlight can cause major damage unless mechanisms are in place to repair - or at least limit - the damage. Humans lacking the capacity to repair ultraviolet (UV) damage have xeroderma pigmentosa. This disease is so serious that suffers cannot leave their house during the day unless completely covered, and must even shade the windows in their homes.

Once you leave the protected surface of Earth, things can get more hostile. One of the major problems that organisms might face during interplanetary transfer (inside a rock blasted off of a planet by a large impact event for example), living on Mars, or even at high altitudes on Earth is the high levels of UV (ultraviolet) radiation.

"A "tetrad" of *Deinococcus radiodurans* cells.

In space there is cosmic and galactic radiation to contend with as well. The dangers of UV and ionizing radiation range from inhibition of photosynthesis up to damage to nucleic acids. Direct damage to DNA or indirect damage through the production of reactive oxygen molecules creates can alter the sequence or even break DNA strands.

Several bacteria including two Rubrobacter species and the green alga *Dunaliella bardawil*, can endure high levels of radiation. *Deinococcus radiodurans*, on the other hand is a champ and can withstand up to 20 kGy of gamma radiation up to 1,000 joules per s02. meter of UV radiation. Indeed, *D. radiodurans* can be exposed to levels of radiation that blow its genome into pieces only to have the organism repair its genome and be back to normal operations in a day.

This extraordinary tolerance is accomplished through a unique repair mechanism which involves reassembling damaged (fragmented) DNA. Scientists at the Department of Energy are looking to augment *D. radiodurans* genome such that it can be used to clean up mixed toxic and radioactive spills. So eager are biotechnologist to understand just how *D. radiodurans* does what it does that its genome was among the first organisms to be fully sequenced.

**Gravity**

Gravity is a constant force in our lives; who has not imagined what it would be like to be an astronaut escaping gravity even temporarily? The universe offers a variety of gravitational experiences, from the near absence of gravity's effects in space (more accurately referred to as microgravity) to the oppressive gravitational regimes of planets substantially larger than ours.

Gravitational effects are more pronounced as the mass of an organism increases. That being said, flight experiments have revealed that even individual cells respond to changes in gravity. Cell cultures carried aboard various spacecraft including kidney cells and white blood cells showed marked alterations in their behavior, some of which is directly due to the absence of the effects of a strong gravity field. Indeed, recent work conducted aboard Space Shuttle missions has shown that there is a genetic component (as yet understood) to kidney cell responses to microgravity exposure.

**Pressure**

Pressure increases with depth, be it in a water column or in rock. Hydrostatic (water) pressure increases at a rate of about one-tenth of an atmosphere per meter depth, whereas lithostatic (rock) pressure increases at about twice that rate. Pressure decreases with altitude, so that by 10 km above sea level atmospheric pressure is almost a quarter of that at sea level.

A deep ocean hydrothermal vent belching sulfide-rich hot water. The black "smoke" is created as sulfide minerals form in the mixing process between vent water and colder ocean water. These minerals settle and can accumulate to great thicknesses.

The boiling point of water increases with pressure, so water at the bottom of the ocean remains liquid at 400¡C. Because liquid water normally does not occur above ~100°C, increased pressure should increase the optimal temperature for microbial growth, but surprisingly pressure only extends temperature range by a few degrees suggesting that it is temperature itself that is the limiting factor.

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The Marianas trench is the world's deepest sea floor at 10,898 m, yet it harbors organisms that can grow at temperature and pressure we experience everyday. It has also yielded obligately piezophilic species (i.e. organisms that are pressure loving and can only grow under high pressure) that can only grow at the immense pressures found at the ocean's greatest depths.

**Other extreme conditions**

A bit of creative thinking suggests other physical and chemical extremes not considered here, including unusual atmospheric compositions, redox potential, toxic or xenobiotic (manmade) compounds, and heavy metal concentration. There are even organisms such as *Geobacter metallireducens* that can survive immersion in high levels of organic solvents such as those found in toxic waste dumps. Others thrive inside the cooling water within nuclear reactors. While these organisms have received relatively little attention from the extremophile community, the search for life elsewhere may well rely on a better understanding of these extremes.

# 2017

**Extremophiles and Astrobiology**

The study of extremophiles holds far more than Guinness Book of World Records-like fascination. Seemingly bizarre organisms are central to our understanding of where life may exist and where our own terrestrial life may one day travel. Did life on Earth originate in a hydrothermal vent? Will extremophiles be the pioneers that make Mars habitable for our own more parochial species?

Happily, extremophile research has lucrative side. Industrial processes and laboratory experiments may be far more efficient at extremes of temperature, salinity and pH, and so on. Natural products made in response to high levels of radiation or salt have been sold commercially. Glory too goes to those working with extremophiles. At least one Nobel Prize, that for the invention of the polymerase chain reaction (PCR), would not have been possible without an enzyme from a thermophile. As the world of molecular biology has become increasingly reliant on products from extremophiles, they will continue be the silent partner in future awards.

Current work on extremophiles in space focuses on four major environments: manned-flight vehicles, interplanetary space (because of the potential for panspermia), Mars and Europa because of the possibility of liquid water - and thus life.


This Mars Global Surveyor spacecraft photo covers an area approximately 3 kilometers (1.9 miles) wide by 6.7 km (4.1 mi) high. The image shows gullies eroded into the wall of a meteor impact crater in Noachis Terra. Channels and associated aprons of debris that are thought to have been formed by groundwater seepage, surface runoff, and debris flow.

**Mars: Habitable?**

Mars is, at first blush, inhospitable. Temperatures are, for the most part, frigid, exposure to ultraviolet radiation is high, and the surface is highly oxidizing, precluding the presence of organic compounds on the surface. The atmospheric pressure is very low (similar to that of Earth's uppermost atmosphere) so liquid water is unstable on the surface. Yet hydrogeological evidence from Mars Global Surveyor hints that liquid water may even flow today under the surface. Previous evidence seems to show that it once flowed much more freely on the surface in ancient times.

Could Mars harbor subsurface life, similar to the subsurface or hydrothermal communities found on Earth? If so, it would be protected from surface radiation, damaging oxidants, and have access to liquid water. Mars is rich in carbon dioxide, the raw material used by plants to produce organic carbon. Life has been found at the depths of Earth's oceans and several kilometers below the surface inside of rocks. If it did arise during a warmer, wetter period in Mars' history, perhaps it managed to migrate into warmer, more clement regions of the planet's interior before the surface became uninhabitable.

**The Large Moons of Jupiter: Underground Oceans**

With evidence mounting that one or more of the large moons of Jupiter (Europa, Ganymede, Callisto) have ice-covered lakes, the possibility of life on these moons becomes a subject of scientific discourse. One of these, Europa, has an ice layer too thick to allow enough light to get through to allow photosynthesis, the process that drives much of terrestrial life including those under the perennially ice-covered lakes of Antarctica. However, Chris Chyba from the SETI Institute has suggested that chemistry in the ocean's ice cover, driven by charged particles accelerated in Jupiter's magnetosphere, could produce sufficient organic and oxidant molecules for a Europan biosphere to be sustained. The Galileo spacecraft has detected a weak magnetic field on Callisto, suggesting that salt water may lie beneath an ice-covered surface. Supportive evidence exists as well for an ocean with Ganymede. Several of Saturn's moons and other outer solar system bodies may also hold the potential for having a subsurface ocean.

**Naked in Space: The Ultimate Exposure**

Panspermia ("seeds spread far"), the idea that life can travel through space from one hospitable location to another is no longer wild speculation. Space is extremely cold, subject to unfiltered solar radiation, solar wind,

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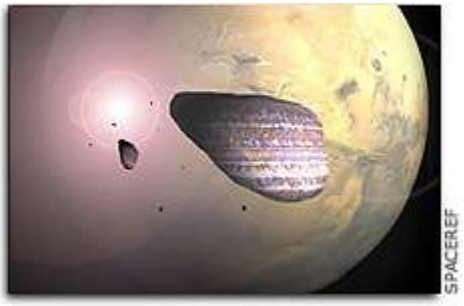
galactic radiation, space vacuum, and to negligible gravity. But this treacherous realm can be crossed by life. [Table II]

**TABLE II. Physical conditions prevailing in the interplanetary space environment**

| Parameter | Interplanetary Space |
|---|---|
| Pressure (Pa) | $10^{-14}$ |
| Solar electromagnetic radiation range | all |
| Cosmic ionizing radiation Gy/yr) | $\leq$ 0.1 |
| Gravity | < 10-6* |
| Temperature (K) | 4* |




Over the history of the solar large impact events may have served as a steady means of transporting rocks (which arrived as meteorites) between one world or another. Whether or not any of these rocks ever actually contained viable life forms is not known. However recent studies suggest that this is possible.

We know from Mars meteorites such as the (now) famous ALH84001 sample that a natural vehicle exists for interplanetary transport. These meteorites contain organic compounds from Mars, showing that such compounds can survive the journey. Moreover, studies have shown that given a rock of sufficient size, conditions within a rock thrown off of Mars - and then later entering Earth's atmosphere - can remain cool enough such that not just organic material - but also microbes contained within - could (theoretically) survive the trip.

The criticism that life cannot endure extended periods in space is now being tested experimentally in space simulation facilities in the U.S. and Germany, and through unmanned flight experiments. NASA's Long Duration Exposure Facility and the European Space Agency's BioPan space experiments showed that microbes can survive direct exposure to the raw conditions of space. Survivors to date include spores of *Bacillus subtilis* and halophiles in the active (vegetative) state. Hopes for further experiments of this nature rest on both unmanned flights and the ESA Exposed Facility planned for the International Space Station.

**Summary**

Earth provides us with a wondrous array of life's adaptations. Indeed, by studying the extremophiles here on Earth, we may get the first clear indication of what ET could be like - or at least the range of things they might eat and breathe.

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17MBP105A
I MSC MICROBIOLOGY
MARINE MICROBIOLOGY

| | | | | | | |
|---|---|---|---|---|---|---|
| Unit II Particulate (organic) material which is only partly disintegrated is called as_____ __ | demeris | divergent | detritus | debris | | detritus |
| An organism which grows preferentially in high salinities. | Halophile | Barophile | Chemophile | Divergephile | | Halophile |
| Living together of two organisms with protecting each other by producing chemicals is called as | Antagonism | Commensalism | Symbiosis | Mutualism | | Antagonism |
| Which were the investigators lived at the same time? | Koch and Pasteur | Darwin and Woese | Van Leeuenhoek and Ricketts | Berg and Hooke | | Koch and Pasteur |

| | | | | | |
|---|---|---|---|---|---|
| The unifying feature of the archaea that distinguishes them from the bacteria is | habitats which are extreme environments with regard to acidity | absence of a nuclear membrane temperature | presence of a cell wall containing a characteristic outer membrane | cytoplasmic ribosomes that are 70S | habitats which are extreme environments with regard to acidity |
| Which instrument is used for sterilization below 100° C | Flame | Autoclave | Filters | Desiccators | Flame |
| _____ is the last phase in growth curve | Log | Lag | stationary | death | death |
| RNA to DNA is called as | replication | biosynthesis | translation | reverse transcription | reverse transcription |
| The | cocci | bacilli | spirilla | comma | comma |
| Single or clusters of flagella at both poles is known as | monotrichous | peritrichous | amphitrichous | peritrichous | amphitrichous |

| | | | | | | |
|---|---|---|---|---|---|---|
| Which of the following bacterial genera (that produces endospore) have medical importance? | E coli | Bacillus | Salmonella typhi | vibrio | | Bacillus |
| Swimming towards a chemical of bacteria is termed as | positive chemotaxis | negative chemotaxis | phototaxis | magnetotaxis | | positive chemotaxis |
| _____ group of bacteria grows in high pressure | Halophiles | Barophiles | thermophiles | psychrophiles | | Barophiles |
| The group of gram positive bacteria having high G+C contents are called as | cyanobacteria | Nanobacteria | Firmicutes | Actinobacteria | | Actinobacteria |

| | | | | | | |
|---|---|---|---|---|---|---|
| Which of the following articles cannot be sterilized in an autoclave ? | Gloves | Culture media | Dressing material | sugars | | sugars |
| . Which of the following disinfectants act by disrupting microbial membranes? | Cationic detergents | Halogens | Heavy metals | Aldehydes | | Cationic detergents |
| Which of the following is best to sterilize heat labile solutions? | Dry heat | Autoclave | Membrane filtration | Pasteurization | | Membrane filtration |
| All the | archaea | fungi | protozoa | humans | | archaea |

| | | | | | | |
|---|---|---|---|---|---|---|
| _____ ___are organism which grows at high pressure rather than at atmospheric pressure. | Barophile | halophile | thermophile | neutrophil | | Barophile |
| Which cell type is considered to have the oldest ancestor? | Archaea | Bacteria | Eukarya | they all share the same ancestor | | Archaea |
| Before most molecules can enter the Krebs citric acid cycle, they must be converted to | citric acid | oxaloacetic acid | NADH or FADH | acetyl-CoA | | acetyl-CoA |

| | | | | | | |
|---|---|---|---|---|---|---|
| The concept of putting microbes to help clean up the environment is called | pasteurization | bioremediation | fermentation | biolistics | | bioremediation |
| Which of the following is not employed as an oxidation method? | Oxidation ponds | Trickling filters | Contact aerators | aeration ponds | | Contact aerators |
| The filtering medium of trickling filters is coated with microbial flora, known as | zoological film | geological film | biofilm | physiological film | | zoological film |

| | | | | | |
|---|---|---|---|---|---|
| Some cyanobacteria produce potent neurotoxins that, if ingested, will kill humans. These cyanobacteria are most likely to contaminate | water rich in organic carbon wastes but poor in phosphate | water those are anoxic | water rich in phosphate wastes but poor in organic carbon | water rich in organic matters | water rich in organic carbon wastes but poor in phosphate |
| The biogas production process takes place at the temperature | lesser than 25°C | 25-40°C | 45-60°C | 45-70°C | 45-60°C |
| What is an anaerobic digester? | New diet drink | Microbe that eats hazardous waste | Method to convert agricultural waste into a biogas | methanol production | Method to convert agricultural waste into a biogas |
| The use of microbes | bioinformatics | biolistics | biotechnology | bioremediation | bioremediation |
| Activated | bacteria | Yeasts and | protozoa | virus | bacteria |
| Bacteria which need oxygen for growth are called | Thermophilic bacteria | Microaerophilic bacteria | Facultative anaerobic bacteria | Anaerobic bacteria | Microaerophilic bacteria |

| | | | | | | |
|---|---|---|---|---|---|---|
| pH required for the growth of bacteria is | 6.8 – 7.2 | 5.6– 8.2 | 3.0 – 6.0 | 8.0– 14.0 | | 6.8 – 7.2 |
| Drug resistance in bacteria is mainly determined by factor | F | R | Col | Lysogenic factor | | R |
| The ion that is required in trace amounts for the growth of bacteria is | Calcium | Magnesium | Cobalt | Sodium | | Cobalt |
| Which one of the following is produced in the greatest numbers during one turn of the Krebs cycle? | NADH | Acetyl-CoA | FADH2 | ATP | | FADH2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Aerobic respiration differs from anaerobic respiration in which of the following respects? | Anaerobic respiration is glycolysis | Aerobic respiration requires the electron transport chain | The final electron acceptors are different | Aerobic respiration produces less ATP | | Anaerobic respiration is glycolysis |
| Acid loving group of organism are called as | Acidophiles | alkalinophiles | barophiles | halophiles | | Acidophiles |
| The group of gram positive bacteria having low G+C contents are called as | cyanobacteria | Nanobacteria | Firmicutes | Actinobacteria | | Firmicutes |
| The predominant kind of bacteria causing spoilage in fish at chilling temperature is | species of *Pseudomonas* | *Micrococcus* | *Bacillus* | vibrio | | species of *Pseudomonas* |

| | | | | | | |
|---|---|---|---|---|---|---|
| Preservation of foods by using salts and sugars works by | raising pH | lowering osmotic pressure | creating a hypertonic environment | creating a hypotonic environment | | raising pH |
| Removal of solids is generally considered as a | Primary treatment | Secondary treatment | Tertiary treatment | treatment | | Primary treatment |
| All of the following species are considered coliforms except | Enterobacter aerogenes | Klebsiella pneumoniae | Salmonella typhi | Escherichia coli | | Salmonella typhi |
| The sulfur pearl of Namibia, Thiomargarita namibiensis | is the world's largest bacterium | stores high concentrations of nitrate in a huge internal vacuole which takes up 98% of the organisms volume | micro bacteria | is the world's smallest bacterium | | is the world's largest bacterium |
| Chemical precipitation of phosphorus is | primary treatment | secondary treatment | tertiary treatment | fourth treatment | | secondary treatment |

| | | | | | |
|---|---|---|---|---|---|
| Which of the following is correct? | Ultramicrobacteria are nanobacteria | Ultramicrobacteria or nanobacteria are so numerous that they are a major food source for heterotrophic flagellates | ultrabacteria not bacteria | ultramicrobacteria are less | Ultramicrobacteria or nanobacteria are so numerous that they are a major food source for heterotrophic flagellates |
| Coliforms are used as indicator organisms because | they are absent wherever enteric pathogens are present | a testing procedure with great specificity is easy to perform | no change | they present everywhere | a testing procedure with great specificity is easy to perform |
| Hyphomycete fungi | produce nonmotile tetaradiate conidia | produce motile tetaradiate conidia | no cilia no flagella | no true fungi | produce nonmotile tetaradiate conidia |

| | | | | | | |
|---|---|---|---|---|---|---|
| Loss of carbon through the microbial loop in oligotrophic environments is _____ __ to/than the loss of carbon through the microbial loop in copiotrophic environments | greater | Lesser | approximately equal | half | | approximately equal |

| | | | | | | |
|---|---|---|---|---|---|---|
| Metabolism of dissolved organic material released by phytoplankton allows heterotrophic bacteria to become part of the particulate organic matter that is passed up the food web to be metabolized and released as mineral | microbial loop | Winogradsky column | Redfield ratio | Gibbs free energy | | microbial loop |
| Which of the following is correct? | All members of Hyphomycetes are also members of Fungi, but not all members of Fungi are members of Hyphomycetes | All members of Fungi are also members of Hyphomycetes, but not all members of Hyphomycetes are members of Fungi | All members of Hyphomycetes are members of Fungi, and all members of Fungi are members of Hyphomycetes | No member of Hyphomycetes is a member of Fungi | | All members of Hyphomycetes are also members of Fungi, but not all members of Fungi are members of Hyphomycetes |

| | | | | | | |
|---|---|---|---|---|---|---|
| The amount of oxygen dissolved in hypolimnion water in the winter is _____ __ to/than the amount of oxygen dissolved in hypolimnion water in the summer | greater | Lesser | approximately equal to | half | | approximately equal to |
| Members of all of the following genera of bacteria typically are found in a maturing Winogradsky column except | Clostridium | Rhodospirillum | Chlorobium | Escherichia | | Rhodospirillum |

| | | | | | | |
|---|---|---|---|---|---|---|
| Because it can be used with a variety of media and allow a resuscitation step the _____ technique has become the common and often preferred method of evaluating the microbiological characteristics of water. | most probable number | Winogradsky | MUG | membrane filtration | | most probable number |
| Which of the following is correct? | A Winogradsky column is used to filter out microorganisms from a water sample taken from a deep black smoker | A Winogradsky column is used to demonstrate interactions and gradients that occur in aquatic environments | no winogradsky | A Winogradsky column is used to filter out microorganisms from non-aquatic environments | | A Winogradsky column is used to demonstrate interactions and gradients that occur in aquatic environments |
| Trickling filter | primary treatment | secondary treatment | tertiary treatment | no treatment | | secondary treatment |

| | | | | | | |
|---|---|---|---|---|---|---|
| Which of the following species in water reveal the presence of fecal pollution of human or animal origin? | E.coli | Fecal Streptococci | Clostridium perfringens | Salmonella | | E.coli |
| Activated sludge undergoes | Primary treatment | Secondary treatment | Tertiary treatment | fourth treatment | | Secondary treatment |
| The rate of flux of oxygen in air is _____ __ to/than the rate of flux of oxygen in water. | greater | Lesser | approximately equal | half | | greater |

| | | | | | | |
|---|---|---|---|---|---|---|
| _____ is an environment like _____ where microorganisms are functioning in an extremely thin film of water and where oxygen-containing air is close to them. | low oxygen diffusion environment; soils | high oxygen diffusion environment; soils | low oxygen diffusion environment; lakes | high oxygen diffusion environment; lakes | | high oxygen diffusion environment; soils |
| The acetate-utilizing methanogens are responsible for | 20% of methane produced in a biogas reactor | 50% of methane produced in a biogas reactor | 70% of methane produced in a biogas reactor | 85% of methane produced in a biogas reactor | | 50% of methane produced in a biogas reactor |
| Which of the following is responsible for the corrosion problem? | Iron bacteria | Sulfur bacteria | Slime forming bacteria | iron, sulfur and slime bacteria | | iron, sulfur and slime bacteria |

| | | | | | | |
|---|---|---|---|---|---|---|
| Water testing relies on the detection of certain indicator organisms known as | acid-fast bacteria | Bacteroids | coliforms | dinoflagellates | | coliforms |
| | the overpopulation of algae | the overabundance of toxic proteins | the depletion of oxygen | the buildup of sediment on the river bottom | | the depletion of oxygen |

# 2017

## I M.Sc Microbiology – Marine microbiology

| LECTURE PLAN - UNIT -111 | | | |
|---|---|---|---|
| **S. no** | Lecture duration(Hr) | **Topics covered** | **Supporting materials** |
| 1 | 1 | Xenobiotics | T2 647-650 |
| 2 | 1 | Biodegradation | T2 650-652 |
| 3 | 1 | Degradative plasmids, hydrocarbons | T2 653-655 |
| 4 | 1 | Oil pollution and surfactants | T2 656 |
| 5 | 1 | Pesticides | T2 657 |
| 6 | 2 | Bioremediation | T2 667-682 |
| 7 | 1 | Role of microbes in marine | T2 667-682 |
| 8 | 1 | Marine nutrient cycles | T2 667-682 |
| 9 | 1 | Disease of marine microbes | T2 667-682 |
| 10 | 1 | Marine biodiversity impacts | T2 667-682 |
| 11 | 1 | Revision of Unit III | |
| 12 | 1 | Voluntary seminar | |
| 13 | 1 | Unit III test | |
| **Textbooks :** | | T1-Microbiology- pelczar – McGraw Hill publishing<br>T2-Microbiology- presscott – McGraw hill publishing<br>T3-Microbial genetics – David friefelder- | |
| **Reference books:** | | | |
| **Website:** | | W1- www.microbiology.com<br>W2 – www.marinestudy.com | |
| **Journals:** | | | |

Prepared by : Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

# 2017

Xenobiotic

A **xenobiotic** is a foreign chemical substance found within an organism that is not normally naturally produced by or expected to be present within. It can also cover substances that are present in much higher concentrations than are usual. Specifically, drugs such as antibiotics are xenobiotics in humans because the human body does not produce them itself, nor are they part of a normal food.

Natural compounds can also become xenobiotics if they are taken up by another organism, such as the uptake of natural human hormones by fish found downstream of sewage treatment plant outfalls, or the chemical defenses produced by some organisms as protection against predators.

The term **xenobiotics**, however, is very often used in the context of pollutants such as dioxins and polychlorinated biphenyls and their effect on the biota, because xenobiotics are understood as substances foreign to an entire biological system, i.e. artificial substances, which did not exist in nature before their synthesis by humans. The term xenobiotic is derived from the Greek words ξένος (xenos) = foreigner, stranger and βίος (bios, vios) = life, plus the Greek suffix for adjectives -τικός, -ή, -ό (tic).

Xenobiotics may be grouped as carcinogens, drugs, environmental pollutants, food additives, hydrocarbons, and pesticides.

**Xenobiotic metabolism**

The body removes xenobiotics by xenobiotic metabolism. This consists of the deactivation and the excretion of xenobiotics, and happens mostly in the liver. Excretion routes are urine, feces, breath, and sweat. Hepatic enzymes are responsible for the metabolism of xenobiotics by first activating them (oxidation, reduction, hydrolysis and/or hydration of the xenobiotic), and then conjugating the active secondary metabolite with glucuronic acid, sulphuric acid, or glutathione, followed by excretion in bile or urine. An example of a group of enzymes involved in xenobiotic metabolism is hepatic microsomal cytochrome P450. These enzymes that metabolize xenobiotics are very important for the pharmaceutical industry, because they are responsible for the breakdown of medications.

Organisms can also evolve to tolerate xenobiotics. An example is the co-evolution of the production of tetrodotoxin in the rough-skinned newt and the evolution of tetrodotoxin resistance in its predator, the Common Garter Snake. In this predator–prey pair, an evolutionary arms race has produced high levels of toxin in the newt and correspondingly high levels of resistance in the snake.[2] This evolutionary response is based on the snake evolving modified forms of the ion channels that the toxin acts upon, so becoming resistant to its effects.

**Xenobiotics in the environment**

Xenobiotic substances are an issue for sewage treatment systems, since they are many in number, and each will present its own problems as to how to remove them (and whether it is worth trying to). It can be dangerous to the health.

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Some xenobiotics are resistant to degradation. For example, they may be synthetic organochlorides such as plastics and pesticides, or naturally occurring organic chemicals such as polyaromatic hydrocarbons (PAHs) and some fractions of crude oil and coal. However, it is believed that microorganisms are capable of degrading almost all the different complex and resistant xenobiotics found on the earth.[4] Many xenobiotics produce a variety of biological effects, which is used when they are characterized using bioassay. Before they can be registered for sale in most countries, xenobiotic pesticides must undergo extensive evaluation for risk factors, such as toxicity to humans, ecotoxicity, or persistence in the environment. For example, during the registration process, the herbicide, cloransulam-methyl was found to degrade relatively quickly in soil.[5]

**Inter-species organ transplantation**

The term **xenobiotic** is also used to refer to organs transplanted from one species to another. For example, some researchers hope that hearts and other organs could be transplanted from pigs to humans. Many people die every year whose lives could have been saved if a critical organ had been available for transplant. Kidneys are currently the most commonly transplanted organ. Xenobiotic organs would need to be developed in such a way that they would not be rejected by the immune system.

**Biodegradation and Bioremediation**

Biodegradation or biological degradation is the phenomenon of biological transformation of organic compounds by living organisms, particularly the microorganisms.

Biodegradation basically involves the conversion of complex organic molecules to simpler (and mostly non-toxic) ones. The term biotransformation is used for incomplete biodegradation of organic compounds involving one or a few reactions. Biotransformation is employed for the synthesis of commercially important products by microorganisms.

Bioremediation refers to the process of using microorganisms to remove the environmental pollutants i.e. the toxic wastes found in soil, water, air etc. The microbes serve as scavengers in bioremediation. The removal of organic wastes by microbes for environmental clean-up is the essence of bioremediation. The other names used (by some authors) for bioremediation are bio-treatment, bio-reclamation and bio-restoration.

It is rather difficult to show any distinction between biodegradation and bioremediation. Further, in biotechnology, most of the reactions of biodegradation/bioremediation involve xenobiotic.

**Xenobiotic:**

Xenobiotic (xenos-foregin) broadly refer to the unnatural, foreign and synthetic chemicals such as pesticides, herbicides, refrigerants, solvents and other organic compounds. Microbial degradation of xenobiotic assumes significance, since it provides an effective and economic means of disposing of toxic chemicals, particularly the environmental pollutants.

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**Pseudomonas — The Predominant Microorganism For Bioremediation:**

Members of the genus Pseudomonas (a soil microorganism) are the most predominant microorganisms that degrade xenobiotic. Different strains of Pseudomonas, that are capable of detoxifying more than 100 organic compounds, have been identified. The examples of organic compounds are several hydrocarbons, phenols, organophosphates, polychlorinated biphenyls (PCBs) and polycylic aromatics and naphthalene.

About 40-50 microbial strains of micro¬organisms, capable of degrading xenobiotics have been isolated. Besides Pseudomonas, other good examples are Mycobacterium, Alcaligenes, and Nocardia. A selected list of microorganisms and the xenobiotics degraded is given in Table 59.1.

**TABLE 59.1 A selected list of microorganisms and the pollutants (xenobiotics) that are degraded by bioremediation**

| *Microorganism* | *Pollutant chemicals* |
|---|---|
| *Pseudomonas* sp | Aliphatic and aromatic hydrocarbons—alkylaminoxides, alkylammonium benzene, naphthalene, anthracene xylene, toluene, polychlorinated biphenyls (PCBs), malathion, parathion, organophosphates. |
| *Mycobacterium* sp | Benzene, branched hydrocarbons, cycloparaffins |
| *Alcaligenes* sp | Polychlorinated biphenyls, alkyl benzene, halogenated hydrocarbons. |
| *Nocardia* sp | Naphthalene, alkylbenzenes, phenoxyacetate. |
| *Arthrobacter* sp | Benzene, polycyclic aromatics, phenoxyacetate, pentachlorophenol. |
| *Corynebacterium* sp | Halogenated hydrocarbons, phenoxyacetate. |
| *Bacillus* sp | Long chain alkanes, phenylurea. |
| *Candida* sp | Polychlorinated biphenyls |
| *Aspergillus* sp | Phenols |
| *Xanthomonas* sp | Polycyclic hydrocarbons |
| *Streptomyces* sp | Halogenated hydrocarbons, phenoxyacetate. |
| *Fusarium* sp | Propanil |
| *Cunninghamella* sp | Polycyclic aromatics, polychlorinated biphenyls. |

**Consortia of microorganisms for biodegradation:**

A particular strain of microorganism may degrade one or more compounds. Sometimes, for the degradation of a single compound, the synergetic action of a few microorganisms (i.e. a consortium or cocktail of microbes)

may be more efficient. For instance, the insecticide parathion is more efficiently degraded by the combined action of *Pseudomonas aeruginosa* and *Pseudomonas stulzeri.*

**Co-metabolism in biodegradation:**

In general, the metabolism (breakdown) of xenobiotics is not associated with any advantage to the microorganism. That is the pollutant chemical cannot serve as a source of carbon or energy for the organism. The term co-metabolism is often used to indicate the non-beneficial (to the microorganism) biochemical pathways concerned with the biodegradation of xenobiotics. However, co- metabolism depends on the presence of a suitable substrate for the microorganism. Such compounds are referred to co-substrates.

**Factors Affecting Biodegradation:**

Several factors influence biodegradation. These include the chemical nature of the xenobiotic, the capability of the individual microorganism, nutrient and O2 supply, temperature, pH and redox potential. Among these, the chemical nature of the substrate that has to be degraded is very important.

Some of the relevant features are given hereunder:

i. In general, aliphatic compounds are more easily degraded than aromatic ones.

ii. Presence of cyclic ring structures and length chains or branches decrease the efficiency of biodegradation.

iii. Water soluble compounds are more easily degraded.

iv. Molecular orientation of aromatic compounds influences biodegradation i.e. ortho > para > meta.

v. The presence of halogens (in aromatic compounds) inhibits biodegradation.

Besides the factors listed above, there are two recent developments to enhance the biodegradation by microorganisms.

**Bio-stimulation:**

This is a process by which the microbial activity can be enhanced by increased supply of nutrients or by addition of certain stimulating agents (electron acceptors, surfactants).

**Bio-augmentation:**

It is possible to increase biodegradation through manipulation of genes. More details on this genetic manipulation i.e. genetically engineered microorganisms (GEMs), are described later. Bio-augmentation can also be achieved by employing a consortium of micro¬organisms.

**Enzyme Systems for Biodegradation:**

Several enzyme systems (with independent enzymes that work together) are in existence in the microorganisms for the degradation of xenobiotics. The genes coding for the enzymes of bio-degradative pathways may be present in the chromosomal DNA or more frequently on the plasmids. In certain

microorganisms, the genes of both chromosome and plasmid contribute for the enzymes of biodegradation. The microorganism Pseudomonas occupies a special place in biodegradation.

selected list of xenobiotics and the plasmids containing the genes for their degradation is given in Table 59.2.

**TABLE 59.2 A selected list of xenobiotics and the plasmids containing genes (in *Plasmodium*) for biodegradation**

| *Xenobiotic* | *Name of plasmid in* Pseudomonas |
|---|---|
| Naphthalene | NAH |
| Xylene | XYL |
| Xylene and toluene | TOL, pWWO, XYL-K |
| Salicylate | SAL |
| Camphor | CAM |
| 3-Chlorobenzene | pAC25 |

**Recalcitrant Xenobiotics:**

There are certain compounds that do not easily undergo biodegradation and therefore persist in the environment for a long period (sometimes in years). They are labeled as recalcitrant.

There may be several reasons for the resistance of xenobiotics to microbial degradation:

i. They may chemically and biologically inert (highly stable).

ii. Lack of enzyme system in the microorganisms for biodegradation.

iii. They cannot enter the microorganisms being large molecules or lack of transport systems.

iv. The compounds may be highly toxic or result in the formation highly toxic products that kill microorganisms.

There are a large number of racalcitrant xenobiotic compounds e.g. chloroform, freons, insecticides (DDT, lindane), herbicides (dalapon) and synthetic polymers (plastics e.g. polystyrene, polyethylene, polyvinyl chlorine).

It takes about 4-5 years for the degradation of DDT (75-100%) in the soil. A group of microorganisms (Aspergillus flavus, Mucor aternans, Fusarium oxysporum and Trichoderma viride) are associated with the slow biodegradation of DDT.

**Bio-magnification:**

The phenomenon of progressive increase in the concentration of a xenobiotic compound, as the substance is passed through the food chain is referred to as bio-magnification or bioaccumulation. For instance, the insecticide DDT is absorbed repeatedly by plants and microorganism.

When they are eaten by fish and birds, this pesticide being recalcitrant, accumulates, and enters the food chain. Thus, DDT may find its entry into various animals, including man. DDT affects the nervous systems, and it has been banned in some countries.

**Types of Bioremediation**:

The most important aspect of environmental biotechnology is the effective management of hazardous and toxic pollutants (xenobiotics) by bioremediation. The environmental clean-up process through bioremediation can be achieved in two ways—in situ and ex situ bioremediation.

**In Situ Bioremediation:**

In situ bioremediation involves a direct approach for the microbial degradation of xenobiotics at the sites of pollution (soil, ground water). Addition of adequate quantities of nutrients at the sites promotes microbial growth. When these microorganisms are exposed to xenobiotics (pollutants), they develop metabolic ability to degrade them.

The growth of the microorganisms and their ability to bring out biodegradation are dependent on the supply of essential nutrients (nitrogen, phosphorus etc.). In situ bioremediation has been successfully applied for clean-up of oil spillages, beaches etc. There are two types of in situ bioremediation-intrinsic and engineered.

**Intrinsic bioremediation:**

The inherent metabolic ability of the microorganisms to degrade certain pollutants is the intrinsic bioremediation. In fact, the microorganisms can be tested in the laboratory for their natural capability of biodegradation and appropriately utilized.

**Engineered in situ bioremediation:**

The inherent ability of the microorganisms for bioremediation is generally slow and limited. However, by using suitable physicochemical means (good nutrient and O2supply, addition of electron acceptors, optimal temperature), the bioremediation process can be engineered for more efficient degradation of pollutants.

**Advantages of in situ bioremediation:**

1. Cost-effective, with minimal exposure to public or site personnel.
2. Sites of bioremediation remain minimally disrupted.

**Disadvantages of in situ bioremediation:**

1. Very time consuming process.
2. Sites are directly exposed to environmental factors (temperature, O2 supply etc.).
3. Microbial degrading ability varies seasonally.

**Ex Situ Bioremediation:**

The waste or toxic materials can be collected from the polluted sites and the bioremediation with the requisite microorganisms (frequently a consortium of organisms) can be carried out at designed places. This process is certainly an improvement over in situ bioremediation, and has been successfully used at some places.

**Advantages of ex situ bioremediation**:

1. Better controlled and more efficient process.
2. Process can be improved by enrichment with desired microorganisms.
3. Time required in short.

**Disadvantages of ex situ bioremediation:**

1. Very costly process.
2. Sites of pollution are highly disturbed.
3. There may be disposal problem after the process is complete.

**Metabolic Effects of Microorganisms on Xenobiotics**:

Although it is the intention of the biotechnologist to degrade the xenobiotics by microorganisms to the advantage of environment and ecosystem, it is not always possible. This is evident from the different types of metabolic effects as shown below.

**Detoxification:**

This process involves the microbial conversion of toxic compound to a non¬toxic one. Biodegradation involving detoxification is highly advantageous to the environment and population.

**Activation:**

Certain xenobiotics which are not toxic or less toxic may be converted to toxic or more toxic products. This is dangerous.

**Degradation:**

The complex compounds are degraded to simpler products which are generally harmless.

**Conjugation:**

The process of conjugation may involve the conversion of xenobiotics to more complex compounds. This is however, not very common.

**Types of Reactions in Bioremediation:**

Microbial degradation of organic compounds primarily involves aerobic, anaerobic and sequential degradation.

**Aerobic bioremediation:**

Aerobic biodegradation involves the utilization of O2 for the oxidation of organic compounds. These compounds may serve as substrates for the supply of carbon and energy to the microorganisms. Two types of enzymes namely mono-oxygenases and- di-oxygenases are involved in aerobic biodegradation. Mono-

oxygenases can act on both aliphatic and aromatic compounds while di-oxygenases oxidize aliphatic compounds.

**Anaerobic bioremediation:**

Anaerobic biodegradation does not require O2 supply. The growth of anaerobic microorganisms (mostly found in solids and sediments), and consequently the degradation processes are slow. However, anaerobic biodegradation is cost- effective, since the need for continuous O2 supply is not there. Some of the important anaerobic reactions and examples of organic compounds degraded are listed below.

Hydrogenation and dehydrogenation — benzoate, phenol, catechol.

Dehaiogenation — Polychlorinated biphenyls (PCBs), chlorinated ethylene's. The term de-chlorination is frequently used for dehaiogenation of chlorinated compounds.

Carboxylation and decarboxylation — toluene, cresol and benzoate.

**Sequential Bioremediation:**

In the degradation of several xenobiotics, both aerobic and anaerobic processes are involved. This is often an effective way of reducing the toxicity of a pollutant. For instance, tetra chloromethane and tetrachloroethane undergo sequential degradation.

**Biodegradation of Hydrocarbons:**

Hydrocarbon are mainly the pollutants from oil refineries and oil spills. These pollutants can be degraded by a consortium or cocktail of microorganisms e.g. *Pseudomonas, Corynebacterium, Arthrobacter, Mycobacterium* and *Nocardia.*

**Biodegradation of Aliphatic Hydrocarbons:**

The uptake of aliphatic hydrocarbons is a slow process due to their low solubility in aqueous medium. Both aerobic and anaerobic processes are operative for the degradation of aliphatic hydrocarbons. For instance, unsaturated hydrocarbons are degraded in both anaerobic and aerobic environments, while saturated ones are degraded by aerobic process. Some aliphatic hydrocarbons which are reclacitrant to aerobic process are effectively degraded in anaerobic environment e.g. chlorinated aliphatic compounds (carbon tetrachloride, methyl chloride, vinyl chloride).

**Biodegradation of Aromatic Hydrocarbons:**

Microbial degradation of aromatic hydrocarbons occurs through aerobic and anaerobic processes. The most important microorganism that participates in these processes is Pseudomonas.

The biodegradation of aromatic compounds basically involves the following sequence of reactions:

1. Removal of the side chains.

2. Opening of the benzene ring.

Most of the non-halogenated aromatic compounds undergo a series of reactions to produce catechol or protocatechuate. The bioremediation of toluene, L-mandelate, benzoate, benzene, phenol, anthracene, naphthalene, phenanthrene and salicylate to produce catechol is shown in Fig. 59.1. Likewise, Fig. 59.2, depicts the bioremediation of quinate, p-hydroxymandelate, p-hydroxybenzoyl formate, p-toluate, benzoate and vanillate to produce protocatechuate.

Catechol and protocatechuate can undergo oxidative cleavage pathways. In ortho-cleavage pathway, catechol and protocatechuate form acetyl CoA (Fig. 59.3), while in meta-cleavage pathway (Fig. 59.4), they are converted to pyruvate and acetaldehyde. The degraded products of catechol and protocatechuate are readily metabolised by almost all the organisms.

**Biodegradation of Pesticides and Herbicides:**

Pesticides and herbicides are regularly used to contain various plant diseases and improve the crop yield. In fact, they are a part of the modern agriculture, and have significantly contributed to green revolution. The common herbicides and pesticides are propanil (anilide), propham (carbamate), atrazine (triazine), picloram (pyridine), dichlorodiphenyl trichloroethane (DDT) monochloroacetate (MCA), monochloropropionate (MCPA) and glyphosate (organophosphate). Most of the pesticides and herbicides are toxic and are recalcitrant (resistant to biodegradation). Some of them are surfactants (active on the surface) and retained on the surface of leaves.

**Biodegradation of Halogenated Aromatic Compounds:**

Most commonly used herbicides and pesticides are aromatic halogenated (predominantly chlorinated) compounds. The bio-degradative path¬ways of halogenated compounds are comparable with that described for the degradation of non-halogenated aromatic compounds (Figs. 59.1, 59.2, 59.3 and 59.4). The rate of degradation of halogenated compounds is inversely related to the number of halogen atoms that are originally present on the target molecule i.e. compounds with higher number of halogens are less readily degraded.

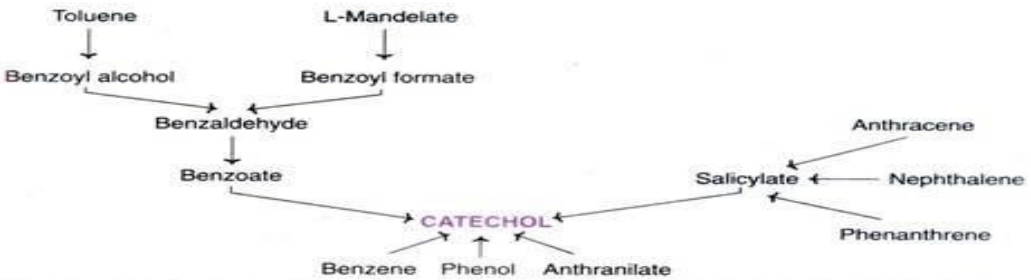


*Fig. 59.1 : Bioremediation of certain aromatic compounds by bacteria to produce catechol.*

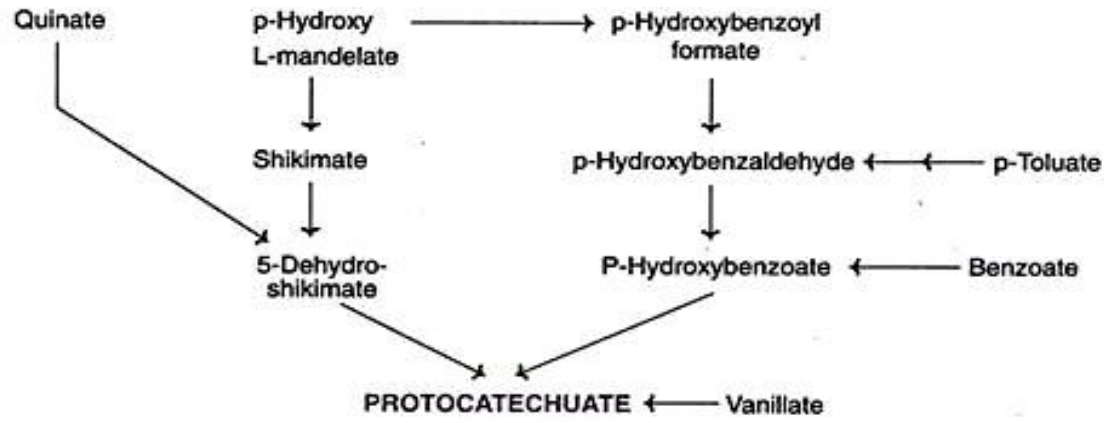


*Fig. 59.2 : Bioremediation of certain organic compounds by bacteria to produce protocatechuate.*

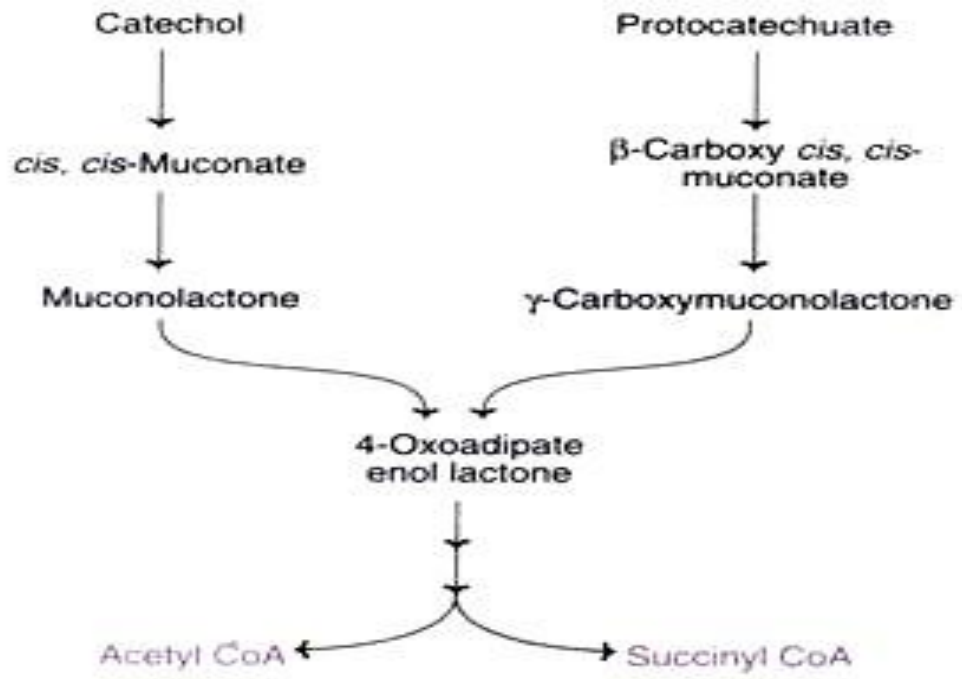


***Fig. 59.3** : Conversion of catechol and protocatechuate to acetyl CoA and succinate by **ortho–cleavage pathway.***

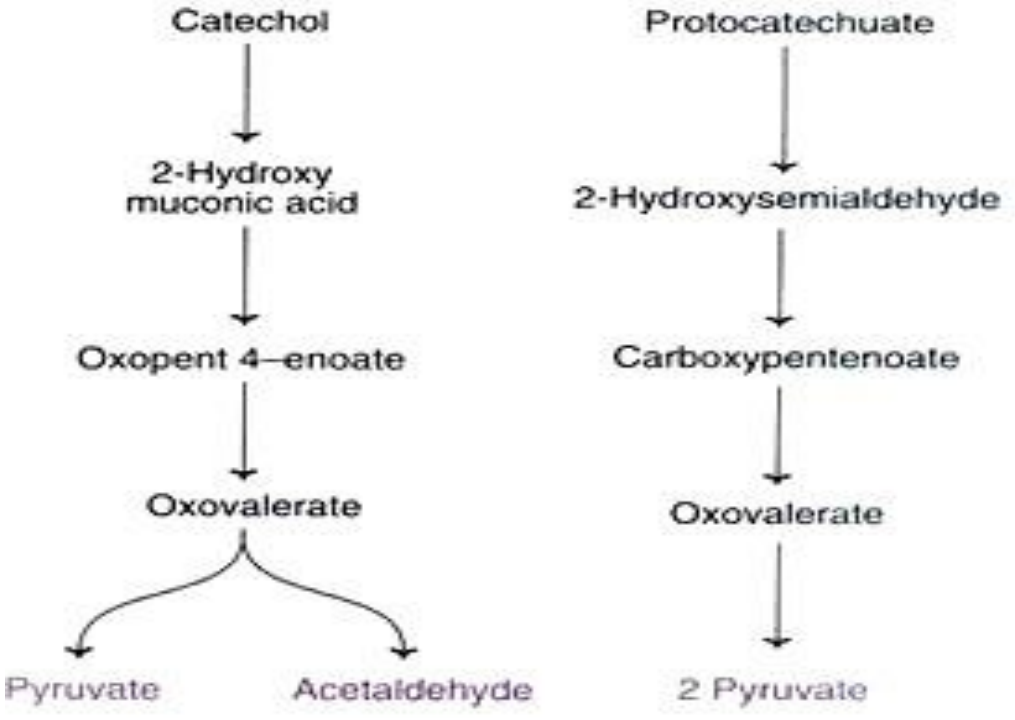


***Fig. 59.4** : Conversion of catechol and protocatechuate to pyruvate and acetaldehyde by **meta–cleavage pathway.***

Dehalogenation (i.e. removal of a halogen substituent from an organic compound) of halogenated compounds is an essential step for their detoxification. Dehalogenation is frequently catalysed by the enzyme di-oxygenase. In this reaction, there is a replacement of halogen on benzene with a hydroxyl group.

Most of the halogenated compounds are also converted to catechol and protocatechuate which can be metabolised (Fig. 59.4). Besides *Pseudomonas*, other microorganisms such as *Azotobacter*, *Bacillus* and *E. coli* are also involved in the microbial degradation of halogenated aromatic compounds.

**Biodegradation of Polychlorinated Biphenyls (PCBs):**

The aromatic chlorinated compounds possessing biphenyl ring (substituted with chlorine) are the PCBs e.g. pentachlorobiphenyl. PCBs are commercially synthesized, as they are useful for various purposes — as pesticides, in electrical conductivity (in transformers), in paints and adhesives. They are inert, very stable and resistant to corrosion.

However, PCBs have been implicated in cancer, damage to various organs and impaired reproductive function. Their commercial use has been restricted in recent years, and are now used mostly in electrical transformers. PCBs accumulate in soil sediments due to hydrophobic nature and high bioaccumulation potential. Although they are resistant to biodegradation, some methods have been recently developed for anaerobic and aerobic oxidation by employing a consortium of microorganisms. Pseudomonas, Alkali genes, Corynebacterium and Acinetobacter. For more efficient degradation of PCBs, the microorganisms are grown on biphenyls, so that the enzymes of biodegradation of PCBs are induced.

**Biodegradation of Some Other Important Compounds:**

**Organo-nitro Compounds:**

Some of the toxic organo-nitro compounds can be degraded by microorganisms for their detoxification.

**2, 4, 6-Trinitrotoluene (TNT):**

Certain bacterial and fungal species belonging to Pseudomonas and Clostrium can detoxify TNT.

**Nitrocellulose:**

Hydrolysis, followed by anaerobic nitrification by certain bacteria, degrades nitrocellulose.

**Synthetic detergents:**

They contain some surfactants (surface active agents) which are not readily biodegradable. Certain bacterial plasmid can degrade surfactants.

**Genetic Engineering for More Efficient Bioremediation:**

Although several microorganisms that can degrade a large number of xenobiotics have been identified, there are many limitations in bioremediation:

i. Microbial degradation of organic compounds is a very slow process.

ii. No single microorganism can degrade all the xenobiotics present in the environmental pollution.

iii. The growth of the microorganisms may be inhibited by the xenobiotics.

iv. Certain xenobiotics get adsorbed on to the particulate matter of soil and become unavailable for microbial degradation.

It is never possible to address all the above limitations and carry out an ideal process of bioremediation. Some attempts have been made in recent years to create genetically engineered microorganisms (CEMs) to enhance bio¬remediation, besides degrading xenobiotics which are highly resistant (recalcitrant) for breakdown. Some of these aspects are briefly described.

**Genetic Manipulation by Transfer of Plasmids:**

The majority of the genes responsible for the synthesis of bio-degradative enzymes are located on the plasmids. It is therefore logical to think of genetic manipulations of plasmids. New strains of bacteria can be created by transfer of plasmids (by conjugation) carrying genes for different degradative pathways.

If the two plasmids contain homologous regions of DNA, recombination occurs between them, resulting in the formation of a larger fused plasmid (with the combined functions of both plasmids). In case of plasmids which do not possess homologous regions of DNA, they can coexist in the bacterium (to which plasmid transfer was done).

The first successful development of a new strain of bacterium (Pseudomonas) by manipulations of plasmid transfer was done by Chakrabarty and his co-workers in 1970s. They used different plasmids and constructed a new bacterium called as superbug that can degrade a number of hydrocarbons of petroleum simultaneously.

United States granted patent to this superbug in 1981 (as per the directive of American Supreme Court). Thus, superbug became the first genetically engineered microorganism to be patented. Superbug has played a significant role in the development of biotechnology industry, although it has not been used for large scale degradation of oil spills.

**Creation of Superbug by Transfer of Plasmids:**

Superbug is a bacterial strain of Pseudomonas that can degrade camphor, octane, xylene and naphthalene. Its creation is depicted in Fig. 59.5.

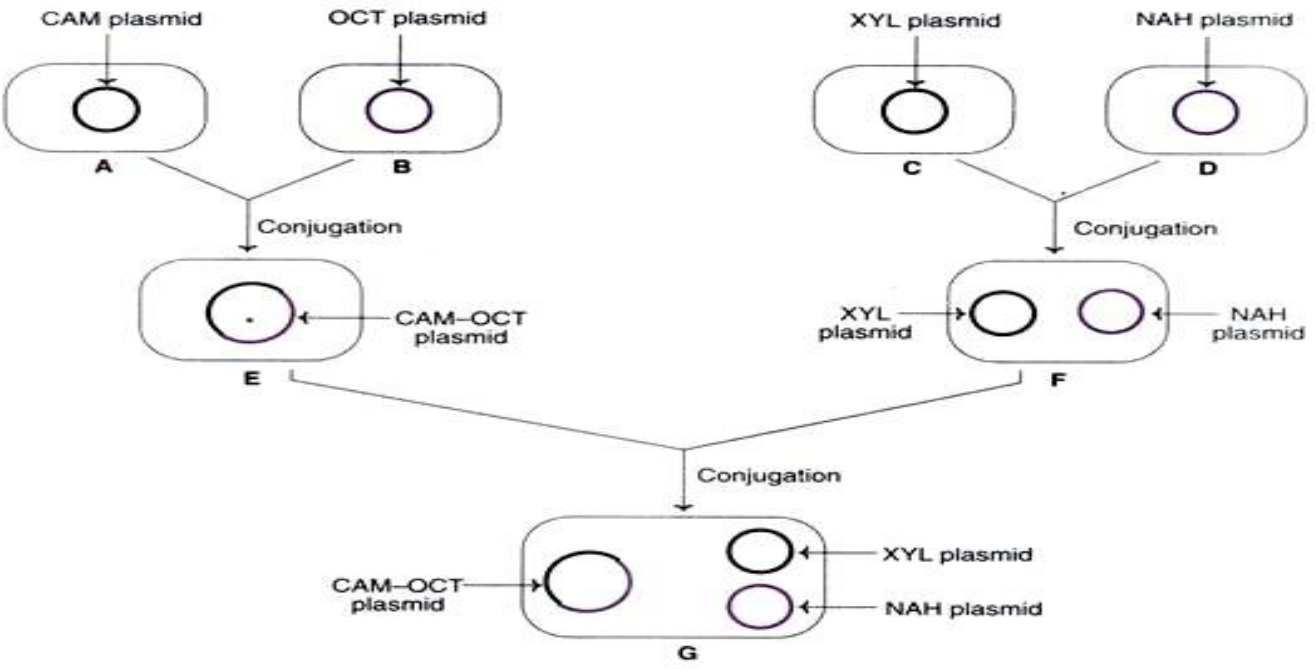


*Fig. 59.5 : Creation of the superbug by transfer of plasmids (A, B, C, D, E, F and G are the different strains of bacteria containing the plasmids shown. Strain G is the superbug.)*

The bacterium containing CAM (camphor- degrading) plasmid was conjugated with another bacterium with OCT (octane-degrading) plasmid. These plasmids are not compatible and therefore, cannot coexist in the same bacterium. However, due to the presence of homologous regions of DNA, recombination occurs between these two plasmids resulting in a single CAM-OCT plasmid. This new bacterium possesses the degradative genes for both camphor and octane.

Another bacterium with XYL (xylene-degrading) plasmid is conjugated with NAH (naphthalene-degrading) plasmid containing bacterium. XYL and NAH plasmids are compatible and therefore can coexist in the same bacterium. This newly, produced bacterium contains genes for the degradation of xylene and naphthalene.

The next and final step is the conjugation of bacterium containing CAM-OCT plasmid with the other bacterium containing XYL and NAH plasmids. The newly created strain is the superbug that carries CAM-OCT plasmid (to degrade camphor and octane), XYL (xylene-degrading) plasmid and NAH (naphthalene-degrading) plasmid.

**Development of Salicylate—Toluene Degrading Bacteria by Plasmid Transfer:**

Some attempts have been made for the creation of a new strain of the bacterium Pseudomonas putida to simultaneously degrade toluene and salicylate. Toluene-degrading (TOL) plasmid was transferred by conjugation to another bacterium that is capable of degrading salicylate (due to the presence of SAL plasmid).

**Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE**

The newly developed strain of Pseudomonas can simultaneously degrade both toluene and salicylate. And this occurs even at a low temperature (0-5°C). However, the new bacterium is not in regular use, as more research is being conducted on its merits and demerits.

**Genetic Manipulation by Gene Alteration:**

Work is in progress to manipulate the genes for more efficient biodegradation. The plasmid pWWO of Pseudomonas codes for 12 different enzymes responsible for the meta-cleavage pathway (for the conversion of catechol and protocatechuate to pyruvate and acetaldehyde, for degradation of certain aromatic compounds. Some success has been reported to alter the genes of plasmid pWWO for more efficient degradation of toluene and xylene.

**Genetically Engineered Microorganisms (GEMs) in Bioremediation:**

Superbug is the first genetically engineered microorganism. Several workers world over have been working for the creation of GEMs, specifically designed for the detoxification of xenobiotics. A selected list of GEMs with a potential for the degradation of xenobiotics is given in Table 59.3. Almost all these CFMs have been created by transferring plasmids.

**TABLE 59.3 A selected list of genetically engineered microorganisms (GEMs) with the potential xenobiotics that can be degraded**

| *Genetically engineered microorganism (GEMs)* | *Xenobiotic* |
|---|---|
| *Pseudomonas diminuta* | Parathion |
| *P. oleovorans* | Alkane |
| *P. cepacia* | 2, 4, 5-Trichlorophenol |
| *P. putida* | Mono- and dichloro-aromatic compounds |
| *Alcaligenes* sp | 2, 4-Dichlorophenoxy acetic acid |
| *Acinetobacter* sp | 4-Chlorobenzene |

**Bio-surfactant Producing GEM:**

A genetically engineered Pseudomonas aeruginosa has been created (by Chakarabarty and his group). This new strain can produce a glycolipid emulsifier (a bio-surfactant) which can reduce the surface tension of an oil water interface. The reduced interfacial tension promotes biodegradation of oils.

**GEM for Degradation of Vanillate and SDS:**

A new strain of Pseudomonas sp (strain ATCC 1915) has been developed for the degradation of vanillate (waste product from paper industry) and sodium dodecyl sulfate (SDS, a compound used in detergents).

**GEMs and Environmental Safety:**

The genetically engineered microorganisms (GEMs) have now become handy tools of biotechnologists. The risks and health hazards associated with the use of GEMs are highly controversial and debatable issues.

The fear of the biotechnologists and even the general public is that the new organism (GEM), once it enters the environment, may disturb the ecological balance and cause harm to the habitat. Some of the GEMs may turn virulant and become genetic bombs, causing great harm to humankind.

Because of the risks involved in the use of GEMs, so far no GEM has been allowed to enter the environmental fields. Thus, the use of GEMs has been confined to the laboratories, and fully controlled processes of biodegradation (usually employing bioreactors). Further, several pre-cautionary measures are taken while creating GEMs, so that the risks associated with their use are minimal.

Some researchers are of the opinion that GEMs will create biotechnological wonders for the environmental management of xenobiotics, in the next few decades. This may be possible only if the associated risks of each GEM is thoroughly evaluated, and fully assured of its biosafety.

**Bioremediation of Contaminated Soils and Waste Lands:**

to industrialization and extensive use of insecticides, herbicides and pesticides, the solids and waste lands world over are getting polluted. The most common pollutants are hydrocarbons, chlorinated solvents, polychlorobiphenyls and metals.

Bioremediation of soils and waste lands by the use of microorganisms is gaining importance in the recent years. In fact, some success has been reported for the detoxification of certain pollutants (e.g. hydrocarbons) in the soil by microorganisms. Bioremediation of soils can be done by involving two principles-bio-stimulation and bio-augmentation.

**Bio-stimulation in Soil Bioremediation:**

Bio-stimulation basically involves the stimulation of microorganisms already present in the soil, by various means.

This can be done by many ways:

i. Addition of nutrients such as nitrogen and phosphorus.

ii. Supplementation with co-substrates e.g. methane added to degrade trichloroethylene.

iii. Addition of surfactants to disperse the hydrophobic compounds in water.

Addition of nutrient and co-substrates promote microbial growth while surfactants expose the hydrophobic molecules. In all these situations, the result is that there occurs bio-stimulation by effective bioremediation of polluted soil or waste land.

**Bio-augmentation in Soil Bioremediation:**

Addition of specific microorganisms to the polluted soil constitutes bio-augmentation. The pollutants are very complex molecules and the native soil microorganisms alone may not be capable of degrading them effectively.

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The examples of such pollutants include polychlorobiphenyls (PCBs), trinitrotoluene (TNT), polyaromatic hydrocarbons (PAHs) and certain pesticides.

Based on the research findings at the laboratory level (with regard to biodegradation), it is now possible to add a combination of microorganisms referred to as consortium or cocktail of microorganisms, to achieve bio-augmentation. With the development of genetically engineered microorganisms (GEMs), they can be also used to bio-augment soils for very efficient bioremediation. But the direct use of GEMs in the soils is associated with several risks and health hazards.

**Techniques of Soil Bioremediation:**

The most commonly used methods for the bioremediation are soils are in situ bioremediation, land farming and slurry phase bioreactors.

**In Situ Bioremediation of Soils:**

In situ bioremediation broadly involves the biological clean-up of soils without excavation. This technique is used for the bioremediation of sub-surfaces of soils, buildings and road ways that are polluted. Sometimes, water (oxygenated) is cycled through the sub-surfaces for increasing the efficiency of microbial degradation. There are two types of in situ soil bioremediation techniques- bioventing and phytoremediation.

**Bioventing:**

This is very efficient and cost- effective technique for the bioremediation of petroleum contaminated soils. Bioventing involves aerobic biodegradation of pollutants by circulating air through sub-surfaces of soil. Although, it takes some years, bioventing can be used for the degradation of soluble paraffin's and polyaromatic hydrocarbons. The major limitation of this technique is air circulation which is not always practicable.

**Phytoremediation:**

Bioremediation by use of plants constitutes phytoremediation. Specific plants are cultivated at the sites of polluted soil. These plants are capable of stimulating the biodegradation of pollutants in the soil adjacent to roots (rhizosphere) although phytoremediation is a cheap and environmental friendly clean-up process for the biodegradation of soil pollutants, it takes several years.



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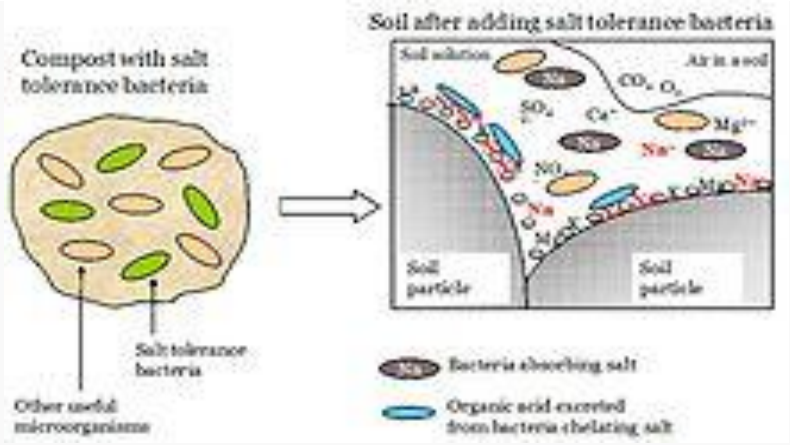


Mechanism of salt removal from tsunami affected soil by bioremediation

**Bioremediation** is a waste management technique that involves the use of organisms to remove or neutralize pollutants from acontaminated site.[1] According to the United States EPA, bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances". Technologies can be generally classified as *in situ* or *ex situ*. *In situ*bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation related technologies are phytoremediation, bioventing, bioleaching, landfarming,bioreactor, composting, bioaugmentation, rhizofiltr ation, and biostimulation.

Bioremediation may occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc.,that help in enhancing the growth of the pollution-eating microbes within the medium (biostimulation). For example, the US Army Corps of Engineers demonstrated that windrowing and aeration of petroleum-contaminated soils enhanced bioremediation using the technique of landfarming.[2] Depleted soil nitrogen status may encourage biodegradation of some nitrogenous organic chemicals,[3] and soil materials with a high capacity to adsorb pollutants may slow down biodegradation owing to limited bioavailability of the chemicals to microbes.[4] Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as **bioremediators**.

However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead are not readily absorbed or captured by microorganisms. A recent experiment, however, suggests that fish bones have some success absorbing lead from contaminated soil. Bone char has been shown to bioremediate small amounts of cadmium, copper, and zinc. A recent

experiment, suggests that the removals of pollutants (nitrate, silicate, chromium and sulphide) from tannery wastewater were studied in batch experiments using marine microalgae. The assimilation of metals such as mercury into the food chain may worsen matters.Phytoremediation is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above-ground parts, which are then harvested for removal The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use. Some damaged artifacts at museums contain microbes which could be specified as bio remediating agents. In contrast to this situation, other contaminants, such as aromatic hydrocarbons as are common in petroleum, are relatively simple targets for microbial degradation, and some soils may even have some capacity to autoremediate, as it were, owing to the presence of autochthonous microbial communities capable of degrading these compounds.

The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes.

**Photosynthetic Pigments**

**Pigments are colorful compounds.**

**Pigments** are chemical compounds which reflect only certain wavelengths of visible light. This makes them appear "colorful". Flowers, corals, and even animal skin contain pigments which give them their colors. More important than their reflection of light is the ability of pigments to **absorb** certain wavelengths.

Because they interact with light to absorb only certain wavelengths, pigments are useful to plants and other **autotrophs** --organisms which make their own food using **photosynthesis**. In plants, algae, andcyanobacteria, pigments are the means by which the energy of sunlight is captured for photosynthesis. However, since each pigment reacts with only a narrow range of the spectrum, there is usually a need to produce several kinds of pigments, each of a different color, to capture more of the sun's energy.

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**There are three basic classes of pigments.**

- **Chlorophylls** are greenish pigments which contain a **porphyrin ring**. This is a stable ring-shaped molecule around which electrons are free to migrate. Because the electrons move freely, the ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll "captures" the energy of sunlight.

There are several kinds of chlorophyll, the most important being chlorophyll "a". This is the molecule which makes photosynthesis possible, by passing its energized electrons on to molecules which will manufacture sugars. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll "a". A

second kind of chlorophyll is chlorophyll "b", which occurs only in "green algae" and in the plants. A third form of chlorophyll which is common is (not surprisingly) called chlorophyll "c", and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates. The differences between the chlorophylls of these major groups was one of the first clues that they were not as closely related as previously thought.

 **Carotenoids** are usually red, orange, or yellow pigments, and include the familiar compound carotene, which gives carrots their color. These compounds are composed of two small six-carbon rings connected by a "chain" of carbon atoms. As a result, they do not dissolve in water, and must be attached to membranes within the cell. Carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to chlorophyll. For this reason, they are called **accessory pigments**. One very visible accessory pigment is **fucoxanthin** the brown pigment which colors kelps and other brown algae as well as the diatoms.

 **Phycobilins** are water-soluble pigments, and are therefore found in the cytoplasm, or in the stroma of the chloroplast. They occur only inCyanobacteria and Rhodophyta.

two classes of phycobilins which may be extracted from these "algae". The vial on the left contains the bluish pigment**phycocyanin**, which gives the Cyanobacteria their name. The vial on the right contains the reddish pigment **phycoerythrin**, which gives the red algae their common name.

Phycobilins are not only useful to the organisms which use them for soaking up light energy; they have also found use as research tools. Both pycocyanin and phycoerythrin **fluoresce** at a particular wavelength. That is, when they are exposed to strong light, they absorb the light energy, and release it by emitting light of a very narrow range of wavelengths. The light produced by this fluorescence is so distinctive and reliable, that phycobilins may be used as chemical "tags". The pigments are chemically bonded to antibodies, which are then put into a solution of cells. When the solution is sprayed as a stream of fine droplets past a laser and computer sensor, a machine can identify whether the cells in the droplets have been "tagged" by the antibodies. This has found extensive use in cancer research, for "tagging" tumor cells.

**Plants**

Green plants have six closely related photosynthetic pigments (in order of increasing polarity):

- Carotene - an orange pigment
- Xanthophyll - a yellow pigment
- Phaeophytin *a* - a gray-brown pigment
- Phaeophytin *b*- a yellow-brown pigment

- Chlorophyll *a* - a blue-green pigment
- Chlorophyll *b* - a yellow-green pigment

Chlorophyll a is the most common of the six, present in every plant that performs photosynthesis. The reason that there are so many pigments is that each absorbs light more efficiently in a different part of the electromagnetic spectrum. Chlorophyll a absorbs well at a wavelength of about 400-450 nm and at 650-700 nm; chlorophyll b at 450-500 nm and at 600-650 nm. Xanthophyll absorbs well at 400-530 nm. However, none of the pigments absorbs well in the green-yellow region, which is responsible for the abundant green we see in nature.

**Bacteria**

Like plants, the cyanobacteria use water as an electron donor for photosynthesis and therefore liberate oxygen; they also use chlorophyll as a pigment. In addition, most cyanobacteria use phycobiliproteins, water-soluble pigments which occur in the cytoplasm of the chloroplast, to capture light energy and pass it on to the chlorophylls. (Some cyanobacteria, the prochlorophytes, use chlorophyll b instead of phycobilin.) It is thought that the chloroplasts in plants and algae all evolved from cyanobacteria.

Several other groups of bacteria use the bacteriochlorophyll pigments (similar to the chlorophylls) for photosynthesis. Unlike the cyanobacteria, these bacteria do not produce oxygen; they typically use hydrogen sulfide rather than water as the electron donor.

Recently, a very different pigment has been found in some marine γ-proteobacteria: proteorhodopsin. It is similar to and probably originated from bacteriorhodopsin (see below under archaea). Bacterial chlorophyll b has been isolated from Rhodopseudomonas spp. but its structure is not yet known

**Algae**

Green algae, red algae and glaucophytes all use chlorophylls. Red algae and glaucophytes also use phycobiliproteins, but green algae do not.

**Archaea**

Halobacteria use the pigment bacteriorhodopsin which acts directly as a proton pump when exposed to light.

**Photosynthesis**

The primary source of energy for nearly all life is the Sun. The energy in sunlight is introduced into the biosphere by a process known as photosynthesis, which occurs in plants, algae and some types of bacteria. Photosynthesis can be defined as the physico-chemical process by which photosynthetic organisms use light energy to drive the synthesis of organic compounds.

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# 2017

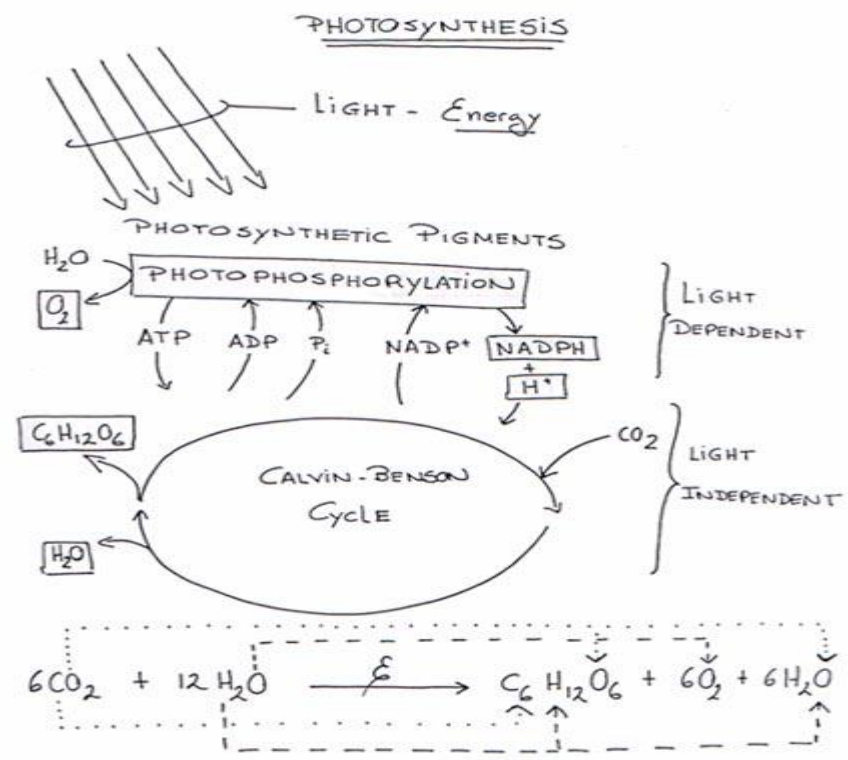
Virtually all oxygen in the atmosphere is thought to have been generated through the process of photosynthesis It is a very complicated biological system. Basically it is the process that converts energy from sunlight to chemical forms of energy that can be used.

Plants, algae, as well as cyanobacteria are responsible for a major part of photosynthesis in oceans. These organisms convert CO2 (carbon dioxide) to organic material by reducing this gas to carbohydrates in a rather complex set of reactions. Electrons for this reduction reaction ultimately come from water, which is then converted to oxygen and protons. Energy for this process is provided by light, which is absorbed by pigments (primarily chlorophylls and carotenoids). Chlorophylls absorb blue and red light and carotenoids absorb blue-green light , but green and yellow light are not effectively absorbed by photosynthetic pigments in plants; therefore, light of these colors is either reflected by leaves or passes through the leaves. This is why plants are green. Other photosynthetic organisms, such as cyanobacteria, known as blue-green algae, and red algae, have additional pigments called phycobilins that are red or blue and that absorb the colors of visible light that are not effectively absorbed by chlorophyll and carotenoids. Yet other organisms, such as the purple and green bacteria, contain bacteriochlorophyll that absorbs in the infrared, in addition to in the blue part of the spectrum. These bacteria do not evolve oxygen, but perform photosynthesis under anaerobic (oxygen-less) conditions. All plants, algae, and cyanobacteria which photosynthesize contain the pigment chlorophyll "a." A second kind of chlorophyll is chlorophyll "b", which occurs only in green algae" and in the plants. A third form of chlorophyll which is common is called chlorophyll "c", and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates.

Photosynthetic pigments come in a huge variety. Some are chlorophyll, carotenoids, and phycobilins, and they differ from each other in their precise chemical structure. Light energy is absorbed by individual pigments, but is not used immediately by these pigments for energy conversion. Instead, the light energy is transferred to chlorophylls that are in a special protein environment where the actual energy conversion event occurs, the light energy is used to transfer an electron to a neighboring pigment. The purpose is to maintain a high rate of electron transfer in the reaction center, even at lower light intensities.

Plants have developed means to convert some of the absorbed light energy to heat rather than to use the absorbed light necessarily for photosynthesis. Because they interact with light to absorb only certain wavelengths, pigments are useful to plants and other autotrophs--organisms which make their own food using photosynthesis. White light is separated into the different colors (wavelengths) of light by passing it through a prism. Wavelength is defined as the distance from peak to peak. Energy is inversely proportional to the wavelength: longer wavelengths have less energy than do shorter ones. Without photosynthesis, the oxygen in the atmosphere would be depleted within several thousand years. It should be emphasized that plants respire

just like any other higher organism, and that during the day this respiration is masked by a higher rate of photosynthesis.




In eukaryotes, photosynthesis takes place in the chloroplast, which has long been known to have prokaryotic features. Chloroplasts are thought to have evolved from a cyanobacterium (or close relative) that was in a symbiotic relationship with a eukaryotic, non-photosynthetic cell and was engulfed inside this cell. The cyanobacterium and the eukaryotic cell presumably were in a mutually beneficial relationship (endosymbiosis), with the photosynthetic organism sharing some of its produced carbohydrates with the host, and the host providing the photosynthetic bacterium with other compounds. The prokaryote slowly gave up its independence as well as its cell wall, and some of its genetic information was transferred to the nucleus of its eukaryotic host. The resulting chloroplast maintains a small, prokaryote-like circular DNA of its own (DNA is material carrying genetic information); this DNA contains the genetic blueprint to make many of the membrane proteins needed in the chloroplast, which apparently are not easily targeted to and/or transported into the chloroplast. Occasionally, photosynthetic organisms are found where the chloroplast has retained a little more of the original cyanobacterial features. For example, in algae such as Cyanophora paradoxa plastids (called cyanelles) are found that resemble cyanobacteria in their overall morphology as well as in the fact that they are surrounded by a cell wall. Even though plants are the most visible representatives of photosynthetic organisms, it should be emphasized that many other types of photosynthetic organisms exist.

**Accessory pigments** are light-absorbing compounds, found in photosynthetic organisms, that work in conjunction with chlorophyll *a*. They include other forms of this pigment, such as chlorophyll *b* in green

algal and higher plant antennae, while other algae may contain chlorophyll *c* or *d*. In addition, there are many non-chlorophyll accessory pigments, such as carotenoids or phycobiliproteins, which also absorb light and transfer that light energy to photosystem chlorophyll. Some of these accessory pigments, in particular the carotenoids, also serve to absorb and dissipate excess light energy, or work as antioxidants. The large, physically associated group of chlorophylls and other accessory pigments is sometimes referred to as a *pigment bed*, though this term is no longer supported by what we know of photosystem and antenna complex structures.
The different chlorophyll and non-chlorophyll pigments associated with the photosystems all have different absorption spectra, either because the spectra of the different chlorophyll pigments are modified by their local protein environment or because the accessory pigments have intrinsic structural differences. The result is that, in vivo, a composite absorption spectrum of all these pigments is broadened and flattened such that a wider range of visible and infrared radiation is absorbed by plants and algae. Most photosynthetic organisms do not absorb green light well, thus most remaining light under leaf canopies in forests or under water with abundant plankton is green, a spectral effect called the "green window". Organisms such as some cyanobacteria and red algae contain accessory phycobiliproteins that absorb green light reaching these habitats.

In aquatic ecosystems, it is likely that the absorption spectrum of water, along with gilvin and tripton (dissolved and particulate organic matter, respectively), determinesphototrophic niche differentiation. The six shoulders in the light absorption of water between wavelengths 400 and 1100 nm correspond to troughs in the collective absorption of at least twenty diverse species of phototrophic bacteria. Another effect is due to the overall trend for water to absorb low frequencies, while gilvin and tripton absorb higher ones. This is why open ocean appears blue and supports yellow species such as *Prochlorococcus*, which contains divinyl-chlorophyll *a* and *b*. *Synechococcus*, colored red withphycoerythrin, is adapted to coastal bodies, while phycocyanin allows Cyanobacteria to thrive in darker inland waters.

**Anabolism** (from Greek: ἀνά, "upward" and βάλλειν, "to throw") is the set of metabolic pathways that construct molecules from smaller units.[1] These reactions require energy. One way of categorizing metabolic processes, whether at the cellular, organ or organism level, is as "anabolic", or as "catabolic" which is the opposite. Anabolism is powered by catabolism, where large molecules are broken down into smaller parts and then used up in cellular respiration. Many anabolic processes are powered by the hydrolysis of adenosine triphosphate (ATP).

Anabolic processes tend toward "building up" organs and tissues. These processes produce growth and differentiation of cells and increase in body size, a process that involvessynthesis of complex molecules.

Examples of anabolic processes include the growth and mineralization of bone and increases in muscle mass. Endocrinologists have traditionally classified hormones as anabolic or catabolic, depending on which part of metabolism they stimulate. The classic anabolic hormones are the anabolic steroids, which stimulate protein synthesis, muscle growth, and insulin. The balance between anabolism and catabolism is also regulated by circadian rhythms, with processes such as glucose metabolism fluctuating to match an animal's normal periods of activity throughout the day

**autotrophy**

(in certain plants and bacteria) the process of making food from inorganic substances, using photosynthesis

**au·to·troph**

An organism capable of synthesizing its own food from inorganic substances, using light or chemical energy. G reen plants, algae, and certain bacteria are autotrophs.

An **autotroph**[α] ("self-feeding", from the Greek *autos* "self" and *trophe* "nourishing") or **producer**, is an organism that produces complex organic compounds (such as carbohydrates, fats, and proteins) from simple substances present in its surroundings, generally using energy from light (photosynthesis) or inorganic chemical reactions (chemosynthesis).[1] They are the producers in a food chain, such as plants on land or algae in water, in contrast to heterotrophs as consumers of autotrophs. They do not need a living source of energy or organic carbon. Autotrophs can reduce carbon dioxide to make organic compounds for biosynthesis and also create a store of chemical energy. Most autotrophs use water as the reducing agent, but some can use other hydrogen compounds such as hydrogen sulfide. Some autotrophs, like green plants and algae, are phototrophs, which means they convert electromagnetic energy from sunlight into chemical energy in the form ofreduced carbon.

Autotrophs can be photoautotrophs or chemoautotrophs. Phototrophs use light as an energy source, while chemotrophs utilize electron donors as a source of energy, whether from organic or inorganic sources; however in the case of autotrophs, these electron donors come from inorganic chemical sources. Such chemotrophs are lithotrophs. Lithotrophs use inorganic compounds, such as hydrogen sulfide, elemental sulfur, ammonium and ferrous iron, as reducing agents for biosynthesis and chemical energy storage. Photoautotrophs and lithoautotrophs use a portion of the ATP produced during photosynthesis or the oxidation of inorganic compounds to reduce $NADP^+$ to NADPH to form organic compounds.

The German term *autotroph* was coined by Albert Bernhard Frank in 1892

## Variants

Some organisms rely on organic compounds as a source of carbon, but are able to use light or inorganic compounds as a source of energy. Such organisms are not defined as autotrophic, but rather as heterotrophic. An organism that obtains carbon from organic compounds but obtains energy from light is called a ***photoheterotroph***, while an organism that obtains carbon from organic compounds but obtains energy from the oxidation of inorganic compounds is termed a ***chemoheterotroph***, ***chemolithoheterotroph***, or ***lithoheterotroph***.

Evidence suggests that some fungi may also obtain energy from radiation. Such radiotrophic fungi were found growing inside a reactor of the Chernobyl nuclear power plant.

## Ecology

Autotrophs are fundamental to the food chains of all ecosystems in the world. They take energy from the environment in the form of sunlight or inorganic chemicals and use it to create energy-rich molecules such as carbohydrates. This mechanism is called primary production. Other organisms, called heterotrophs, take in autotrophs as food to carry out functions necessary for their life. Thus, heterotrophs — all animals, almost all fungi, as well as most bacteria and protozoa — depend on autotrophs, or primary producers, for the energy and raw materials they need. Heterotrophs obtain energy by breaking down organic molecules (carbohydrates, fats, and proteins) obtained in food. Carnivorous organisms rely on autotrophs indirectly, as the nutrients obtained from their heterotroph prey come from autotrophs they have consumed.

Most ecosystems are supported by the autotrophic primary production of plants that capture photons initially released by the sun. The process of photosynthesis splits a water molecule ($H_2O$), releasing oxygen ($O_2$) into the atmosphere, and reducing carbon dioxide ($CO_2$) to release the hydrogen atoms that fuel the metabolic process of primary production. Plants convert and store the energy of the photon into the chemical bonds of simple sugars during photosynthesis. These plant sugars are polymerized for storage as long-chain carbohydrates, including other sugars, starch, and cellulose; glucose is also used to make fats and proteins. When autotrophs are eaten by heterotrophs, i.e., consumers such as animals, the carbohydrates, fats, and proteins contained in them become energy sources for the heterotrophs.[5] Proteins can be made using nitrates, sulfates, and phosphates in the soil.

## Bacterial Photosynthesis

Many prokaryotes (bacteria and cyanobacteria) possess phototrophic modes of metabolism. The types of photosynthesis in the two groups of prokaryotes differ mainly in the type of compound that serves as the hydrogen donor in the reduction of $CO_2$ to glucose. Phototrophic organisms differ from heterotrophic

organisms in that they utilize the glucose synthesized intracellularly for biosynthetic purposes (as in starch synthesis) or for energy production, which usually occurs through cellular respiration.

Unlike phototrophs, heterotrophs require glucose (or some other preformed organic compound) that is directly supplied as a substrate from an exogenous source. Heterotrophs cannot synthesize large concentrations of glucose from $CO_2$by specifically using $H_2O$ or ($H_2S$) as a hydrogen source and sunlight as energy. Plant metabolism is a classic example of photolithotrophic metabolism: plants need $CO_2$ and sunlight; $H_2O$ must be provided as a hydrogen source and usually $NO_3^-$ is the nitrogen source for protein synthesis. Organic nitrogen, supplied as fertilizer, is converted to $NO_3^-$ in all soils by bacteria via the process of ammonification and nitrification. Although plant cells are phototrophic, they also exhibit a heterotrophic mode of metabolism in that they respire. For example, plants use classic respiration to catabolize glucose that is generated photosynthetically. Mitochondria as well as the soluble enzymes of the glycolytic pathway are required for glucose dissimilation, and these enzymes are also found in all plant cells. The soluble Calvin cycle enzymes, which are required for glucose synthesis during photosynthesis, are also found in plant cells. It is not possible to feed a plant by pouring a glucose solution on it, but water supplied to a plant will be "photolysed" by chloroplasts in the presence of light; the hydrogen(s) generated from $H_2O$ is used by Photosystems I and II (PSI and PSII) to reduce $NADP^+$ to NADPH + $H^+$. With the ATP generated by PSI and PSII, these reduced pyridine nucleotides, $CO_2$ is reduced intracellularly to glucose. This metabolic process is carried out in an integrated manner by Photosystems I and II ("Z" scheme) and by the Calvin cycle pathway. A new photosynthetic, and nitrogen fixing bacterium, *Heliobacterium chlorum*, staining Gram positive was isolated, characterized, and found to contain a new type of chlorophyll, i.e., bacteriochlorophyll 'g'. 16S r-RNA sequence analyses showed this organism to be phylogenetically related to members of the family *Bacillaceae*, although all currently known phototrophes are Gram negative( Table 4.4). A few*Heliobacteriium* strains did show the presence of endospores. Another unusual phototrophe is the Gram negative*Halobacterium halobium* (now named *Halobacterium salinarium*), an archaebacterium growing best at 30°C in 4.0–5.0 M (or 25%, w/v) NaCl. This bacterium is a facultative phototrophe having a respiratory mode; it also possesses a purple membrane within which bacteriorhodopsin serves as the active photosynthetic pigment. This purple membranae possesses a light driven proton translocation pump which mediates photosynthetic ATP synthesis via a proton extrusion reaction (see Mitchell Hypothesis). Table 4-4 summarizes the characteristics of known photosynthetic bacteria.

TABLE 4-4 Characteristics Commonly Exhibited by Phototrophic Bacteria[a]

| Photosynthetic Type | Characteristics | Representative Families and Genera |
|---|---|---|
| Purple bacteria<br>Sulfur-type (formerly *Thiorhodaceae*) photolithotrophic bacteria | Obligate phototrophs<br>Strict anaerobes<br>$H_2S$ (or $H_2$) serve as H source<br>Possess S granules when $H_2S$ used<br>Contain bacteriochlorophyll *a* or *b* | Chromatiaceae (*Chromatium, Thiospirillum, Thiosarcina, Thiocapsa*) |
| Non-sulfur-type (formerly *Athiorhodaceae*), photoorganotrophic bacteria | Facultative phototrophs (have respiratory mechanism and will grow heterotrophically)<br>Oxygen-tolerant anaerobes<br>Most require one or more B vitamins<br>Simple organic compounds serve as H source<br>Contain bacteriochlorophyll *a* or *b* | Rhodospirillaceae (*Rhodopseudomonas, Rhodospirillum, Rhodomicrobium*) |
| Green bacteria<br>Photolithotrophic bacteria | Obligate phototrophs<br>Strict anaerobes<br>Contains chlorobium chlorophyll, which is currently referred to as bacteriochlorophyll type c and d<br>Many require vitamin $B_{12}$<br>$S_2$ deposited extracellularly | Chlorobiaceae (*Chlorobium, Chloropseudomonas*) |

[a]All are Gram negative; if motile, they exhibit polar flagellation. Most species are anaerobic, although some purple nonsulfur bacteria (family Athiorhodaceae) are facultative phototrophs and can grow as heterotrophs by using the anaerobic respiratory mode of metabolism; they are therefore oxygen tolerant. For further information, see Bergey's Manual of Determinative Bacteriology, 8th ed, part 1.

Characteristics Commonly Exhibited by Phototrophic Bacteria[a].

**Autotrophy**

Bacteria that grow solely at the expense of inorganic compounds (mineral ions), without using sunlight as an energy source, are called autotrophs, chemotrophs, chemoautotrophs, or chemolithotrophs. Like photosynthetic organisms, all autotrophs use $CO_2$ as a carbon source for growth; their nitrogen comes from inorganic compounds such as $NH_3$, $NO_3^-$, or $N_2$ Interestingly, the energy source for such organisms is the oxidation of specific inorganic compounds. Which inorganic compound is oxidized depends on the bacteria in question (Table 4-5). Many autotrophs will not grow on media that contain organic matter, even agar.

TABLE 4-5 Inorganic Oxidation Reactions Used by Autotrophic Bacteria as Energy Sources

| Chemosynthetic Type | Inorganic Compounds Oxidized as Energy (−E) Source | Representative Families, Genera, and Species[a] | Nitrogen Cycle Reaction |
|---|---|---|---|
| $NH_3$ oxidizers (aerobic) | $NH_3 \xrightarrow{(-E)} NO_2^-$ (−E) = chemical energy or ATP produced | Nitrobacteriaceae *(Nitrosomonas, Nitrosococcus, Nitrosospira)* | Nitrification<br>Nitrification<br>Nitrification<br>Nitrification |
| $NO_2^-$ oxidizers (aerobic) | $NO_2^- \xrightarrow{(-E)} NO_3^-$ | Nitrobacteriaceae *(Nitrobacter, Nitrococcus)* | Nitrification<br>Nitrification<br>Nitrification |
| Sulfur oxidizers[b] (aerobic)<br>Iron oxidizers (aerobic) | $S_2 \xrightarrow{(-E)} SO_4^{2-}$; $Fe^{2+} \xrightarrow{(-E)} Fe^{3+}$; use both reactions | *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*; *Ferrobacillus*, *Leptothrix* | |
| Sulfur compound oxidizers Denitrification (anaerobic) | $S_2O_3$ oxidized; $NO_3^-$ reduced | *Thiobacillus denitrificans* | |

[a] All are Gram-negative species (see *Bergey's Manual of Determinative Bacteriology*, 8th ed, part 12).
[b] Strict autotrophic modes of metabolism are not present in sulfur and sulfur compound-oxidizing bacteria. For example, heterotrophic sulfur compound oxidizers are known, the aerobic species being able to oxidize $H_2S \xrightarrow{(-E)} S_2$ (e.g., *Beggiatoa* and *Thiothrix* species).

**Table 4-5 Inorganic Oxidation Reactions Used by Autotrophic Bacteria as Energy Sources.**

Also found among the autotrophic microorganisms are the sulfur-oxidizing or sulfur-compound-oxidizing bacteria, which seldom exhibit a strictly autotrophic mode of metabolism like the obligate nitrifying bacteria (see discussion of nitrogen cycle below). The representative sulfur compounds oxidized by such bacteria are $H_2S$, $S_2$, and $S_2O_3$. Among the sulfur bacteria are two very interesting organisms; *Thiobacillus ferrooxidans*, which gets its energy for autotrophic growth by oxidizing elemental sulfur or ferrous iron, and *T. denitrificans*, which gets its energy by oxidizing $S_2O_3$anaerobically, using $NO_3^-$ as the sole terminal electron acceptor. T denitrificans reduces $NO_3$ to molecular $N_2$, which is liberated as a gas; this biologic process is called denitrification.

All autotrophic bacteria must assimilate $CO_2$, which is reduced to glucose from which organic cellular matter is synthesized. The energy for this biosynthetic process is derived from the oxidation of inorganic compounds discussed in the previous paragraph. Note that all autotrophic and phototrophic bacteria possess essentially the same organic cellular constituents found in heterotrophic bacteria; from a nutritional viewpoint, however, the autotrophic mode of metabolism is unique, occurring only in bacteria.

**Anerobic Respiration**

Some bacteria exhibit a unique mode of respiration called anaerobic respiration. These heterotrophic bacteria that will not grow anaerobically unless a specific chemical component, which serves as a terminal electron acceptor, is added to the medium. Among these electron acceptors are $NO_3^-$, $SO_4^{2-}$, the organic compound fumarate, and $CO_2$. Bacteria requiring one of these compounds for anaerobic growth are said to be anaerobic respirers.

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A large group of anaerobic respirers are the nitrate reducers (Table 4-6). The nitrate reducers are predominantly heterotrophic bacteria that possess a complex electron transport system(s) allowing the $NO_3^-$ ion to serve anaerobically as a terminal acceptor of electrons

($NO_3^- \xrightarrow{2e} NO_2^-$; $NO_3^- \xrightarrow{5e} N_2$; or $NO_3^- \xrightarrow{8e} NH_3$) The organic compounds that serve as specific electron donors for these three known nitrate reduction processes are shown in Table 4-6. The nitrate reductase activity is common in bacteria and is routinely used in the simple nitrate reductase test to identify bacteria

TABLE 4-6 Nitrate Reducers

| Physiologic Types of Nitrate Reductases | Electron Donor(s) | Representative Organisms |
|---|---|---|
| Respiratory ($NO_3^- \rightarrow NO_2^-$) | Formate<br>NADH | *Escherichia coli*<br>*Klebsiella acrogenes* |
| Denitrifying | NADH | *Pseudomonas aeroginosa* |
| ($NO_3^- \rightarrow N_2$) | Pyruvate<br>NADH, succinate | *Clostridium perfringens*<br>*Paracoccus denitrificans* |
| Assimilatory ($NO_3^- \rightarrow NH_3$) | Lactate<br>$H_2$, formate<br>NADH, succinate<br>NADH<br>NADH, lactate, glycerol-phosphate | *Staphylococcus aureus*<br>*Vibrio succinogenes*<br>*Bacillus stearothermophilus*<br>*Enterobacter aerogenes*<br>*Escherichia coli* |

**Table 4-6 Nitrate Reducers.**

$$4AH_2 + HNO_3 \xrightarrow{\text{Nitrate reduction}} 4A + NH_3 + 3H_2O + \text{energy}$$

($AH_2$ = organic substrate, which serves as electron donor)

A second group of anaerobic respirers, the sulfate reducers, utilizes $SO_4^{2-}$ ion in similar fashion

($SO_4^{2-} \xrightarrow{8e} H_2S$):

$$4AH_2 + H_2SO_4 \xrightarrow{\text{Sulfate reduction}} 4A + H_2S + 4H_2O + \text{energy}$$

The third group, the fumarate respirers, are anaerobic bacteria that require exogenous HOOC — CH = CH — COOH for growth. Fumarate is reduced to succinate (HOOC — $CH_2$ — $CH_2$ — COOH), which is secreted as a by-product.

$$AH_2 + HOOC{=}\overset{H}{\overset{\|}{C}} - \overset{H}{\overset{\|}{C}}{-}COOH \xrightarrow{\text{Fumarate reduction}} A + HOOC - CH_2 - CH_2 - COOH + \text{energy}$$

Organisms of still another specialized group of anaerobic respirers, the methanogens, produce methane gas $CO_2 \xrightarrow{8e} CH_4$ as a metabolic end product of microbial growth. $H_2$ gas is the growth substrate; $CO_2$ is the terminal electron acceptor.

$$4H_2 + CO_2 \xrightarrow{CO_2 \text{ reduction}} CH_4 + 2H_2O + \text{energy}$$

The methanogens are among the most anaerobic bacteria known, being very sensitive to small concentrations of molecular $O_2$. They are also archaebacteria, which typically live in unusual and deleterious environments.

All of the above anaerobic respirers obtain chemical energy for growth by using these anaerobic energy-yielding oxidation reactions.

**The Nitrogen Cycle**

Nowhere can the total metabolic potential of bacteria and their diverse chemical-transforming capabilities be more fully appreciated than in the geochemical cycling of the element nitrogen. All the basic chemical elements (S, O, P, C, and H) required to sustain living organisms have geochemical cycles similar to the nitrogen cycle.

The nitrogen cycle is an ideal demonstration of the ecologic interdependence of bacteria, plants, and animals. Nitrogen is recycled when organisms use one form of nitrogen for growth and excrete another nitrogenous compound as a waste product. This waste product is in turn utilized by another type of organism as a growth or energy substrate. Figure 4-10shows the nitrogen cycle.

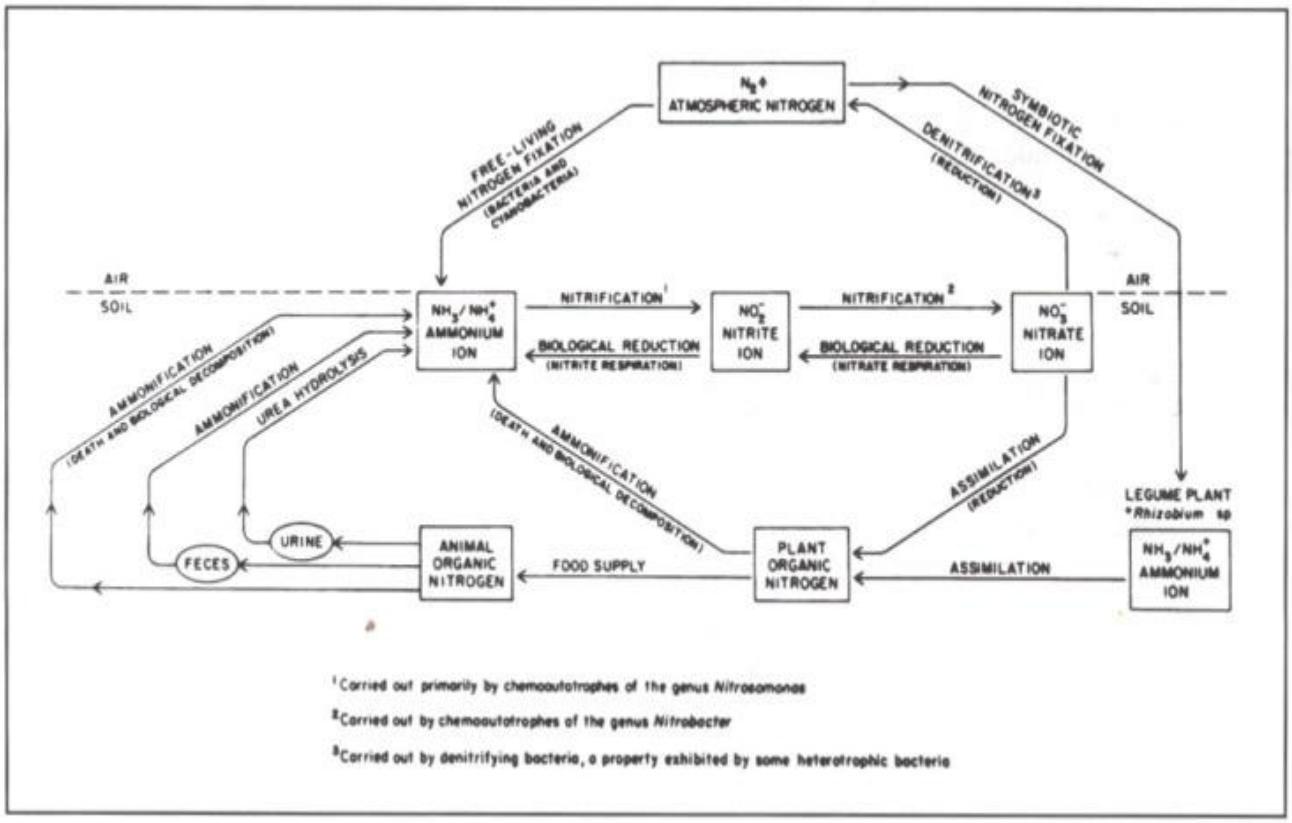


**Figure 4-10 The nitrogen cycle.**

When the specific breakdown of organic nitrogenous compounds occurs, that is, when proteins are degraded to amino acids (proteolysis) and then to inorganic $NH_3$, by heterotrophic bacteria, the process is called ammonification. This is an essential step in the nitrogen cycle. At death, the organic constituents of the tissues and cells decompose biologically to inorganic constituents by a process called mineralization; these inorganic end products can then serve as nutrients for other life forms. The $NH_3$ liberated in turn serves as a utilizable nitrogen source for many other bacteria. The breakdown of feces and urine also occurs by ammonification.

The other important biologic processes in the nitrogen cycle include nitrification (the conversion of $NH_3$ to $NO_3$by autotrophes in the soil; denitrification (the anaerobic conversion of $NO_3$ to $N_2$ gas) carried out by many heterotrophs); and nitrogen fixation ($N_2$to $NH_3$, and cell protein). The latter is a very specialized prokaryotic

process called diazotrophy, carried out by both free-living bacteria (such as *Azotobacter*, *Derxia*, *Beijeringeia*, and *Azomona* species) and symbionts (such as *Rhizobium* species) in conjunction with legume plants (such as soybeans, peas, clover, and bluebonnets). All plant life relies heavily on $NO_3^-$ as a nitrogen source, and most animal life relies on plant life for nutrients.

### Heterotrophic Metabolism

Heterotrophic metabolism is the biologic oxidation of organic compounds, such as glucose, to yield ATP and simpler organic (or inorganic) compounds, which are needed by the bacterial cell for biosynthetic or assimilatory reactions.

### Respiration

Respiration is a type of heterotrophic metabolism that uses oxygen and in which 38 moles of ATP are derived from the oxidation of 1 mole of glucose, yielding 380,000 cal. (An additional 308,000 cal is lost as heat.)

### Fermentation

In fermentation, another type of heterotrophic metabolism, an organic compound rather than oxygen is the terminal electron (or hydrogen) acceptor. Less energy is generated from this incomplete form of glucose oxidation, but the process supports anaerobic growth.

### Krebs Cycle

The Krebs cycle is the oxidative process in respiration by which pyruvate (via acetyl coenzyme A) is completely decarboxylated to $CO_2$. The pathway yields 15 moles of ATP (150,000 calories).

### Glyoxylate Cycle

The glyoxylate cycle, which occurs in some bacteria, is a modification of the Krebs cycle. Acetyl coenzyme A is generated directly from oxidation of fatty acids or other lipid compounds.

### Electron Transport and Oxidative Phosphorylation

In the final stage of respiration, ATP is formed through a series of electron transfer reactions within the cytoplasmic membrane that drive the oxidative phosphorylation of ADP to ATP. Bacteria use various flavins, cytochrome, and non-heme iron components as well as multiple cytochrome oxidases for this process.

### Mitchell or Proton Extrusion Hypothesis

The Mitchell hypothesis explains the energy conservation in all cells on the basis of the selective extrusion of $H^+$ ions across a proton-impermeable membrane, which generates a proton motive force. This energy allows for ATP synthesis both in respiration and photosynthesis.

### Bacterial Photosynthesis

Bacterial photosynthesis is a light-dependent, anaerobic mode of metabolism. Carbon dioxide is reduced to glucose, which is used for both biosynthesis and energy production. Depending on the hydrogen source used to reduce $CO_2$, both photolithotrophic and photoorganotrophic reactions exist in bacteria.

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## Autotrophy

Autotrophy is a unique form of metabolism found only in bacteria. Inorganic compounds are oxidized directly (without using sunlight) to yield energy (e.g., $NH_3$, $NO_2^-$, $S_2$, and $Fe^{2+}$). This metabolic mode also requires energy for $CO_2$reduction, like photosynthesis, but no lipid-mediated processes are involved. This metabolic mode has also been called chemotrophy, chemoautotrophy, or chemolithotrophy.

## Anaerobic Respiration

Anaerobic respiration is another heterotrophic mode of metabolism in which a specific compound other than $O_2$ serves as a terminal electron acceptor. Such acceptor compounds include $NO_3^-$, $SO_4^{2-}$, fumarate, and even $CO_2$ for methane-producing bacteria.

## The Nitrogen Cycle

The nitrogen cycle consists of a recycling process by which organic and inorganic nitrogen compounds are used metabolically and recycled among bacteria, plants, and animals. Important processes, including ammonification, mineralization, nitrification, denitrification, and nitrogen fixation, are carried out primarily by bacteria.

**Carbon fixation** or **carbon assimilation** refers to the conversion process of inorganic carbon (carbon dioxide) to organic compounds by living organisms. The most prominent example is photosynthesis, although chemosynthesis is another form of carbon fixation that can take place in the absence of sunlight. Organisms that grow by fixing carbon are called autotrophs. Autotrophs include photoautotrophs, which synthesize organic compounds using the energy of sunlight, and lithoautotrophs, which synthesize organic compounds using the energy of inorganic oxidation. Heterotrophs are organisms that grow using the carbon fixed by autotrophs. The organic compounds are used by heterotrophs to produce energy and to build body structures. "Fixed carbon", "reduced carbon", and "organic carbon" are equivalent terms for various organic compounds.

**Photosynthesis: Pathway of Carbon Fixation**

Photosynthesis is the synthesis of organic molecules using the energy of light. For the sugar glucose (one of the most abundant products of photosynthesis) the equation is:

**$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$**

A description of the experiments that led to this equation are described in Discovering the Secrets of Photosynthesis.

Light provides the energy to:

- transfer electrons from water to **nicotinamide adenine dinucleotide phosphate** (**$NADP^+$**) forming **NADPH**;

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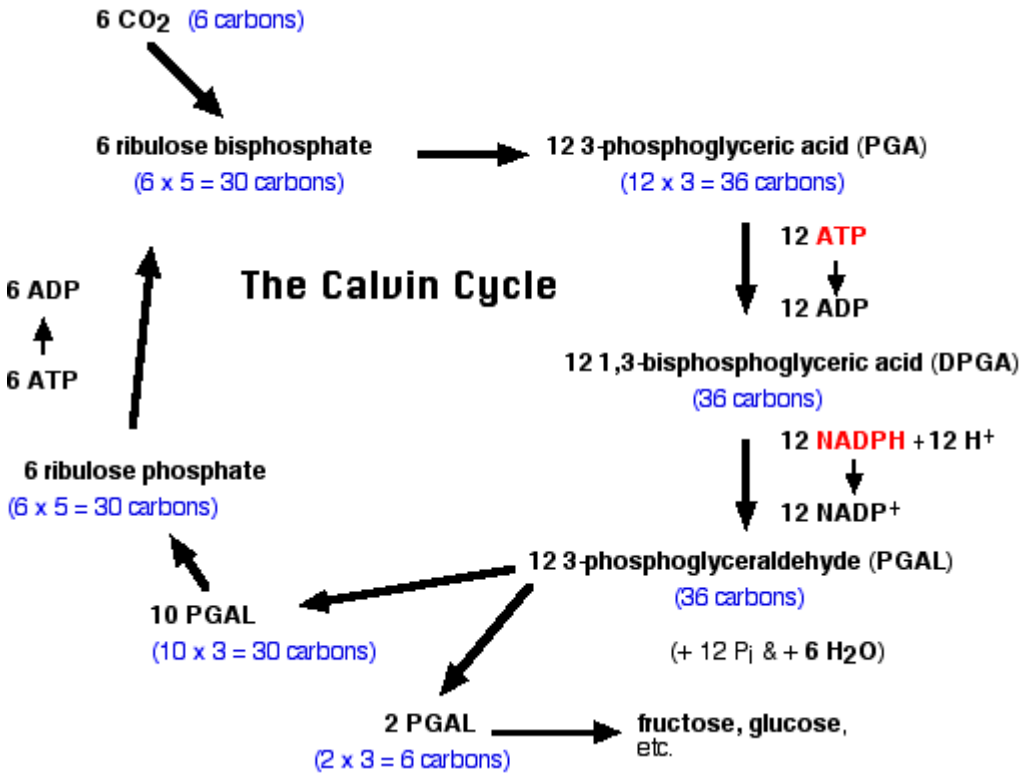
- generate **ATP**.

The details of these processes are described in Photosynthesis: The Role of Light.

**ATP** and **NADPH** provide the energy and electrons to reduce carbon dioxide ($CO_2$) to organic molecules.

**The Steps**

- $CO_2$ combines with the phosphorylated 5-carbon sugar **ribulose bisphosphate**.
- This reaction is catalyzed by the enzyme **ribulose bisphosphate carboxylase oxygenase** (**RUBISCO**)(an enzyme which can fairly claim to be the most abundant protein on earth).
- The resulting 6-carbon compound breaks down into two molecules of **3-phosphoglyceric acid** (**PGA**).
- The PGA molecules are further phosphorylated (by **ATP**) and are reduced (by **NADPH**) to form **phosphoglyceraldehyde** (**PGAL**).
- Phosphoglyceraldehyde serves as the starting material for the synthesis of **glucose** and **fructose**.
- Glucose and fructose make the disaccharide **sucrose**, which travels in solution to other parts of the plant (e.g., fruit, roots).
- Glucose is also the monomer used in the synthesis of the **polysaccharides starch** and **cellulose**.




The graphic shows the steps in the fixation of carbon dioxide during photosynthesis. All of these reactions occur in the **stroma** of the chloroplast. These steps were worked out by Melvin Calvin and his colleagues at the University of California and, for this reason, are named the Calvin cycle.

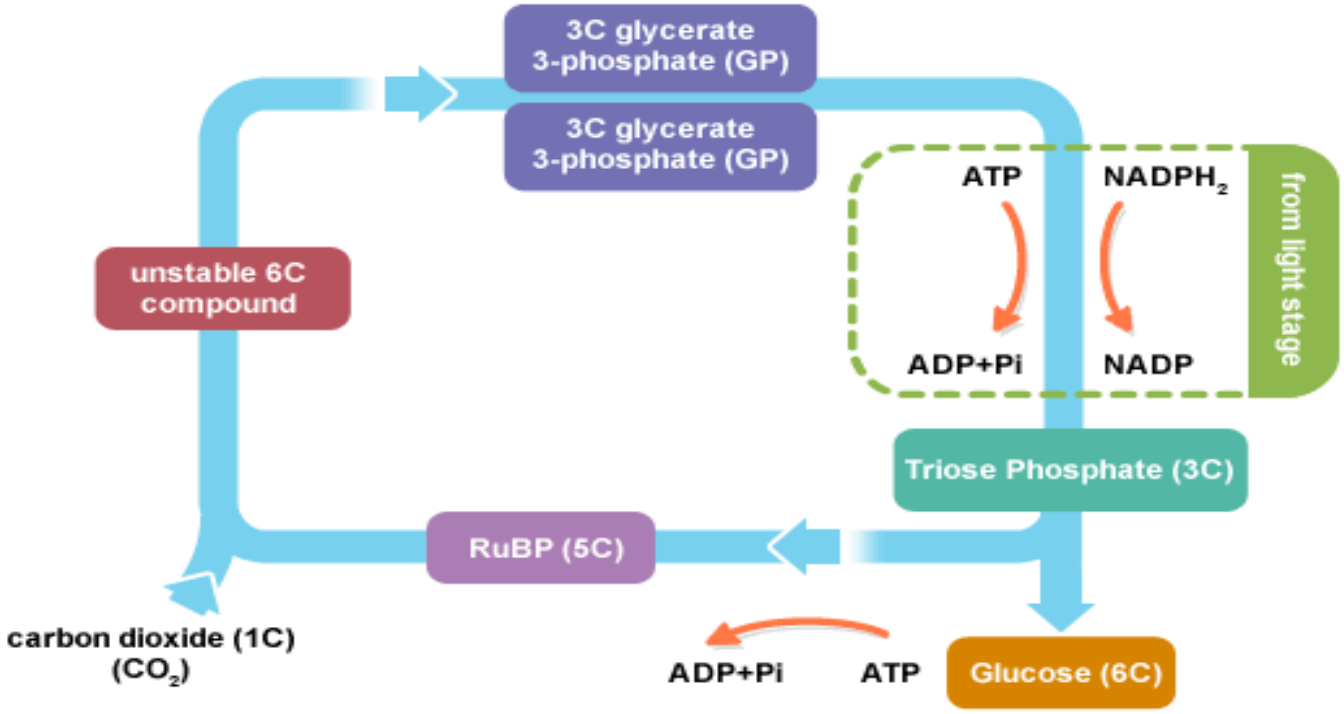
**Experiment**

The experimental apparatus is shown at the right. After various intervals of illumination, a suspension of unicellular algae is inactivated and the contents of the cells extracted. The compounds in a drop of the extract are then separated by paper chromatography.

The identity of each substance may be determined simply by comparing its position with the positions occupied by known substances under the same conditions. Or, a fragment containing the spot can be cut from the sheet and chemically analyzed.

To determine which, if any, of the substances separated on the chromatogram are radioactive, a sheet of X-ray film is placed next to the chromatogram. If dark spots appear on the film (because of radiation emitted by the $^{14}C$ atoms), their position can be correlated with the positions of the chemicals in the chromatogram. Using this technique of **autoradiography**, Calvin found that $^{14}C$ turned up in glucose molecules within 30 seconds after the start of photosynthesis. When he permitted photosynthesis to proceed for only 5 seconds, however, the radioactivity was concentrated in several other, smaller, molecules.

**Carbon fixation (The Calvin Cycle)**




Carbon fixation stage in photosynthesis

**The carbon fixation stage occurs in the stroma and results in the production of *glucose*.**

It is a result of an enzyme controlled sequence of reactions requiring ATP and hydrogen ($NADPH_2$) from the light stage, and carbon dioxide. It involves the reduction of carbon dioxide, that is the addition of hydrogen, to form carbohydrate.

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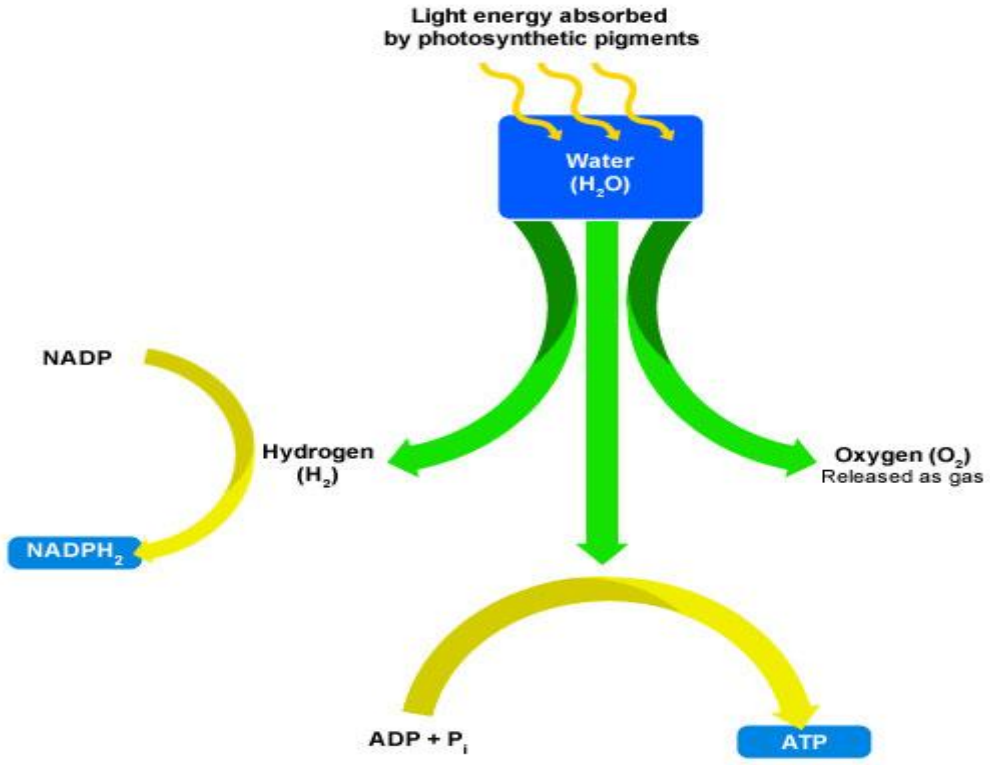
- $CO_2$ is accepted by the 5C compound **ribulose 1,5-biphosphate (RuBP)** to form an unstable 6C compound.
- The 6C compound formed immediately splits into two *molecules* of a 3C compound called **glycerate 3-phosphate (GP)**.
- Energy is used to convert GP into **triose phosphate**, a 3 carbon compound. It is at this point that the ATP and hydrogen produced in the light dependent stage are used.
- Triose phosphate doubles up to form **glucose**. Glucose may then be used in either respiration to provide energy, stored as *starch*, or used to synthesise*cellulose* for cell walls.
- The cycle is completed when the leftover GP molecules are met with a carbon acceptor and converted into RuBP, which is then joined with carbon dioxide to re-start the cycle.

All the major biological molecules in plants are derived from the photosynthetic process:

- Proteins
- Fats
- Carbohydrates
- Nucleic acids

**The light dependent stage**




**This stage takes place in the granum.**

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Light energy is absorbed by chlorophyll and is used to regenerate adenosine tri-phosphate or *ATP* and split water. This is called the **photolysis of water**. Photolysis of water results in the release of oxygen, as a by-product, and the release of hydrogen. The hydrogen released from the water molecule is transferred to the hydrogen acceptor. NADP becomes reduced to form $NADPH_2$.

**The products of the light stage are ATP and $NADPH_2$.**

The hydrogen and ATP are used in the carbon fixation reactions:

- ATP provides the energy for the reactions.
- $NADPH_2$ provides the hydrogen for the reduction of *carbon dioxide* to form *carbohydrate*.

**The limiting factors of photosynthesis**

There are three main limiting factors in photosynthesis:

- **Lack of carbon dioxide**. If there is no carbon dioxide available RuBP cannot be converted into GP. As a result the RuBP starts to build up and no more glucose will be produced.
- **Low temperatures**. These limit photosynthesis since the enzymes controlling the reactions are below their optimum temperature.
- **Lack of light**. In the absence of light neither the ATP or the $NADPH_2$ will be produced and so the GP cannot be converted into glucose. This results in the GP building up and the RuBP being used up.

| | $C_3$ pathways | | $C_4$ pathways |
|---|---|---|---|
| 1. | The primary acceptor of $CO_2$ is RUBP – a six-carbon compound. | 1. | The primary acceptor of $CO_2$ is phosphoenol pyruvate – a three-carbon compound. |
| 2. | The first stable product is 3-phosphoglycerate. | 2. | The first stable product is oxaloacetic acid. |
| 3. | It occurs only in the mesophyll cells of the leaves. | 3. | It occurs in the mesophyll and bundle-sheath cells of the leaves. |
| 4. | It is a slower process of carbon fixation and photo-respiratory losses are high. | 4. | It is a faster process of carbon fixation and photo-respiratory losses are low. |

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| LIGHT REACTION (light dependent reaction) | DARK REACTION (light independent reaction) |
|---|---|
| It occurs in thylakoid membranes of chloroplasts in a leaf. | It occurs in the stroma of the living chloroplast. |
| Light energy is absorbed by photosystems PS-I and PS-II | Carbon dioxide present in the atmosphere is absorbed. |
| Cyclic and non-cyclic photophosphorylation occur in the cells | C3 pathway is observed in green plants. In desert plants, we can observe c4 pathway. |
| End products are ATP and NADP.H2. | End products of light reaction are used up to synthesise sugar. |
| Oxygen is released as a by-product of the photolysis if water. | Main product is sugar in the form of glucose |

| C3 Photosynthesis | C4 Photosynthesis | CAM Photosynthesis |
|---|---|---|
| • **The typical photo synthesis.**<br>• **Use the Calvin Cycle and incorporate $CO_2$ into organic material.**<br>• **Produces THREE CARBON COMPOUND (G3P).**<br>• **Stomata open during the day**<br>• **RUBISCO enzyme used**<br>• **Photosynthesis in leaf**<br>**Used under cool and moist conditions with normal light conditions.** | • Photosynthesis is faster in desert's high heat.<br>• Stomata open during the day<br>• PEP CARBOXYLE LASE enzyme used because it allows $CO_2$ to be taken into the plant quickly and delivers to RUBISCO<br>• Photosynthesis in inner cells (REQ special Kranz Anatomy).<br>Photosynthesizes faster under high light/temperatures because $CO_2$ by passes oxygen and photorespiration and goes directly to RUBISCO.<br>Better water efficiency because $CO_2$ goes directly to RUBISCO | • Named after the first family it was found in.<br>• CAM – Crassulacean Acid Metabolism<br>• Stomata open at NIGHT. Closed during day.<br>• $CO_2$ is converted to an acid and stored during the nighttime<br>• In the day, the acid is broken down back to $CO_2$ for use.<br>• Better water efficiency with low water because closed stomata during the day saves water from evotranspiration<br>• Can CAM-Idle, or close stomata during both DAY AND NIGHT.<br>CAM plants included cactuses, agaves, orchids, and bromeliads. |

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| | | | | | |
|---|---|---|---|---|---|
| Unit III | | | | | |
| The magnitude of BOD of wastewater is related to | bacterial count | amount of organic material | amount of inorganic material | viral count | amount of organic material |
| Biogas production is | a temperature-dependent process | a temperature independent process | an oxygen dependent process | none of the above | an oxygen dependent process |
| Iron bacteria can produce | slime | undesirable odors and tastes | no taste | extreme acidity | undesirable odors and tastes |
| Biomass | provides the U.S. with about 50% of its energy | consists largely of wood, animal, and human waste | is unlikely to be a major source of energy globally | offers the consumer high quality energy with low environmental impact | offers the consumer high quality energy with low environmental impact |
| Which is not a form of biomass energy? | Incineration of solid waste | Composting to produce methane | Ethanol and methanol production for auto fuel | Photovoltaic production of hydrogen | Incineration of solid waste |

| | | | | | |
|---|---|---|---|---|---|
| Which of the following statement is not correct? | The use of 25-40°C temperatures allows the biogas production to be more stable | The use of 25-40°C temperatures does not destroy potentially harmful bacteria | The use of 25-40°C temperatures destroys potentially harmful bacteria | None of the above | The use of 25-40°C temperatures does not destroy potentially harmful bacteria |
| Which of the following is not the biofertilisers producing bacteria? | Nostoc | Anabaena | volvox | Clostridium | Clostridium |
| Which of the following is capable of oxidizing sulfur to sulfates? | Thiobacillus thiooxidans | Desulfotomaculum | Rhodospirillum | Rhodomicrobium | Thiobacillus thiooxidans |
| The | nitrate | nitrate | nitro | nitrate | nitro |

| | | | | | | |
|---|---|---|---|---|---|---|
| The groups of symbiotic bacteria, which have the ability to fix nitrogen | derive their food and minerals from the legume, and in turn they supply the legume with some or all of its nitrogen | grow together for a mutual benefit is called symbiosis and so these bacteria are called symbiotic nitrogen-fixing bacteria | these bacteria are from the genus, Rhizobium | no change | | derive their food and minerals from the legume, and in turn they supply the legume with some or all of its nitrogen |
| An example of a symbiotic nitrogen fixer is | Azotobacter | Beijerinckia | Clostridium | Rhizobium | | Azotobacter |
| Which of the following statement is not true about composition of biogas? | It is composed almost exclusively of methane and carbon dioxide | It also contains with traces of H2S, N2, H2and CO | It also contains with traces of O2 and Cl2 | no O2 and NO Co2 | | It also contains with traces of O2 and Cl2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| The groups of bacteria which have the ability to fix nitrogen from air to soil are | symbiotic | Nonsymbiotic | both (a) and (b) | non symbiotic | | symbiotic |
| Which are the main source of biofertilisers? | Cyanobacteria | Bacillus | Streptococcus | coliform | | Cyanobacteria |
| Degree of compost maturity can be assesed by | infrared technique | germination test | both (a) and (b) | no test | | germination test |
| The diagnostic enzyme for nitrogen-fixing organisms is | nitrogenase | nitrate reductase | nitrate oxidase | no enzyme | | nitrate reductase |
| Syntrophism involves | exchange of nutrients between two species | exchange of nutrients among species | no exchange of nutrients between two species | no exchange of nutrients among species | | exchange of nutrients among species |
| Assimilativ | plants | Fungi | prokaryot | virus | | prokaryot |

| | | | | | | |
|---|---|---|---|---|---|---|
| Which of the following bacteria is associated with food poisoning due to consumption of sea fish? | Vibrio parahaemolyticus | V alginolyticus | V vulnificus | V. chlorae | | Vibrio parahaemolyticus |
| Which of the following conditions can be caused by Plesiomonas? | Septicaemia | Gastroenteritis | Cellulites | edema | | Septicaemia |
| Which of the following does not cause wound infection following exposure to sea water or infected shellfish? | Vibrio vulnificus | V. alginolyticus | V. cholerae | Aeromonas | | V. cholerae |

| | | | | | | |
|---|---|---|---|---|---|---|
| The DNA coding for the production of cholera toxin in Vibrio cholerae is on the | phage | Plasmid | chromosome | transposon | | plasmid |
| Which of the following toxin resembles cholera toxin? | Stable toxin of E. coli | Diphtheria toxin | Labile toxin of Escherichia coli | Tetanus toxin | | Stable toxin of E. coli |
| Which of the following bacteria lack a cell wall and are therefore resistant to penicillin? | Cyanobacteria | Mycoplasmas | Bdellovibrios | Spirochetes | | Mycoplasmas |
| A cluster of polar flagella is called | lophotrichous | Amphitrichous | monotrichous | peritrichous | | lophotrichous |

| | | | | | | |
|---|---|---|---|---|---|---|
| The protein from which hook and filaments of flagella are composed of, is | keratin | Flagellin | gelatin | casein | | flagellin |
| The cocci which mostly occur in single or pairs are | Streptococci | Diplococci | Tetracocci | None of these | | Diplococci |
| Which of | Gram- | Gram- | Both (a) | None of | | Gram- |
| Peptidoglycan accounts for _____ __ of the dry weight of cell wall in many gram positive bacteria | 50% or more | About 10% | 11%+ 0.22% | About 20% | | 50% or more |
| Bacteria | move | Reproduc | stick to | grow in | | move |
| Which of the following is true about cell wall of gram-positive bacteria? | It consists of multiple layers | It is thicker than that associated with gram-negative bacteria | It contains teichoic acids | Less lipid | | It contains teichoic acids |

| | | | | | | |
|---|---|---|---|---|---|---|
| The cell walls of many gram positive bacteria can be easily destroyed by the enzyme known as | lipase | Lysozyme | pectinase | peroxidase | | lysozyme |
| For most bacteria, the optimum pH for growth lies between | 6.5-7.5 | 3.5-4.5 | 4.5-5.5 | 5.5-6.5 | | 6.5-7.5 |
| Suspension cultures consist of cells and cell aggregates, growing dispersed in | liquid medium | solid nutrient medium | solid or liquid medium | none of these | | liquid medium |
| The protoplast can be used to | modify genetic information | create plant hybrid | study plant viral infections | no alteration | | modify genetic information |
| The cell wall of | gram-positive bacteria are thicker than gram-negative bacteria | gram-negative bacteria are thicker than gram-positive bacteria | both have same thickness but composition is different | less lipid | | gram-positive bacteria are thicker than gram-negative bacteria |

| | | | | | |
|---|---|---|---|---|---|
| Peptidoglycan is also known as | N-acetyl muramic acid | murein mucopeptide | N acetylglucosamine | mesodiaminopimetic acid | murein mucopeptide |
| The cocci which forms a pair | Staphylococci | Diplococcic | Tetracocci | Streptococci | diplococcic |
| Chemotaxis is a phenomenon of | swimming away of bacteria | swimming towards bacteria | swimming away or towards of bacteria in presence of chemical compound | swim in media | swimming away or towards of bacteria in presence of chemical compound |
| The structure responsible for motility of bacteria is | pilli | Flagella | sheath | capsules | flagella |
| The next to last step in peptidoglycan biosynthesis is | synthesis of the NAM-peptide subunit | removal of the subunit from bactoprenol | linking the sugar of the disaccharide-peptide unit to the growing peptidoglycan chain | cross-linking the peptide side chains of peptidoglycan | linking the sugar of the disaccharide-peptide unit to the growing peptidoglycan chain |
| The cocci which forms a chain is | Streptococci | Diplococci | Staphylococci | Tetracocci | Streptococci |

| | | | | | | |
|---|---|---|---|---|---|---|
| The arrangement, in which flagella are distributed all round the bacterial cell, is known as | lophotrichous | Amphitrichous | peritrichous | monotrichous | | peritrichous |
| Periplasm is | the area between the inner and outer membranes of gram-negative bacteria | the area between the inner and outer membranes of Gram-positive bacteria | the interior portion of mitochondria | the area outside the cell membrane that is influenced by the polymers | | the area between the inner and outer membranes of gram-negative bacteria |
| Which of the following has peptidoglycan as a major constituent of cell wall? | Gram-negative bacteria | Gram-positive bacteria | Fungi | virus | | Gram-positive bacteria |
| The common word for bacteria which are helically curved rods is | cooci | Pleomorphic | bacillus | spirilla | | spirilla |

| | | | | | | |
|---|---|---|---|---|---|---|
| The bacteria deficient in cell wall is | Treponema | Mycoplasma | Staphylococcus | Klebsiella | | Mycoplasma |
| Which of the following is not true about peptidoglycan? | It is a polymer consisting of N-acetyl glucosamine, N-acetyl muramic acid and amino acids (alanine, lysine, etc.) | It is present in prokaryotic cell wall | It occurs in the form of a bag shaped macro molecule surrounding the cytoplasm membrane | No NAM NAG | | |
| Single or clusters of flagella at both poles is known as | monotrichous | Peritrichous | amphitrichous | atrichous | | amphitrichous |
| Which of the following bacterial genera (that produces endospore) have medical importance? | Salmonella | Bacillus | proteus | E. coli | | Bacillus |

| | | | | | |
|---|---|---|---|---|---|
| _____media is used for cultivation of bacteria | Nutrient agar | MacConkey agar | EMB agar | MHA | Nutrient agar |
| Single bacteria will form a _____ colony | Multiple | Single | No | infinite | Single |
| Which instrument is used for sterilization above 100° C | Flame | Autoclave | Filters | Desiccators | Autoclave |
| _____ is the first phase in growth curve | Log | Lag | stationary | death | Lag |
| DNA to | replication | Biosynthe | translatio | transcripti | replication |
| Oligonucleotide means containing | 10 nucleotides | more than 10 nucleotides | less than 10 nucleotides | no nucleotides | 10 nucleotides |
| _____ group of bacteria grows in high temperature | Halophiles | Basophiles | thermophiles | psychrophiles | thermophiles |

| | | | | | |
|---|---|---|---|---|---|
| The group of gram positive bacteria having low G+C contents are called as | cyanobacteria | Nanobacteria | Firmicutes | Actinobacteria | Firmicutes |
| BGA expanded as | Blue Green Algae | Blue Grown Algae | Blue non Grown Algae | Blue Gram Algae | Blue Green Algae |
| The time required to kill 90% of the microorganisms in a sample at a specific temperature is the | decimal reduction time | thermal death point | F value | D value | decimal reduction time |
| Which of the following is best used for long term storage of microbial samples when carried out properly? | Storage in a freezer at -10°C | Storage in a freezer at ultra low temperatures (-70°C) | Storage in a refrigerator on an agar slant | Storage on a petri plate at room temperature | Storage in a freezer at ultra low temperatures (-70°C) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Which of the antibiotic is not used as a food preservative ? | Pimaricin | Nisin | Tylosin | β-lactam antibiotic | | Nisin |
| Which antibiotic has a beta-lactam ring? | Cephalosporin | Penicillin | Tetracycline | Streptomycin | | Penicillin |
| In eukaryotic cells, ribosomes are | 70S | 60S | 80S | Not specific | | 80S |
| Porins are located in | the outer membrane of gram-negative bacteria | the peptidoglycan layer of gram-positive bacteria | the cytoplasmic membrane of both gram-negative and gram-positive bacteria | the periplasmic space of gram-negative bacteria | | the outer membrane of gram-negative bacteria |
| For most bacteria, the optimum pH for growth lies between | 6.5-7.5 | 3.5-4.5 | 4.5-5.5 | 5.5-6.5 | | 6.5-7.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Suspension cultures consist of cells and cell aggregates, growing dispersed in | liquid medium | solid nutrient medium | solid or liquid medium | semisolid media | | liquid medium |

# 2017

## I M.Sc Microbiology – Marine microbiology

| LECTURE PLAN - UNIT -1V | | | |
|---|---|---|---|
| **S. no** | Lecture duration(Hr) | **Topics covered** | **Supporting materials** |
| 1 | 1 | Photosynthetic pigments | T2 167-168 |
| 2 | 1 | Accessory pigments | T2 168 |
| 3 | 1 | Chlorophyll | T2 168 |
| 4 | 1 | Carotenoids | T2 169 |
| 5 | 1 | Rhodopsin | T2 170 |
| 6 | 1 | Phycobilliprotein | T2 171 |
| 7 | 1 | carbohydrates | T2 190-200 |
| 8 | 1 | Anabolism | T2 190-200 |
| 9 | 1 | Photosynthesis | T2 200-210 |
| 10 | 1 | Autotrophic generation of ATP | T2 200-210 |
| 11 | 1 | Fixation of CO2 | T2 210-220 |
| 12 | 1 | Calvin cycle | T2 210-220 |
| 13 | 2 | C3 and C4 pathway | T2 220-240 |
| 14 | 1 | Unit IV test | |
| **Textbooks :** | | T1-Microbiology- pelczar – McGraw Hill publishing<br>T2-Microbiology- presscott – McGraw hill publishing<br>T3-Microbial genetics – David friefelder- | |
| **Reference books:** | | | |
| **Website:** | | W1- www.microbiology.com<br>W2 – www.marinestudy.com | |
| **Journals:** | | | |

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# 2017

**Sampling tools for the marine environment**

This article provides an overview of the sampling tools and fishing techniques used in the marine environment. These relate to species occuring on the sea surface, the water column and on the seabed.

## Introduction

All methods of physical capture are inherently selective. Small fish may pass through large-meshed nets; large fish may out-swim trawls; gill nets will catch fish mainly of a certain size range. Fish may react differently to fishing gear with respect to species, size, biological state, environmental conditions including ambient light and the acoustic noise field, among many other factors.

[2]This is why organisms are subdivided out of practical necessity, in that the sampling approach and sample size that are appropriate for one group are often inappropriate for another. The disparity in appropriate techniques for different sizes of groups of organisms has contributed greatly to the paucity of studies on more than one taxonomic grouping at a given locale.

Unfortunately, where conflicting conclusions have been drawn patterns in different groups of organisms, it is rarely possible to know whether the patterns truly vary among groups or merely reflect differences in sampling efforts. The choice of a suitable sampler is a compromise between a variety of factors.

Sampling tools for pelagic organisms

## Midwater or pelagic trawl

[1]A midwater or pelagic trawl is a set of gear that is used to catch fish that are between the sea surface and bottom, generally staying clear of the bottom. Occasionally, midwater trawls are configured with floats to perform catching in the shallow-surface layer.

[3]A midwater trawl consists of a cone shaped body, normally made of four panels, ending in a codend with lateral wings extending forward from the opening.

[1]Midwater and bottom trawls (see further) have many parts in common, if differing in dimensions and shapes due to their different fishing objects and hydrodynamic regimes of operation. Midwater trawls are designed to catch fish in the midwater column, hence must be capable of rapid maneuvering while maintaining an open net mouth. This is reflected in differences in the body of the net, rigging, and even trawl doors.

Composting is nature's way of recycling. Composting biodegrades organic waste. i.e. food waste, manure, leaves, grass trimmings, paper, wood, feathers, crop residue etc., and turns it into a valuable organic fertilizer.

Composting is a natural biological process, carried out under controlled aerobic conditions (requires oxygen). In this process, various microorganisms, including bacteria and fungi, break down organic matter into simpler substances. The effectiveness of the composting process is dependent upon the environmental conditions present within the composting system i.e. oxygen, temperature, moisture, material disturbance, organic matter and the size and activity of microbial populations.

Composting is not a mysterious or complicated process. Natural recycling (composting) occurs on a continuous basis in the natural environment. Organic matter is metabolized by microorganisms and consumed by invertebrates. The resulting nutrients are returned to the soil to support plant growth.

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# 2017

Composting is relatively simple to manage and can be carried out on a wide range of scales in almost any indoor or outdoor environment and in almost any geographic location. It has the potential to manage most of the organic material in the waste stream including restaurant waste, leaves and yard wastes, farm waste, animal manure, animal carcasses, paper products, sewage sludge, wood etc. and can be easily incorporated into any waste management plan.

Since approximately 45 - 55% of the waste stream is organic matter, composting can play a significant role in diverting waste from landfills thereby conserving landfill space and reducing the production of leachate and methane gas. In addition, an effective composting program can produce a high quality soil amendment with a variety of end uses.

The essential elements required by the composting microorganisms are carbon, nitrogen, oxygen and moisture. If any of these elements are lacking, or if they are not provided in the proper proportion, the microorganisms will not flourish and will not provide adequate heat. A composting process that operates at optimum performance will convert organic matter into stable compost that is odor and pathogen free, and a poor breeding substrate for flies and other insects. In addition, it will significantly reduce the volume and weight of organic waste as the composting process converts much of the biodegradable component to gaseous carbon dioxide.

**The composting process is carried out by three classes of microbes -**

• Psychrophiles - low temperature microbes
• Mesophiles -medium temperature microbes
• Thermophiles - high temperature microbes

Generally, composting begins at mesophilic temperatures and progresses into the thermophilic range. In later stages other organisms including Actinomycetes, Centipedes, Millipedes, Fungi, Sowbugs, Spiders and Earthworms assist in the process.

**Temperature**

Temperature is directly proportional to the biological activity within the composting system. As the metabolic rate of the microbes accelerates, the temperature within the system increases. Conversely, as the metabolic rate of the microbes decreases, the system temperature decreases. Maintaining a temperature of 130°F or more for 3 to 4 days favors the destruction of weed seeds, fly larvae and plant pathogens.

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At a temperature of 155 degrees F, organic matter will decompose about twice as fast as at 130 degrees F. Temperatures above 155 degrees F may result in the destruction of certain microbe populations. In this case temperature may rapidly decline. Temperature will slowly rise again as the microbe population regenerates.
Moisture content, oxygen availability, and microbial activity all influence temperature. When the pile temperature is increasing, it is operating at optimum performance and should be left alone. As the temperature peaks, and begins to decrease, the pile should be turned to incorporate oxygen into the compost. Subsequently , the pile should respond to the turning and incorporation of oxygen, and temperature should again cycle upwards. The turning process should be continued until the pile fails to re-heat. This indicates that the compost material is biologically stable.

Composting microorganisms thrive in moist conditions. For optimum performance, moisture content within the composting environment should be maintained at 45 percent. Too much water can cause the compost pile to go anaerobic and emit obnoxious odors. Too little will prevent the microorganisms from propagating.

**Particle Size**

The ideal particle size is around 2 to 3 inches. In some cases, such as in the composting of grass clippings, the raw material may be too dense to permit adequate air flow or may be too moist. A common solution to this problem is to add a bulking agent (straw, dry leaves, paper, cardboard) to allow for proper air flow. Mixing materials of different sizes and textures also helps aerate the compost pile.

**Turning**

During the composting process oxygen is used up quickly by the microbes as they metabolize the organic matter. As the oxygen becomes depleted the composting process slows and temperatures decline. Aerating the compost by turning should ensure an adequate supply of oxygen to the microbes.

**Composting Period**

The composting period is governed by a number of factors including, temperature, moisture, oxygen, particle size, the carbon-to-nitrogen ratio and the degree of turning involved. Generally, effective management of these factors will accelerate the composting process.

**Carbon to Nitrogen Ratio**

The microbes in compost use carbon for energy and nitrogen for protein synthesis. The proportion of these two elements required by the microbes averages about 30 parts carbon to 1 part nitrogen. Accordingly, the ideal

ratio of Carbon to Nitrogen (C:N) is 30 to 1 (measured on a dry weight basis). This ratio governs the speed at which the microbes decompose organic waste.

Most organic materials do not have this ratio and, to accelerate the composting process, it may be necessary to balance the numbers.

The C:N ratio of materials can be calculated by using table 1 below. Example, if you have two bags of cow manure (C:N = 20:1) and one bag of corn stalks (C:N = 60:1) then combined you have a C:N ration of (20:1 + 20:1 + 60:1)/3 = (100:1)/3 = 33:1

Table 1 lists the Carbon/Nitrogen Ratios of Some Common Organic Materials

Table 1.

| Material | C:N Ratio |
|---|---|
| Vegetable wastes | 12-20:1 |
| Alfalfa hay | 13:1 |
| Cow manure | 20:1 |
| Apple pomace | 21:1 |
| Leaves | 40-80:1 |
| Corn stalks | 60:1 |
| Oat straw | 74:1 |
| Wheat straw | 80:1 |
| Paper | 150-200:1 |
| Sawdust | 100-500:1 |
| Grass clippings | 12-25:1 |
| Coffee grounds | 20:1 |
| Bark | 100-130:1 |
| Fruit wastes | 35:1 |
| Poultry manure (fresh) | 10:1 |

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| | |
|---|---|
| **Horse manure** | **25:1** |
| **Newspaper** | **50-200:1** |
| **Pine needles** | **60-110:1** |
| **Rotted manure** | **20:1** |

The C:N ratios listed above are for guidelines only.

**Composters for smaller volumes**

Plastic bin (well ventilated)

Metal or plastic drum (base removed – well ventilated)

**Composters for larger volumes**

Rotating drum (in vessel)

Enclosure (made from 4 x 4 pallets lined with chicken wire)

Open pile – windrow (covered with plastic or tarp)

**In-Vessel**

An in-vessel, aerobic mechanical composter can be constructed from a steel drum, or tank designed to rotate at three to five revolutions per hour. Rotation can be carried out with a simple hand crank or a timed electrical mechanical device. This type of composter can produce a stabilized compost in three to four days and can be an environmentally appropriate, low management alternative to bin composting.

**Aerated Bin**

An aerated bin can be constructed using 4' × 4' pallets fastened together to form a box and lined with wire mesh. To limit the degree of turning and permit air to flow through the pile the structure can be elevated or, in the alternative, perforated pipes can be incorporated into the structure. One side of the structure should be detachable to facilitate loading, mixing and unloading. The composter should be waterproof and located in and area that is protected from the wind.

Static compost piles and windrows should be large enough to retain heat and small enough to facilitate air to its center. As a rule of thumb, the minimum dimensions of a pile should be 3 feet by 3 feet by 3 feet.

**Turning Units**

Turning units are ideally suited for batch composting and are extremely practical for building and turning active compost. Turning units allow convenient mixing for aeration and accelerated composting.

**Composting Methods**

**Hot Composting**

Hot composting is the most efficient method for producing quality compost in a relatively short time. In

addition, it favors the destruction of weed seeds, fly larvae and pathogens. While hot composting, using the windrow or bin method, requires a high degree of management, hot composting, using the in-vessel method, requires a lesser degree of management.

**Cold Composting**

This method is ideal for adding organic matter around trees, in garden plots, in eroded areas etc. The time required to decompose organic matter using this method is governed, to a large extent, by environmental conditions and could take two years or more.

**Sheet Composting**

Sheet composting is carried out by spreading organic material on the surface of the soil or untilled ground and allowing it to decompose naturally. Over time, the material will decompose and filter into the soil. This method is ideally suited for forage land, no-till applications, erosion control, roadside landscaping etc. The process does not favor the destruction of weed seeds, fly larvae, pathogens etc. and composting materials should be limited to plant residue and manure. Again, decomposition time is governed by environmental conditions and can be quite lengthy.

**Trench Composting**

Trench composting is relatively simple. Simply dig a trench 6 - 8 inches deep, fill with 3 - 4 inches of organic material and cover with soil. Wait a few weeks and plant directly above the trench. This method does not favor the destruction of weed seeds, fly larvae and pathogens and the composting process can be relatively slow.

**Loading the Bin / Windrow**

Place the raw materials in layers using a balance of high carbon (moist) and low carbon (dry) materials. Each layer should be no more than four to six inches in depth. Spray each layer with a light mist of CBCT Stock Solution (Mix CBCT Concentrate and water at a rate of 1:200). This will initiate and accelerate the composting process and eliminate odors).

**Procedure:**

Step 1. Start with a 4 to 6 inch layer of coarse material set on the bottom of the composter or on top of the soil.

Step 2. Add a 3 to 4 inch layer of low carbon material.

Step 3. Add a 4 to 6 inch layer of high carbon material.

Step 4. Add a 1 inch layer of garden soil or finished compost.

Step 5. Mix the layers of high carbon material, low carbon material, and soil or compost.

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Repeat steps 2 through 5 until the composting bin is filled (maximum 4 feet in height). Cap with dry material.

**Loading the Vessel (in-vessel composting)**

To accelerate the composting process, simply mix the high carbon and low carbon materials together before placing them in the composter. Add the mixture to the composter in small batches, spraying each batch with a light mist of water or CBCT stock solution.

**Adding material during the composting process**

Ideally, new materials should be added to the composting system during turning or mixing. Generally, the addition of moist materials accelerates the composting process while the addition of dry materials slows the process.

**About Compost**

Finished compost can be classified as a 100% organic fertilizer containing primary nutrients as well as trace minerals, humus and humic acids, in a slow release form. Compost improves soil porosity, drainage and aeration and moisture holding capacity and reduces compaction. Compost can retain up to ten times it's weight in water. In addition, compost helps buffer soils against extreme chemical imbalances; aids in unlocking soil minerals; releases nutrients over a wide time window; acts as a buffer against the absorption of chemicals and heavy metals; promotes the development of healthy root zones; suppresses diseases associated with certain fungi; and helps plants tolerate drought conditions.

**Applications**

Compost can be used in a variety of applications. High quality compost can be used in agriculture, horticulture, landscaping and home gardening. Medium quality compost can be used in applications such as erosion control and roadside landscaping. Low quality compost can be used as a landfill cover or in land reclamation projects

**Vermicompost**

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# 2017

Rotary screen harvested vermicompost, composed of worm castings

**Vermicompost** is the product or process of composting using various worms, usually red wigglers, white worms, and other earthworms, to create a heterogeneous mixture of decomposing vegetable or food waste, bedding materials, and **vermicast**, also called worm castings, worm humus or worm manure, is the end-product of the breakdown of organic matter by an earthworm.[1] These castings have been shown to contain reduced levels of contaminants and a higher saturation of nutrients than do organic materials before vermicomposting.[2] Containing water-soluble nutrients, vermicompost is an excellent, nutrient-rich organic fertilizer and soil conditioner.[3] This process of producing vermicompost is called *vermicomposting*.

While vermicomposting is generally known as a nutrient rich source of organic compost used in farming and small scale sustainable, organic farming, the process of vermicasting is undergoing research as a treatment for organic waste in sewage and wastewater plants around the world.

**Suitable species**

One of the earthworm species most often used for composting is the Red Wiggler (*Eisenia fetida* or *Eisenia andrei*); *Lumbricus rubellus* (a.k.a. red earthworm or dilong (China)) is another breed of worm that can be used, but it does not adapt as well to the shallow compost bin as does *Eisenia fetida*. European nightcrawlers (*Eisenia hortensis*) may also be used. Users refer to European nightcrawlers by a variety of other names, including dendrobaenas, dendras, and Belgian nightcrawlers. African Nightcrawlers (*Eudrilus eugeniae*) are another set of popular composters. *Lumbricus terrestris* (a.k.a. Canadian nightcrawlers (US) or common earthworm (UK)) are not recommended, as they burrow deeper than most compost bins can accommodate.[8]

Blueworms (*Perionyx excavatus*) may be used in the tropics.[9]

These species commonly are found in organic-rich soils throughout Europe and North America and live in rotting vegetation, compost, and manure piles. They may be aninvasive species in some areas.[1][10] As they

are shallow-dwelling and feed on decomposing plant matter in the soil, they adapt easily to living on food or plant waste in the confines of a worm bin.

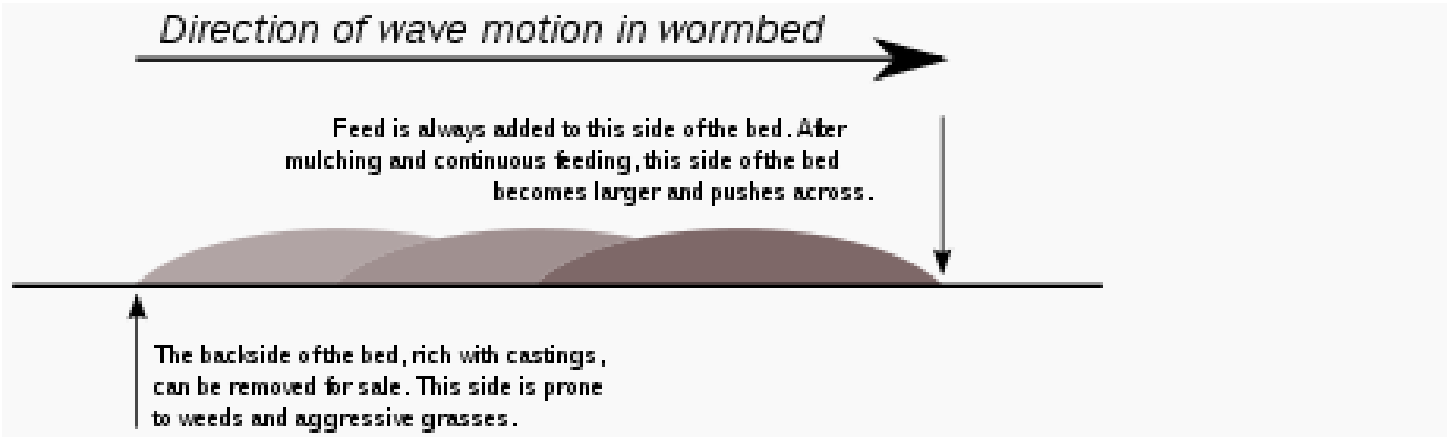
Composting worms are available to order online, from nursery mail-order suppliers or angling shops where they are sold as bait. They can also be collected from compost and manure piles. These species are not the same worms that are found in ordinary soil or on pavement when the soil is flooded by water.

**Large scale**

Large-scale vermicomposting is practiced in Canada, Italy, Japan, Malaysia, the Philippines, and the United States.[11][12] The vermicompost may be used for farming, landscaping, to create compost tea, or for sale. Some of these operations produce worms for bait and/or home vermicomposting.

There are two main methods of large-scale vermiculture. Some systems use a windrow, which consists of bedding materials for the earthworms to live in and acts as a large bin; organic material is added to it. Although the windrow has no physical barriers to prevent worms from escaping, in theory they should not due to an abundance of organic matter for them to feed on. Often windrows are used on a concrete surface to prevent predators from gaining access to the worm population.

The windrow method and compost windrow turners were developed by Fletcher Sims Jr. of the Compost Corporation in Canyon, Texas. The Windrow Composting system is noted as a sustainable, cost-efficient way for farmers to manage dairy waste.[13]




Movement of castings through a worm bed.

The second type of large-scale vermicomposting system is the raised bed or flow-through system. Here the worms are fed an inch of "worm chow" across the top of the bed, and an inch of castings are harvested from below by pulling a breaker bar across the large mesh screen which forms the base of the bed.

Because red worms are surface dwellers constantly moving towards the new food source, the flow-through system eliminates the need to separate worms from the castings before packaging. Flow-through systems are well suited to indoor facilities, making them the preferred choice for operations in colder climates.

Small scale

Demonstration home scale worm bin at a community garden site - painted plywood

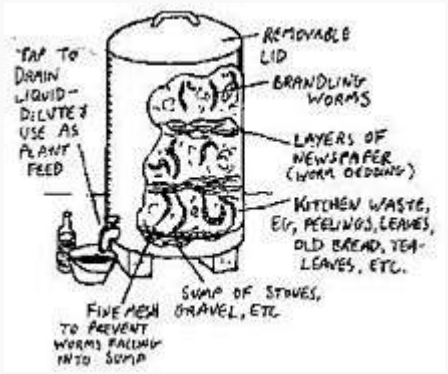



Diagram of a household-scale worm composting bin

For vermicomposting at home, a large variety of bins are commercially available, or a variety of adapted containers may be used. They may be made of old plastic containers, wood, Styrofoam, or metal containers. The design of a small bin usually depends on where an individual wishes to store the bin and how they wish to feed the worms.

Some materials are less desirable than others in worm bin construction. Metal containers often conduct heat too readily, are prone to rusting, and may release heavy metals into the vermicompost. Styrofoam containers may release chemicals into the organic material. Some cedars, Yellow cedar, and Redwood contain resinous oils that may harm worms, although Western Red Cedar has excellent longevity in composting conditions. Hemlock is another inexpensive and fairly rot-resistant wood species that may be used to build worm bins.

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Bins need holes or mesh for aeration. Some people add a spout or holes in the bottom for excess liquid to drain into a tray for collection. The most common materials used are plastic: recycled polyethylene and polypropylene and wood. Worm compost bins made from plastic are ideal, but require more drainage than wooden ones because they are non-absorbent. However, wooden bins will eventually decay and need to be replaced.

Small-scale vermicomposting is well-suited to turn kitchen waste into high-quality soil amendments, where space is limited. Worms can decompose organic matter without the additional human physical effort (turning the bin) that bin composting requires.

Composting worms which are detritivorous (eaters of trash), such as the red wiggler *Eisenia fetidae*, are epigeic (surface dwellers) together with symbiotic associated microbes are the ideal vectors for decomposing food waste. Common earthworms such as *Lumbricus terrestris* are anecic(deep burrowing) species and hence unsuitable for use in a closed system. Other soil species that contribute include insects, other worms and molds.[20]

## Climate and temperature

There may be differences in vermicomposting methods depending on the climate.[21] It is necessary to monitor the temperatures of large-scale bin systems (which can have high heat-retentive properties), as the feedstocks used can compost, heating up the worm bins as they decay and killing the worms.

The most common worms used in composting systems, redworms (*Eisenia foetida*, *Eisenia andrei*, and *Lumbricus rubellus*) feed most rapidly at temperatures of 15–25 °C (59-77 °F). They can survive at 10 °C (50 °F). Temperatures above 30 °C (86 °F) may harm them.[22] This temperature range means that indoor vermicomposting with redworms is possible in all but tropical climates.[23] Other worms like Perionyx excavatus are suitable for warmer climates.[24] If a worm bin is kept outside, it should be placed in a sheltered position away from direct sunlight and insulated against frost in winter.

## Feedstock

There are few food wastes that vermicomposting cannot compost, although meat waste and dairy products are likely to putrefy, and in outdoor bins can attract vermin. Green waste should be added in moderation to avoid heating the bin.

### Small-scale or home systems

Such systems usually use kitchen and garden waste, using "earthworms and other microorganisms to digest organic wastes, such as kitchen scraps". This includes:

- All fruits and vegetables (including citrus and other "high acid" foods)
- Vegetable and fruit peels and ends
- Coffee grounds and filters
- Tea bags (even those with high tannin levels)
- Grains such as bread, cracker and cereal (including moldy and stale)
- Eggshells (rinsed off)
- Leaves and grass clippings (not sprayed with pesticides)

### Large-scale or commercial

Such vermicomposting systems need reliable sources of large quantities of food. Systems presently operating[27] use:

- Dairy cow or pig manure
- Sewage sludge
- Brewery waste
- Cotton mill waste
- Agricultural waste
- Food processing and grocery waste
- Cafeteria waste
- Grass clippings and wood chips

Harvesting

Worms in a bin being harvested

**Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE**

Vermicompost is ready for harvest when it contains few-to-no scraps of uneaten food or bedding[*citation needed*]. There are several methods of harvesting from small-scale systems: "dump and hand sort", "let the worms do the sorting", "alternate containers" and "divide and dump."[30] These differ on the amount of time and labor involved and whether the vermicomposter wants to save as many worms as possible from being trapped in the harvested compost.

The pyramid method of harvesting worm compost is considered the simplest method for single layer bins. It is commonly used in small scale vermiculture. While harvesting, it's also a good idea to try to pick out as many eggs/cocoons as possible and return them to the bin. Eggs are small, lemon-shaped yellowish objects that can usually be seen pretty easily with the naked eye and picked out.

Properties

Vermicompost has been shown to be richer in many nutrients than compost produced by other composting methods.[34] It has also outperformed a commercial plant medium with nutrients added, but levels of magnesium required adjustment, as did pH. However, in one study it has been found that homemade backyard vermicompost was lower in microbial biomass, soil microbial activity, and yield of a species of ryegrass than municipal compost,

It is rich in microbial life which converts nutrients already present in the soil into plant-available forms. Unlike other compost, worm castings also contain worm mucus which helps prevent nutrients from washing away with the first watering and holds moisture better than plain soil.

Increases in the total nitrogen content in vermicompost, an increase in available nitrogen and phosphorus, as well as the increased removal of heavy metals from sludge and soil have been reported. The reduction in the bioavailability of heavy metals has been observed in a number of studies.

Benefits

**Soil**

- Improves soil aeration
- Enriches soil with micro-organisms (adding enzymes such as phosphatase and cellulase)
- Microbial activity in worm castings is 10 to 20 times higher than in the soil and organic matter that the worm ingests [41]
- Attracts deep-burrowing earthworms already present in the soil
- Improves water holding capacity

**Plant growth**

- Enhances germination, plant growth, and crop yield
- Improves root growth and structure
- Enriches soil with micro-organisms (adding plant hormones such as auxins and gibberellic acid)[

**Economic**

- Biowastes conversion reduces waste flow to landfills
- Elimination of biowastes from the waste stream reduces contamination of other recyclables collected in a single bin (a common problem in communities practicing single-stream recycling)
- Creates low-skill jobs at local level
- Low capital investment and relatively simple technologies make vermicomposting practical for less-developed agricultural regions

**Environmental**

- Helps to close the "metabolic gap" through recycling waste on-site
- Large systems often use temperature control and mechanized harvesting, however other equipment is relatively simple and does not wear out quickly[*citation needed*]
- Production reduces greenhouse gas emissions such as methane and nitric oxide (produced in landfills or incinerators when not composted or through methane harvest)[43]

As fertilizer

Mid-scale worm bin (1 m X 2.5 m up to 1 m deep), freshly refilled with bedding

Vermicompost can be mixed directly into the soil, or steeped in water and made into a worm tea by mixing some vermicompost in water, bubbling in oxygen with a small air pump, and steeping for a number of hours or days.

The microbial activity of the compost is greater if it is aerated during this period. The resulting liquid is used as a fertilizer or sprayed on the plants. The dark brown waste liquid, or leachate, that drains into the bottom of some vermicomposting systems as water-rich foods break down, is best applied back to the bin when added moisture is needed due to the possibility of phytotoxin content and organic acids that may be toxic to

plants. The pH, nutrient, and microbial content of these fertilizers varies upon the inputs fed to worms. Pulverized limestone, or calcium carbonate can be added to the system to raise the pH.

Troubleshooting

Worms and fruit fly pupas under the lid of a home worm bin.

### Smells

When closed, a well-maintained bin is odorless; when opened, it should have little smell if any smell is present, it is earthy.[44] Worms require gaseous oxygen.[45] Oxygen can be provided by airholes in the bin, occasional stirring of bin contents, and removal of some bin contents if they become too deep or too wet. If decomposition becomes anaerobic from excess wet feedstock added to the bin, or the layers of food waste have become too deep, the bin will begin to smell of ammonia.

### Moisture

If decomposition has become anaerobic, to restore healthy conditions and prevent the worms from dying, the smelly, excess waste water must be removed and the bin returned to a normal moisture level. To do this, first reduce addition of food scraps with a high moisture content and second, add fresh, dry bedding such as shredded newspaper to your bin, mixing it in well.

### Pest species

Pests such as rodents and flies are attracted by certain materials and odors, usually from large amounts of kitchen waste, particularly meat. Eliminating the use of meat or dairy product in a worm bin decreases the possibility of pests.[46]

In warm weather, fruit and vinegar flies breed in the bins if fruit and vegetable waste is not thoroughly covered with bedding. This problem can be avoided by thoroughly covering the waste by at least 5 centimetres (2.0 in) of bedding. Maintaining the correct pH (close to neutral) and water content of the bin (just enough water where squeezed bedding drips a couple of drops) can help avoid these pests as well.

### Worms escaping

Worms generally stay in the bin, but may try to leave the bin when first introduced, or often after a rainstorm when outside humidity is high.[47] Maintaining adequate conditions in the worm bin and putting a light over the bin when first introducing worms should eliminate this problem.[48]

### Nutrient levels

Commercial vermicomposters test, and may amend their products to produce consistent quality and results. Because the small-scale and home systems use a varied mix of feedstocks, the nitrogen, potassium and phosphorus content of the resulting vermicompost will also be inconsistent. NPK testing may be helpful before the vermicompost or tea is applied to the garden.

In order to avoid over-fertilization issues, such as nitrogen burn, vermicompost can be diluted as a tea 50:50 with water, or as a solid can be mixed in 50:50 with potting soil.[49]
The mucus produced creates a natural time-release fertilizer which cannot burn plants.

**Single-cell protein (SCP**) refers to edible unicellular microorganisms. The biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria may be used as an ingredient or a substitute for protein-rich foods, and is suitable for human consumption or as animal feeds.

Whereas industrial agriculture is marked by a high water footprint,[1] high land use,[2] biodiversity destruction,[2] general environmental degradation[2] and contributes to climate change by emission of a third of all greenhouse gases,[3] production of SCP does not necessarily exhibit any of these serious drawbacks. As of today, SCP is commonly grown on agricultural waste products, and as such inherits the ecological footprint and water footprint of industrial agriculture. However, SCP may also be produced entirely independent of agricultural waste products through autotrophic growth.[4] Thanks to the high diversity of microbial metabolism, autotrophic SCP provides several different modes of growth, versatile options of nutrients recycling, and a substantially increased efficiency compared to crops.[4]

With the world population reaching 9 billion by 2050, there is strong evidence that agriculture will not be able to meet demand[5] and that there is serious risk of food shortage.[6][7]Autotrophic SCP represents options of fail-safe mass food-production which can produce food reliably even under harsh climate conditions.
In 1781, processes for preparing highly concentrated forms of yeast were established. Research on Single Cell Protein Technology started a century ago when Max Delbrück and his colleagues found out the high value of surplus brewer's yeast as a feeding supplement for animals.[8] During World War I and World War II, yeast-

SCP was employed on a large scale in Germany to counteract food shortages during the war. Inventions for SCP production often represented milestones for biotechnology in general: for example, in 1919, Sak in Denmark and Hayduck in Germany invented a method named, "Zulaufverfahren", (fed-batch) in which sugar solution was fed continuously to an aerated suspension of yeast instead of adding yeast to diluted sugar solution once (batch).[8] In post war period, the Food and Agriculture Organization of the United Nations (FAO) emphasized on hunger and malnutrition problems of the world in 1960 and introduced the concept of protein gap, showing that 25% of the world population had a deficiency of protein intake in their diet.[8] It was also feared that agricultural production would fail to meet the increasing demands of food by humanity. By the mid 60's, almost quarter of a million tons of food yeast were being produced in different parts of the world and Soviet Union alone produced some 900,000 tons by 1970 of food and fodder yeast.[8]

In the 1960s, researchers at British Petroleum developed what they called "proteins-from-oil process": a technology for producing single-cell protein by yeast fed by waxy n-paraffins, a byproduct of oil refineries. Initial research work was done by Alfred Champagnat at BP's Lavera Oil Refinery in France; a small pilot plant there started operations in March in 1963, and the same construction of the second pilot plant, at Grangemouth Oil Refinery in Britain, was authorized.[9]

**The term SCP was coined in 1966 by Carroll L. Wilson of MIT**.

The "food from oil" idea became quite popular by the 1970s, with Champagnat being awarded the UNESCO Science Prize in 1976,[11] and paraffin-fed yeast facilities being built in a number of countries. The primary use of the product was as poultry and cattle feed.[12]

The Soviets were particularly enthusiastic, opening large "BVK" (*belkovo-vitaminny kontsentrat*, i.e., "protein-vitamin concentrate") plants next to their oil refineries in Kstovo(1973)[13][14][15] and Kirishi (1974).[16] The Soviet Ministry of Microbiological Industry had eight plants of this kind by 1989. However, due to concerns of toxicity of alkanes in SCP and pressured by the environmentalist movements, the government decided to close them down, or convert to some other microbiological processes.[16]

Production Process

Single-cell proteins develop when microbes ferment waste materials (including wood, straw, cannery, and food-processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta).[17] The problem with extracting single-cell proteins from the wastes is the dilution and cost. They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation, and filtration, or the use of semi-permeable membranes.

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The single-cell protein must be dehydrated to approximately 10% moisture content and/or acidified to aid in storage and prevent spoilage. The methods to increase the concentrations to adequate levels and the de-watering process require equipment that is expensive and not always suitable for small-scale operations. It is economically prudent to feed the product locally and soon after it is produced.

## Advantages

Large-scale production of microbial biomass has many advantages over the traditional methods for producing proteins for food or feed.

1. Microorganisms have a much higher growth rate (algae: 2–6 hours, yeast: 1–3 hours, bacteria: 0.5–2 hours). This also allows to select for strains with high yield and good nutritional composition quickly and easily compared to breeding.
2. Whereas large parts of the crop, such as stems, leaves and roots are not edible, single-cell microorganisms can be used entirely. Whereas parts of the edible fraction of crops contains is undigestible, many microorganisms are digestible at a much higher fraction.[4]
3. Microorganisms usually have a much higher protein content of 30–70% in the dry mass than vegetables or grains.[20] The amino acid profiles of many SCP microorganisms often have excellent nutritional quality, comparable to a hen's egg.
4. Some microorganisms can build vitamins and nutrients which eukaryotic organisms such as plants cannot produce or not produce in significant amounts, including vitamin B12.
5. Microorganisms can utilize a broad spectrum of raw materials as carbon sources including alkanes, methanol, methane, ethanol and sugars. What was considered "waste product" often can be reclaimed as nutrients and support growth of edible microorganisms.
6. Like plants, autotrophic microorganisms are capable to grow on $CO_2$ . Some of them, such as bacteria with the Wood-Ljungdahl-Pathway or the reductive TCA can fix CO2 between 2-3,[21] up to 10 times more efficiently than plants[22] when also considering the effects of photoinhibition.
7. Some bacteria, such as several homoacetogenic clostridia are capable to perform syngas fermentation. This means they can metabolize synthesis gas, a gas mixture of CO, $H_2$ and $CO_2$ that can be made by gasification of residual intractable biowastes such as lignocellulose.
8. Some bacteria are diazotrophic, i.e. they can fix $N_2$ from the air and are thus independent of chemical N-fertilizer, whose production, utilization and degradation causes tremendous harm to the environment, deteriorates public health, and fosters climate change.[23]
9. Many bacteria can utilize $H_2$ for energy supply, using enzymes called hydrogenases. Whereas hydrogenases are normally highly $O_2$-sensitive, some bacteria are capable of performing $O_2$-dependent respiration of $H_2$. This feature allows autotrophic bacteria to grow on $CO_2$ without light at a fast growth

rate. Since $H_2$ can be made efficiently by water electrolysis, in a manner of speaking, those bacteria can be "powered by electricity".[4]

10. Microbial biomass production is independent of seasonal and climatic variations, and can be easily shielded from extreme weather events that are expected to cause crop failures with the ongoing climate-change. Light-independent microorganisms such as yeasts can continue to grow at night.
11. Cultivation of microorganisms generally has a much lower water footprint than agricultural food production. Whereas the global average blue-green water footprint (irrigation, surface, ground and rain water) of crops reaches about 1800 liters per kg crop[1] due to evaporation, transpiration, drainage and runoff, closed bioreactors producing SCP exhibits none of these causes.
12. Cultivation of microorganisms does not require fertile soil and therefore does not compete with agriculture. Thanks to the low water requirements, SCP cultivation can even be done in dry climates with infertile soil and may provide a means of fail-safe food supply in arid countries.
13. Photosynthetic microorganisms can reach a higher solar-energy-conversion efficiency than plants, because in photobioreactors supply of water, $CO_2$ and a balanced light distribution can be tightly controlled.
14. Unlike agricultural products which are processed towards a desired quality, it is easier with microorganisms to direct production towards a desired quality. Instead of extracting amino acids from soy beans and throwing away half of the plant body in the process, microorganisms can be genetically modified to overproduce or even secrete a particular amino acid. However, in order to keep a good consumer acceptance, it is usually easier to obtain similar results by screening for microorganisms which already have the desired trait or train them via selective adaptation.

Disadvantages

Although SCP shows very attractive features as a nutrient for humans, however there are some problems that deter its adoption on global basis:

- Fast growing microorganisms such as bacteria and yeast tend to have a high concentration of nucleic acid, notably RNA. Levels of must be limited in the diets of monogastricanimals to <50 g per day. Ingestion of purine compounds arising from RNA breakdown leads to increased plasma levels of uric acid, which can cause gout and kidney stones. Uric acid can be converted to allantoin, which is excreted in urine. Nucleic acid removal is not necessary from animal feeds but is from human foods. A temperature hold at 64 °C inactivates fungal proteases and allows . However, this problem can be remediated.[20] One common method consists in a heat treatment which kills the cells, inactivates proteases and allows endogenous RNases to hydrolyse RNA with release of nucleotides from cell to culture broth.[24]

- Similar to plant cells, the cell wall of some microorganisms such as algae and yeast contain non-digestible components, such as cellulose. The cells of some kind of SCP should be broken up in order to liberate the cell interior and allow complete digestion.[20]
- Some kind of SCP exhibits unpleasant color and flavors.
- Depending on the kind of SCP and the cultivation conditions, care must be taken to prevent and control contamination by other microorganisms because contaminants may produce toxins such as mycotoxins or cyanotoxins. An interesting approach to address this problem was proposed with the fungus *Scytalidium acidophilum* which grows at a pH as low as 1. This allows to hydrolyse paper wastes to a sugar medium and creates aseptic conditions at low-cost.[18]
- Some yeast and fungal proteins tend to be deficient in methionine.

Production of Single Cell Protein

The production of Single Cell Protein can be done by using waste materials as the substrate, specifically agricultural wastes such as wood shavings, sawdust, corn cobs, and many others. Examples of other waste material substrates are food processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta.

The process of SCP production from any microorganism or substrate would have the following basic steps:

1. Provision of a carbon source; it may need physical and/or chemical pretreatments.

Large scale biomass fermenter

1. Addition, to the carbon source, of sources of nitrogen, phosphorus and other nutrients needed to support optimal growth of the selected microorganism.
2. Prevention of contamination by maintaining sterile or hygienic conditions. The medium components may be heated or sterilized by filtration and fermentation equipments may be sterilized.
3. The selected microorganism is inoculated in a pure state.

4. SCP processes are highly aerobic (except those using algae). Therefore, adequate aeration must be provided. In addition, cooling is necessary as considerable heat is generated.
5. The microbial biomass is recovered from the medium.
6. Processing of the biomass for enhancing its usefulness and/or storability.

The selection of certain microbial strain is very important, some of the criteria are:

1. Performance (growth rate, productivity, yield) on the specific. preferably low-cost substrates to be used
2. Temperature and pH tolerance
3. Oxygen requirements, heat generation during fermentation and foaming characteristics
4. Growth morphology and genetic stability in the fermentation
5. Ease of recovery, and requirements for further downstream processing
6. Structure and composition of the final product, in terms of protein

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| Unit IV<br>The protoplast can be used to | modify genetic information | create plant hybrid | study plant viral infections | no alteration | modify genetic information |
| The cell wall of | gram-positive bacteria are thicker than gram-negative bacteria | gram-negative bacteria are thicker than gram-positive bacteria | both have same thickness but composition is different | no change | gram-positive bacteria are thicker than gram-negative bacteria |
| Peptidoglycan is also known | N-acetyl muramic acid | murein mucopeptide | N acetylglucosamine | mesodiaminopimetic acid | murein mucopeptide |
| Which is most likely to be exposed on the surface of a gram-negative bacterium? | Pore protein (porin) | Protein involved in energy generation | Lipoteichoic acid | Phospholipids | Pore protein (porin) |
| The last step in synthesis of peptidoglycan is | attachment of a peptide to muramic acid | attaching two amino acids to form a cross-link | attachment of a portion of peptidoglycan to a membrane lipid | binding of penicillin to a membrane protein | attaching two amino acids to form a cross-link |

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| Cytoplasmic inclusions include | ribosomes | Flagella | pili | Cell wall | | ribosomes |
| The cocci which forms a bunch and irregular pattern are | Staphylococci | Diplococcic | Tetracocci | Streptococci | | Staphylococci |
| Chemotaxis is a phenomenon of | swimming away of bacteria | swimming towards bacteria | swimming away or towards of bacteria in presence of chemical compound | no swim | | swimming away or towards of bacteria in presence of chemical compound |
| The | pilli | Flagella | sheath | capsules | | pilli |
| The next to last step in peptidoglycan biosynthesis is | synthesis of the NAM-peptide subunit | removal of the subunit from bactoprenol | linking the sugar of the disaccharide-peptide unit to the growing peptidoglycan chain | cross-linking the peptide side chains of peptidoglycan | | cross-linking the peptide side chains of peptidoglycan |
| The cocci which forms a four is | Streptococci | Diplococcic | Staphylococci | Tetracocci | | Tetracocci |

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| The arrangement, in which flagella are distributed all round the bacterial cell, is known as | lophotrichous | Amphitrichous | peritrichous | monotrichous | | Peritrichous |
| Periplasm is | the area between the inner and outer membranes of gram-negative bacteria | the area between the inner and outer membranes of Gram-positive bacteria | the interior portion of mitochondria | the area outside the cell membrane that is influenced by the polymers | | the area between the inner and outer membranes of gram-negative bacteria |
| Which of the following has peptidoglycan as a major constituent of cell wall? | Gram-negative bacteria | Gram-positive bacteria | Fungi | virus | | Gram-positive bacteria |
| The common word for bacteria which are helically curved rods is | cooci | Pleomorphic | bacillus | spirilla | | Spirilla |

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| The bacteria deficient in cell wall is | Treponema | Mycoplasma | Staphylococcus | Klebsiella | | Mycoplasma |
| Which of the following is not true about peptidoglycan? | It is a polymer consisting of N-acetyl glucosamine, N-acetyl muramic acid and amino acids (alanine, lysine, etc.) | It is present in prokaryotic cell wall | It occurs in the form of a bag shaped macro molecule surrounding the cytoplasm membrane | None of the above | | It occurs in the form of a bag shaped macro molecule surrounding the cytoplasm membrane |
| The | cocci | bacilli | spirilla | pleomorp | | Pleomorp |
| Single or clusters of flagella at both poles is known as | monotrichous | peritrichous | amphitrichous | atrichous | | Amphitrichous |
| Which of the following bacterial genera (that produces endospore) have medical importance? | Shigella | Bacillus | vibrio | Coliform | | Bacillus |

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| Pharmacokinetics is: | The study of biological and therapeutic effects of drugs | The study of absorption, distribution, metabolism and excretion of drugs | The study of mechanisms of drug action | The study of methods of new drug development | | The study of absorption, distribution, metabolism and excretion of drugs |
| The main mechanism of drugs absorption in GI tract : | Active transport (carrier-mediated diffusion) | Filtration (aqueous diffusion) | Endocytosis and exocytosis | Passive diffusion (lipid diffusion) | | Passive diffusion (lipid diffusion) |
| What does the term “bioavailability” mean? | Plasma protein binding degree of substance | Permeability through the brain-blood barrier | Fraction of an uncharged drug reaching the systemic circulation following any route administration | Amount of a substance in urine relative to the initial dose | | Fraction of an uncharged drug reaching the systemic circulation following any route administration |
| Which route of drug administration is most likely to lead to the first-pass effect? | Sublingual | Oral | Intravenous | Intramuscular | | Oral |

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| The volume of distribution (Vd) relates: | Single to a daily dose of an administrated drug | An administrated dose to a body weight | An uncharged drug reaching the systemic circulation | The amount of a drug in the body to the concentration of a drug in plasma | The amount of a drug in the body to the concentration of a drug in plasma |
| Metabolic transformation (phase 1) is: | Acetylation and methylation of substances | Transformation of substances due to oxidation, reduction or hydrolysis | Glucuronide formation | Binding to plasma proteins | Transformation of substances due to oxidation, reduction or hydrolysis |
| Which organ involved in first pass effect? | Heart | Kidney | Brain | Liver | Liver |
| Which | Intraveno | Oral | Topical | Dissolutio | Dissolutio |
| Which of the following processes proceeds in the second phase of biotransformation? | Acetylation | Reduction | Oxidation | Hydrolysis | Acetylation |
| Which | Catalase | Polypheno | Cytochoro | Oxygenas | Cytochoro |

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| Cytochorome p450 MO is found mainly in | Heart | Liver | Brain | Kidney | | Liver |
| Dichloroisopropylarterenol blocks | Alpha adrenergic receptors | Beta adrenergic receptors | Both alpha and beta receptors | Gamma adrenergic receptors | | Beta adrenergic receptors |
| Half life (t ½) is the time required to: | Change the amount of a drug in plasma by half during elimination | Metabolize a half of an introduced drug into the active metabolite | Absorb a half of an introduced drug | Bind a half of an introduced drug to plasma proteins | | Change the amount of a drug in plasma by half during elimination |
| Irreversible interaction of an antagonist with a receptor is due to: | Ionic bonds | Hydrogen bonds | Covalent bonds | Sulphur bond | | Covalent bonds |
| The second messenger of G-protein-coupled (metabotropic) receptor: | Adenylyl cyclase | Sodium ions | Phospholipase C | cAMP | | cAMP |

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| Give the definition for a therapeutical dose: | The amount of a substance to produce the minimal biological effect | The amount of a substance to produce effects hazardous for an organism | The amount of a substance to produce the required effect in most patients | The amount of a substance to accelerate an increase of concentration of medicine in an organism | | The amount of a substance to produce the required effect in most patients |
| The substance which changes the activity of an effector element but doesn't belong to second messengers: | cAMP | cGMP | G–protein | Calcium ions | | G–protein |
| An agonist can produce submaximal effects and has moderate efficacy it's called: | Partial agonist | Antagonist | Agonist-antagonist | Full agonist | | Partial agonist |

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| Conjugation is: | Process of drug reduction by special enzymes | Process of drug oxidation by special oxidases | Coupling of a drug with an endogenous substrate | Solubilization in lipids | | Coupling of a drug with an endogenous substrate |
| What is implied by “active transport”? | Transport of drugs trough a membrane by means of diffusion | Transport without energy consumption | Engulf of drug by a cell membrane with a new vesicle formation | Transport against concentration gradient | | Transport against concentration gradient |
| What kind of substances can’t permeate membranes by passive diffusion? | Lipid-soluble | Non-ionized substances | Hydrophobic substances | Hydrophilic substances | | Hydrophilic substances |
| The reasons determining bioavailability are: | Rheological parameters of blood | Amount of a substance obtained orally and quantity of intakes | Extent of absorption and hepatic first-pass effect | Glomerular filtration rate | | Extent of absorption and hepatic first-pass effect |

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| For the calculation of the volume of distribution (Vd) one must take into account: | Concentration of a substance in plasma | Concentration of substance in urine | Therapeutical width of drug action | A daily dose of drug | | Concentration of a substance in plasma |
| Biotransformation of a medicinal substance results in: | Faster urinary excretion | Slower urinary excretion | Easier distribution in organism | Higher binding to membranes | | Faster urinary excretion |
| The organelle that carry Cytochorome p450 MO is | Endoplasmic reticulam | Golgi complex | Mitochondria | Mitochondria | | Endoplasmic reticulam |
| Conjugation of a drug includes the following EXCEPT: | Glucoronidation | Sulfate formation | Hydrolysis | Methylation | | Hydrolysis |

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| The phase II reaction which produce a compound with greater pharmocological actvity | Glucoronic acid conjugation | Conjugation with amino acid | Methylation | Glutathione conjugation | | Methylation |
| Elimination is expressed as follows: | Rate of renal tubular reabsorption | Clearance speed of some volume of blood from substance | Time required to decrease the amount of drug in plasma by one-half | Clearance of an organism from a xenobiotic | | Clearance of an organism from a xenobiotic |
| Acidic drug rapidly absorbed at | Stomach | GI tract | Large intestine | Mouth | | Stomach |
| Coenzyme required by Cytochorome p450 MO is | NADH | NADPH | Lipoic acid | TPP | | NADPH |
| Basic drugs are absorbed in | small intestine | stomach | Large intestine | Pancreas | | small intestine |

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| Which effect may lead to toxic reactions when a drug is taken continuously or repeatedly? | Refractoriness | Cumulative effect | Tolerance | Tachyphylaxis | | Cumulative effect |
| What term is used to describe a more gradual decrease in responsiveness to a drug, taking days or weeks to develop? | Refractoriness | Cumulative effect | Tolerance | Tachyphylaxis | | Tolerance |
| What term is used to describe a decrease in responsiveness to a drug which develops in a few minutes? | Refractoriness | Cumulative effect | Tolerance | Tachyphylaxis | | Tachyphylaxis |
| Which | Water | lipid | ionsoluble | Non | | lipid |

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| Science that deals with drug | Pharmacy | pharmacognosy | pharmacodynamics | pharmacology | Pharmacology |
| Inhibition of MAO causses an | decrease in the deamination of noradrenalin | increasse in the deamination of dopamine | increase in the deamination of noradrenalin | decrease in the deamination of dopamine | decrease in the deamination of noradrenalin |
| Systemic clearance (CLs) is related with: | Only the concentration of substances in plasma | Only the elimination rate constant | Volume of distribution, half life and elimination rate constant | Bioavailability and half life | Volume of distribution, half life and elimination rate constant |
| Elimination rate constant (Kelim) is defined by the following parameter: | Rate of absorption | Maximal concentration of a substance in plasma | Highest single dose | Half life (t ½) | Half life (t ½) |
| The time required to kill 90% of the microorganisms in a sample at a specific temperature is the | decimal reduction time | thermal death point | F value | D value | decimal reduction time |

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| Which of the following is best used for long term storage of microbial samples when carried out properly? | Storage in a freezer at -10°C | Storage in a freezer at ultra low temperatures (-70°C) | Storage in a refrigerator on an agar slant | Storage on a petri plate at room temperature | | Storage in a freezer at ultra low temperatures (-70°C) |
| Which of the antibiotic is not used as a food preservative ? | Pimaricin | Nisin | Tylosin | β-lactam antibiotic | | Nisin |
| Which antibiotic has a beta-lactam ring? | Cephalosporin | Penicillin | Tetracycline | Streptomycin | | Penicillin |
| In eukaryotic cells, ribosomes are | 70S | 60S | 80S | Not specific | | 80S |

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| Porins are located in | the outer membrane of gram-negative bacteria | the peptidoglycan layer of gram-positive bacteria | the cytoplasmic membrane of both gram-negative and gram-positive bacteria | the periplasmic space of gram-negative bacteria | | the outer membrane of gram-negative bacteria |
| For most bacteria, the optimum pH for growth lies between | 6.5-7.5 | 3.5-4.5 | 4.5-5.5 | 5.5-6.5 | | 6.5-7.5 |
| Suspension cultures consist of cells and cell aggregates, growing dispersed in | liquid medium | solid nutrient medium | solid or liquid medium | semisolid media | | liquid medium |

# 2017

## I M.Sc Microbiology – Marine microbiology

| LECTURE PLAN - UNIT -V | | | |
|---|---|---|---|
| **S. no** | Lecture duration(Hr) | **Topics covered** | **Supporting materials** |
| 1 | 1 | Bar coding | W1 |
| 2 | 1 | Genome sequencing | W1 |
| 3 | 1 | Physical mapping | W1 |
| 4 | 1 | Waste composting | T1 643 |
| 5 | 1 | cryopresevation | T1 646 |
| 6 | 1 | SCP production | T1 569 |
| 7 | 1 | bioactive compounds | T1 618 |
| 8 | 1 | Revision of Unit V | |
| 9 | 1 | Unit V test | |
| 10 | 1 | Old Question papers discussion | |
| **Textbooks :** | | T1-Microbiology- pelczar – McGraw Hill publishing<br>T2-Microbiology- presscott – McGraw hill publishing<br>T3-Microbial genetics – David friefelder- | |
| **Reference books:** | | | |
| **Website:** | | W1- www.microbiology.com<br>W2 – www.marinestudy.com | |
| **Journals:** | | | |

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# DNA barcoding

**DNA barcoding** is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. It differs from molecular phylogeny in that the main goal is not to determine patterns of relationship but to identify an unknown sample in terms of a preexisting classification. Although barcodes are sometimes used in an effort to identify unknown species or assess whether species should be combined or separated, the utility of DNA barcoding for these purposes is subject to debate. The most commonly used barcode region, for animals, at least, is a segment of approximately 600 base pairs of the mitochondrial gene cytochrome oxidase I (COI).

Applications include, for example, identifying plant leaves even when flowers or fruit are not available, identifying insect larvae (which may have fewer diagnostic characters than adults and are frequently less well-known), identifying the diet of an animal, based on its stomach contents or faeces and identifying products in commerce (for example, herbal supplements, wood, or skins and other animal parts).

## Choice of locus

A desirable locus for DNA barcoding should be standardized (so that large databases of sequences for that locus can be developed),[6] present in most of the taxa of interest and sequenceable without species-specific PCR primers, short enough to be easily sequenced with current technology and provide a large variation between species yet a relatively small amount of variation within a species.[8]

Although several loci have been suggested, a common set of standardized regions were selected by the respective committees:

- For animals and many other eukaryotes, the mitochondrial COI gene
- For plants, the concatenation of the rbcL and matK chloroplast genes. These provide poor resolution for land plants, and a call was made for regions to be assessed that could complement rbcL and matK.
- For fungi, the internal transcribed spacer (ITS) region

### Mitochondrial DNA

DNA barcoding is based on a relatively simple concept. All eukaryote cells contain mitochondria, and animal mitochondrial DNA (mtDNA) has a relatively fast mutation rate, resulting in the generation of diversity within and between populations over relatively short evolutionary timescales (thousands of generations). Typically, in animals, a single mtDNA genome is transmitted to offspring by each breeding female, and the genetic effective population size is proportional to the number of breeding females. This contrasts with the nuclear genome, which is around 100 000 times larger, where males and females each contribute two full

genomes to the gene pool and effective size is therefore proportional to twice the total population size. This reduction in effective population size leads to more rapid sorting of mtDNA gene lineages within and among populations through time, due to variance in fecundity among individuals (the principle of coalescence). The combined effect of higher mutation rates and more rapid sorting of variation usually results in divergence of mtDNA sequences among species and a comparatively small variance within species. A 658-bp region (the **Folmer region**) of the mitochondrialcytochrome c oxidase subunit I (COI) gene was proposed as a potential 'barcode.

Exceptions, where mtDNA fails as a test of species identity, can occur through occasional recombination (direct evidence for recombination in mtDNA is available in some bivalvessuch as *Mytilus*[13] but it is suspected that it may be more widespread[14]) and through occurrences of hybridization.[15] Male-killing microorganisms,[16] cytoplasmic incompatibility-inducing symbionts (e.g., *Wolbachia*[16]), as well as heteroplasmy, where an individual carries two or more mtDNA sequences, may affect patterns of mtDNA diversity within species, although these do not necessarily result in bar-coding failure. Occasional horizontal gene transfer (such as via cellular symbionts[17]), or other "reticulate" evolutionary phenomena in a lineage can lead to misleading results (i.e., it is possible for two different species to share mtDNA). In particular, mtDNA seems to be particularly prone to interspecific introgression [18] probably due to difference between sexes in mate-choice and dispersal. Additionally, some species may carry divergent mtDNA lineages segregating within populations, often due to historical geographic structure, where these divergent lineages do not reflect species boundaries. As of February 2013, the Barcode of Life Data Systems database included almost 2,000,000 barcode sequences from over 160,000 species of animals, plants, and fungi.

## Identifying flowering plants

The use of the COI sequence "is not appropriate for most species of plants because of a much slower rate of cytochrome c oxidase I gene evolution in higher plants than in animals". A series of experiments was then conducted to find a more suitable region of the genome for use in the DNA barcoding of flowering plants (or the larger group of land plants). One 2005 proposal was the nuclear internal transcribed spacer region and the plastid trnH-psbA intergenic spacer; other researchers advocated other regions such as matK

In 2009, a collaboration of a large group of plant DNA barcode researchers proposed two chloroplast genes, rbcL and matK, taken together, as a barcode for plants. Adding the nuclear internal transcribed spacer ITS2 region was proposed to provide better resolution between species. As of 2015, the search for better DNA barcodes for plants continues, with the proposal that the chloroplast region *ycf1* may be suitable.

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## Artificial DNA

The use of artificial DNA sequences introduced into foodstuff offers an alternative application of DNA barcoding. While the read-out technologies stay the same, this approach enables the barcoding of non-natural properties, such as foodstuff manufacturer.

**Vouchered specimens**

DNA sequence databases like GenBank contain many sequences that are not tied to vouchered specimens (for example, herbarium specimens, cultured cell lines, or sometimes images). This is problematic in the face of taxonomic issues such as whether several species should be split or combined, or whether past identifications were sound. Therefore, best practice for DNA barcoding is to sequence vouchered specimens.

Origin

The use of nucleotide sequence variations to investigate evolutionary relationships is not a new concept. Carl Woese used sequence differences in ribosomal RNA (rRNA) to discover archaea, which in turn led to the redrawing of the evolutionary tree, and molecular markers (e.g., allozymes, rDNA, and mtDNA sequences) have been successfully used in molecular systematics for decades. DNA barcoding provides a standardised method for this process via the use of a short DNA sequence from a particular region of the genome to provide a 'barcode' for identifying species. In 2003, Paul D.N. Hebert from the University of Guelph, Ontario, Canada, proposed the compilation of a public library of DNA barcodes that would be linked to named specimens. This library would "provide a new master key for identifying species, one whose power will rise with increased taxon coverage and with faster, cheaper sequencing".

Case studies

## Identification of birds

In an effort to find a relationship between traditional species boundaries established by taxonomy and those inferred by DNA barcoding, Hebert and co-workers sequenced DNA barcodes of 260 of the 667 bird species that breed in North America (Hebert *et al.* 2004a[26]). They found that every single one of the 260 species had a different COI sequence. 130 species were represented by two or more specimens; in all of these species, COI sequences were either identical or were most similar to sequences of the same species. COI variations between species averaged 7.93%, whereas variation within species averaged 0.43%. In four cases there were deep intraspecific divergences, indicating possible new species. Three out of these four polytypic species are already split into two by some taxonomists. Hebert *et al.*'s (2004a[26]) results reinforce these views and strengthen the case for DNA barcoding. Hebert *et al.* also proposed a standard sequence threshold to define new species, this threshold, the so-called "barcoding gap", was defined as 10 times the mean intraspecific variation for the group under study.

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## Identification of fish

The Fish Barcode of Life Initiative (FISH-BOL),[27] is a global effort to coordinate an assembly of a standardised DNA barcode library for all fish species, one that is derived from voucher specimens with authoritative taxonomic identifications.[28] The benefits of barcoding fishes include facilitating species identification for all potential users, including taxonomists; highlighting specimens that represent a range expansion of known species; flagging previously unrecognized species; and perhaps most importantly, enabling identifications where traditional methods are not applicable. An example is the possible identification of groupers causing Ciguatera fish poisoning from meal remnants.[29]

Since its inception in 2005 FISH-BOL has been creating a valuable public resource in the form of an electronic database containing DNA barcodes for almost 10000 species, images, and geospatial coordinates of examined specimens.[30] The database contains linkages to voucher specimens, information on species distributions, nomenclature, authoritative taxonomic information, collateral natural history information and literature citations. FISH-BOL thus complements and enhances existing information resources, including the Catalog of Fishes, FishBase and various genomics databases .

## Delimiting cryptic species

The next major study into the efficacy of DNA barcoding was focused on the neotropical skipper butterfly, *Astraptes fulgerator* at the Area de Conservación de Guanacaste (ACG) in north-western Costa Rica. This species was already known as a cryptic species complex, due to subtle morphological differences, as well as an unusually large variety ofcaterpillar food plants. However, several years would have been required for taxonomists to completely delimit species. sequenced the COI gene of 484 specimens from the ACG. This sample included "at least 20 individuals reared from each species of food plant, extremes and intermediates of adult and caterpillar color variation, and representatives" from the three major ecosystems where *Astraptes fulgerator* is found. Researchers concluded that *Astraptes fulgerator* consists of 10 different species in north-western Costa Rica. These results, however, were subsequently challenged by Brower who pointed out numerous serious flaws in the analysis, and concluded that the original data could support no more than the possibility of three to seven cryptic taxa rather than ten cryptic species. This highlights that the results of DNA barcoding analyses can be dependent upon the choice of analytical methods used by the investigators, so the process of delimiting cryptic species using DNA barcodes can be as subjective as any other form of taxonomy.

A more recent example used DNA barcoding for the identification of cryptic species included in the ongoing long-term database of tropical caterpillar life generated by Dan Janzen and Winnie Hallwachs in Costa Rica at the ACG. In 2006 researchers examined whether a COI DNA barcode could function as a tool for identification and discovery for the 20 morphospecies of *Belvosia* parasitoid flies (Tachinidae) that have been reared

from caterpillars in ACG. Barcoding not only discriminated among all 17 highly host-specific morphospecies of ACG *Belvosia*, but it also suggested that the species count could be as high as 32 by indicating that each of the three generalist species might actually be arrays of highly host-specific cryptic species.

In 2007 Smith *et al.* expanded on these results by barcoding 2,134 flies belonging to what appeared to be the 16 most generalist of the ACG tachinid morphospecies.[35] They encountered 73 mitochondrial lineages separated by an average of 4% sequence divergence and, as these lineages are supported by collateral ecological information, and, where tested, by independent nuclear markers (28S and ITS1), the authors therefore viewed these lineages as provisional species. Each of the 16 initially apparent generalist species were categorized into one of four patterns: (i) a single generalist species, (ii) a pair of morphologically cryptic generalist species, (iii) a complex of specialist species plus a generalist, or (iv) a complex of specialists with no remaining generalist. In sum, there remained 9 generalist species classified among the 73 mitochondrial lineages analyzed.

, also in 2007, Whitworth *et al.* reported that flies in the related family Calliphoridae could not be discriminated by barcoding.[19] They investigated the performance of barcoding in the fly genus *Protocalliphora*, known to be infected with the endosymbiotic bacteria *Wolbachia*. Assignment of unknown individuals to species was impossible for 60% of the species, and if the technique had been applied, as in the previous study, to identify new species, it would have underestimated the species number in the genus by 75%. They attributed the failure of barcoding to the non-monophyly of many of the species at the mitochondrial level; in one case, individuals from four different species had identical barcodes. The authors went on to state:

The pattern of *Wolbachia* infection strongly suggests that the lack of within-species monophyly results from introgressive hybridization associated with *Wolbachia*infection. Given that *Wolbachia* is known to infect between 15 and 75% of insect species, we conclude that identification at the species level based on mitochondrial sequence might not be possible for many insects.[19] Mwabvu *et al.* (2013) observed a high level of divergence (19.09% for CO1, 520 base pairs) between two morphologically indistinguishable populations of *Bicoxidens flavicollis*millipedes in Zimbabwe, and suggested the presence of cryptic species in *Bicoxidens flavicollis*.[36]

Marine biologists have also considered the value of the technique in identifying cryptic and polymorphic species and have suggested that the technique may be helpful when associations with voucher specimens are maintained,[24] though cases of "shared barcodes" (e.g., non-unique) have been documented in cichlid fishes and cowries[20]

## The Moorea Biocode Project

The Moorea Biocode Project is a barcoding initiative to create the first comprehensive inventory of all non-microbial life in a complex tropical ecosystem, the island of Moorea in Tahiti. Supported by a grant from the Gordon and Betty Moore Foundation, the Moorea Biocode Project is a 3-year project that brings together researchers from the Smithsonian Institution, UC Berkeley, France's National Center for Scientific Research (CNRS), and other partners. The outcome of the project is a library of genetic markers and physical identifiers for every species of plant, animal and fungi on the island that will be provided as a publicly available database resource for ecologists and evolutionary biologists around the world.

The software back-end to the Moore Biocode Project is Geneious Pro and two custom-developed plugins from the New Zealand-based company, Biomatters. The Biocode LIMS and Genbank Submission plugins have been made freely available to the public[38] and users of the free Geneious Basic software will be able to access and view the Biocode database upon completion of the project, while a commercial copy of Geneious Pro is required for researchers involved in data creation and analysis.

## Criticisms

DNA barcoding has met with spirited reaction from scientists, especially systematists, ranging from enthusiastic endorsement to vociferous opposition.[39][40] For example, many stress the fact that DNA barcoding does not provide reliable information above the species level[*citation needed*], while others indicate that it is inapplicable at the species level, but may still have merit for higher-level groups.[19] Others resent what they see as a gross oversimplification of the science of taxonomy. And, more practically, some suggest that recently diverged species might not be distinguishable on the basis of their COI sequences.[41] Due to various phenomena, Funk & Omland (2003[42]) found that some 23% of animal species are polyphyletic if their mtDNA data are accurate, indicating that using an mtDNA barcode to assign a species name to an animal will be ambiguous or erroneous some 23% of the time (see also Meyer & Paulay, 2005[43]). Studies with insects suggest an equal or even greater error rate, due to the frequent lack of correlation between the mitochondrial genome and the nuclear genome or the lack of a barcoding gap (e.g., Hurst and Jiggins, 2005,[17] Whitworth *et al.*, 2007,[19] Wiemers & Fiedler, 2007[44]). Problems with mtDNA arising from male-killing microorganisms and cytoplasmic incompatibility-inducing symbionts (e.g., *Wolbachia*)[16] are also particularly common among insects. Given that insects represent over 75% of all known organisms,[45] this suggests that while mtDNA barcoding may work for vertebrates, it may not be effective for the majority of known organisms.

Moritz and Cicero (2004[46]) have questioned the efficacy of DNA barcoding by suggesting that other avian data is inconsistent with Hebert *et al.*'s interpretation, namely, Johnson and Cicero's (2004[47]) finding that 74% of sister species comparisons fall below the 2.7% threshold suggested by Hebert *et al.* These criticisms are

somewhat misleading considering that, of the 39 species comparisons reported by Johnson and Cicero, only 8 actually use COI data to arrive at their conclusions. Johnson and Cicero (2004[47]) have also claimed to have detected bird species with identical DNA barcodes, however, these 'barcodes' refer to an unpublished 723-bp sequence of ND6 which has never been suggested as a likely candidate for DNA barcoding.

The DNA barcoding debate resembles the phenetics debate of decades gone by. It remains to be seen whether what is now touted as a revolution in taxonomy will eventually go the same way as phenetic approaches, of which was claimed exactly the same decades ago, but which were all but rejected when they failed to live up to overblown expectations.[48] Controversy surrounding DNA barcoding stems not so much from the method itself, but rather from extravagant claims that it will supersede or radically transform traditional taxonomy. Other critics fear a "big science" initiative like barcoding will make funding even more scarce for already underfunded disciplines like taxonomy, but barcoders respond that they compete for funding not with fields like taxonomy, but instead with other big science fields, such as medicine and genomics.[49] Barcoders also maintain that they are being dragged into long-standing debates over the definition of a species and that barcoding is less controversial when viewed primarily as a method of identification, not classification.[2][25]

The current trend appears to be that DNA barcoding needs to be used alongside traditional taxonomic tools and alternative forms of molecular systematics so that problem cases can be identified and errors detected. Non-cryptic species can generally be resolved by either traditional or molecular taxonomy without ambiguity. However, more difficult cases will only yield to a combination of approaches. And finally, as most of the global biodiversity remains unknown, molecular barcoding can only hint at the existence of new taxa, but not delimit or describe them

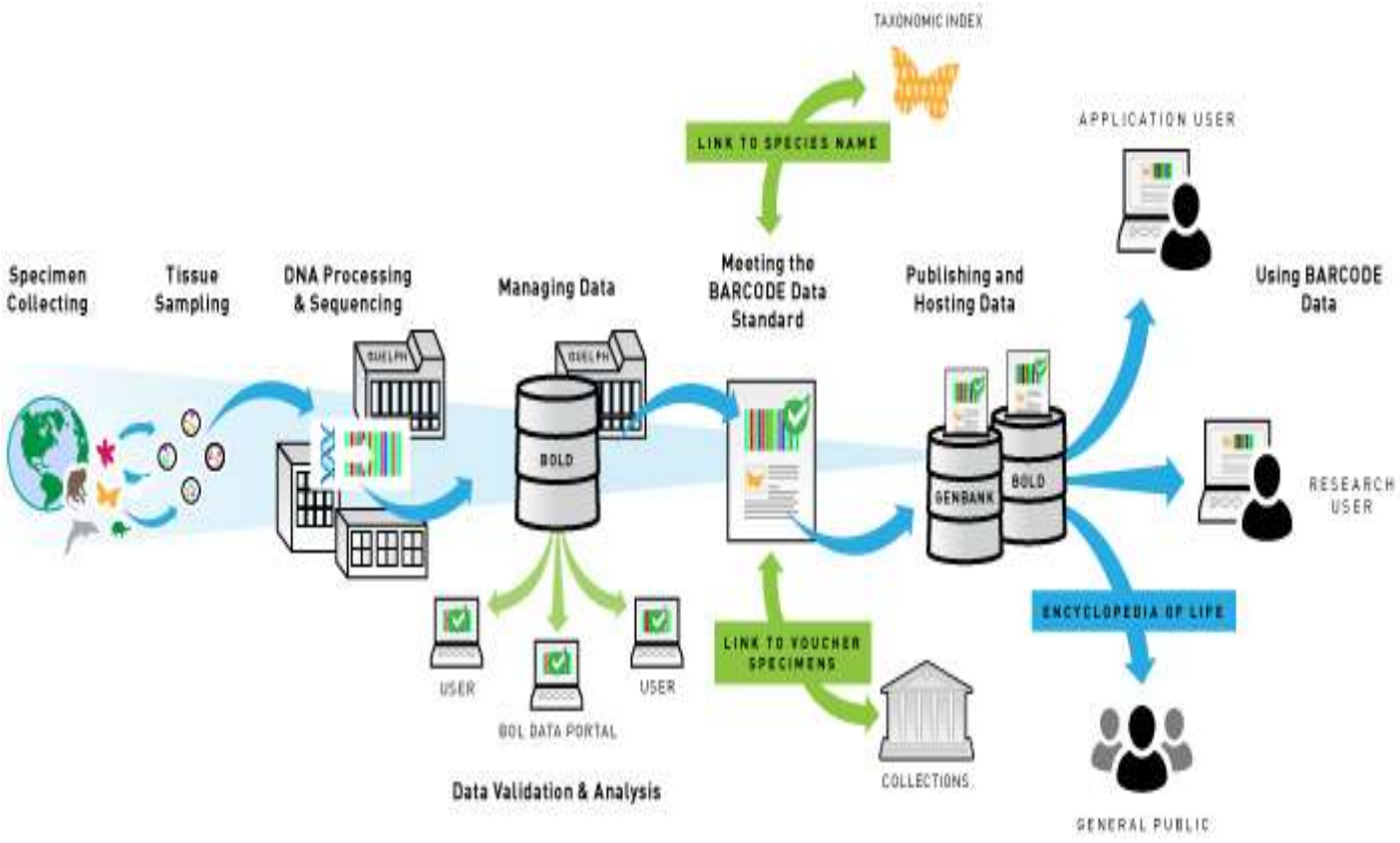
**DNA barcoding software**

Software for DNA barcoding requires integration of a field information management system (FIMS), laboratory information management system (LIMS), sequence analysis tools, workflow tracking to connect field data and laboratory data, database submission tools and pipeline automation for scaling up to eco-system scale projects. Geneious Pro can be used for the sequence analysis components, and the two plugins made freely available through the Moorea Biocode Project, the Biocode LIMS and Genbank Submissionplugins handle integration with the FIMS, the LIMS, workflow tracking and database submission.

The Barcode of Life Data Systems (BOLD) is a web based workbench and database supporting the acquisition, storage, analysis, and publication of DNA barcode records. By assembling molecular, morphological, and distributional data, it bridges a traditional bioinformatics chasm. BOLD is the most prominently used barcoding

software and is freely available to any researcher with interests in DNA barcoding. By providing specialized services, it aids the assembly of records that meet the standards needed to gain BARCODE designation in the global sequence databases. Because of its web-based delivery and flexible data security model, it is also well positioned to support projects that involve broad research alliances.

**DNA Barcoding?**




THE BARCODING PIPELINE

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In 2003, Paul Hebert, researcher at the University of Guelph in Ontario, Canada, proposed "DNA barcoding" as a way to identify species. Barcoding uses a very short genetic sequence from a standard part of the genome the way a supermarket scanner distinguishes products using the black stripes of the Universal Product Code (UPC). Two items may look very similar to the untrained eye, but in both cases the barcodes are distinct.

Until now, biological specimens were identified using morphological features like the shape, size and color of body parts. In some cases a trained technician could make routine identifications using morphological "keys" (step-by-step instructions of what to look for), but in most cases an experienced professional taxonomist

is needed. If a specimen is damaged or is in an immature stage of development, even specialists may be unable to make identifications. Barcoding solves these problems because even non-specialists can obtain barcodes from tiny amounts of tissue. This is not to say that traditional taxonomy has become less important. Rather, DNA barcoding can serve a dual purpose as a new tool in the taxonomists toolbox supplementing their knowledge as well as being an innovative device for non-experts who need to make a quick identification.

The gene region that is being used as the standard barcode for almost all animal groups is a 648 base-pair region in the mitochondrial cytochrome c oxidase 1 gene ("CO1"). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. COI is not an effective barcode region in plants because it evolves too slowly, but two gene regions in the chloroplast, matK and rbcL, have been approved as the barcode regions for plants.

**Barcoding projects have four components:**

- **The Specimens:** Natural history museums, herbaria, zoos, aquaria, frozen tissue collections, seed banks, type culture collections and other repositories of biological materials are treasure troves of identified specimens.
- **The Laboratory Analysis:** Laboratory protocols (pdf; 400Kb) can be followed to obtain DNA barcode sequences from these specimens. The best equipped molecular biology labs can produce a DNA barcode sequence in a few hours. The data are then placed in a database for subsequent analysis.
- **The Database:** One of the most important components of the Barcode Initiative is the construction of a public reference library of species identifiers which could be used to assign unknown specimens to known species. There are currently two main barcode databases that fill this role:
- The International Nucleotide Sequence Database Collaborative is a partnership amongGenBank in the U.S., the Nucleotide Sequence Database of the European Molecular Biology Lab in Europe, and the DNA Data Bank of Japan. They have agreed to CBOL's data standards (pdf; 30Kb) for barcode records.
- Barcode of Life Database (BOLD) was created and is maintained by University of Guelph in Ontario. It offers researchers a way to collect, manage, and analyze DNA barcode data.
- **The Data Analysis:** Specimens are identified by finding the closest matching reference record in the database. CBOL's Data Analysis Working Group has created the Barcode of Life Data Portal which offers researchers new and more flexible ways to store, manage, analyze and display their barcode data.

The concept of DNA barcoding has become one of the most important and significant scientific visions in the last decade. As an emerging and effective tool for species identification, the concept of DNA barcoding has gained worldwide popularity. The ground-breaking concept of DNA barcoding was put forward in the year

2003 by Professor Paul Hebert and collaborators serving at University of Guelph, Canada. Mitochondrial *cytochrome c oxidase* subunit 1 (COI) gene was suggested as unique barcode region for animals ( Hebert et al., 2003). This sequence was validated at the 1st International Conference on DNA Barcode of Life. Henceforth, several studies have shown that the sequence diversity in a ~650 bp region near the 5′ region of the COI gene provides strong species level resolution for different animal groups like birds ( Yoo et al., 2006, Tavares and Baker, 2008 and Schindel et al., 2011), springtails (Hogg and Hebert, 2004), shrimps (Trivedi et al., 2011), fishes ( Ward et al., 2005, Yancy et al., 2008,Bhattacharjee et al., 2012, Laskar et al., 2013 and Trivedi et al., 2014), tortoise (Kundu et al., 2013), oysters (Trivedi et al., 2012), mammals (Lim, 2012), spiders (Greenstone et al., 2005), mosquitoes (Cywinska et al., 2006), ticks (Zhang and Zhang, 2014) etc.

The Consortium for the Barcode of Life (CBOL) was established to support worldwide DNA barcoding and subsequently an international online data management system – the Barcode of Life Data Systems (http://www.barcodinglife.org) came into effect. Survey and assessment of genetically diverse organisms of the earth through DNA barcoding is led by CBOL. A milestone in the field of DNA barcoding was achieved by launching of International Barcode of Life Project (iBOL). Canada was the first country to establish national network for DNA barcoding as The Canadian Barcode of Life Network (BOLNET.ca). Subsequently, several countries and regions have also established barcoding networks as part of the iBOL like Europe (ECBOL; http://www.ecbol.org/), Norway (NorBOL; http://dnabarcoding.no/en/), Mexico (MexBOL; http://www.mexbol.org/) and Japan (JBOLI; http://www.jboli.org/). Besides this, thematic programs like human health (HealthBOL), polar life (PolarBOL) and quarantine and plant pathogens (QBOL, as a part of the ECBOL) are also in place.

**Advantages of DNA barcoding in marine perspective**

More than 70% of our planet is covered by oceans that have higher biodiversity compared to terrestrial or freshwater ecosystems. The massive marine ecosystem is the habitat for a large number of flora and fauna, both macro and micro. Among the 35 animal phyla, 34 phyla have marine representatives while 14 include exclusively marine animals (Briggs, 1994 and Gray, 1997). The occurrence of cryptic species is relatively common in marine ecosystems. Cryptic species are those species that are morphologically similar but genetically distinct. DNA barcoding can be a very effective tool in assessment of these cryptic species. Another problem that persists in the marine and estuarine habitat is the linking of the larval stages with the adult forms. DNA barcoding can accurately link the larval stages of a species in order to unravel the life cycle of different marine species, which is usually difficult and in some cases not possible using the morphological approach. The threat of invasive species to marine biodiversity can be globally assessed through DNA barcoding (Molnar et al., 2008).

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The invasive alien species (IAS) poses severe threat and is capable of inflecting huge economic losses. DNA barcoding can be used to quickly and accurately identify the invasive alien species and prompt preventive measures with subsequent regulatory control can be initiated. Barcoding of indicator species can be fruitful in the monitoring and abatement of marine pollution including coastal pollution. One main aim of DNA barcoding initiative is the discovery of new species. DNA barcoding can be used as an important tool for identification, authentication and safety assessment of sea food, particularly for processed, cooked or smoked products. This molecular identification can even allow us to trace the origin of certain products (Galimberti et al., 2013). A study conducted on the Japanese delicacy tuna sushi from different restaurants in USA, revealed the presence of endangered species, fraud and also a health hazard (Lowenstein et al., 2009). An analysis of 254 Canadian seafood samples revealed that 41% of the samples were mislabeled (Hanner et al., 2011).

DNA barcoding is an important tool in wildlife forensics and conservation. It can be used to identify endangered sea turtles by assessing turtle meat, carcasses or eggs that are illegally traded (Vargas et al., 2009). One important requirement of DNA barcoding is the collection and maintenance of samples as voucher specimens, which allows reliable means of corroborating the identification of the species from which data is accumulated. The voucher specimens provide permanent documentation for investigation of marine biodiversity. DNA barcoding has a great utility in the field of taxonomy (Ali et al., 2014).

DNA barcoding can be very effective for molecular phylogenic studies, geographical distribution and conservation of marine biodiversity. DNA barcoding can be used for pest and disease control as well. With the recent developments in deep sea research and the revelation that several deep sea organisms possess extraordinary pharmaceutical properties, DNA barcoding of deep sea organisms has gained global attention. Census of the Diversity of Abyssal Marine Life (CeDAMar) is devoted to the barcoding of deep sea organisms. The user-friendliness of DNA barcodes is also an added advantage and can be effectively used for marine biodiversity assessment, fisheries management and conservation (Pérez1-Huete and Quezada, 2013).

**Worldwide DNA barcoding initiative for marine organisms**

MarBOL, the Marine Barcode of Life, is an international campaign to barcode marine species. MarBOL (http://www.marinebarcoding.org) is led by an International Steering Committee and an affiliated project of the Census of Marine Life (CoML). CoML is involved in several Ocean Realm Field Projects (Table 1). Already five International Barcode of Life Conferences have been held and the 6th International Barcode of Life Conference is scheduled to be held in Guelph, Ontario, Canada during August 18–22, 2015.

Table 1. Involvement of Census of Marine Life (CoML) in various Ocean Realm Field Projects.

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| S. No. | Ocean Realm Field Projects of CoML | Abbreviations |
|---|---|---|
| 1 | Arctic Ocean Diversity | ArcOD |
| 2 | Biogeography of Chemosynthetic Ecosystems | ChEss |
| 3 | Census of Antarctic Marine Life | CAML |
| 4 | Census of Diversity of Abyssal Marine Life | CeDAMar |
| 5 | Census of Marine Zooplankton | CMarZ |
| 6 | Continental Margin Ecosystems on a Worldwide Scale | CoMargE |
| 7 | Global Census of Coral Reef Ecosystems | CREEFS |
| 8 | Global Census of Marine Life on Seamounts | CenSeam |
| 9 | Gulf of Maine Area Program | GOMA |
| 10 | International Census of Marine Microbes | ICOMM |
| 11 | Natural Geography in Shore Areas | NaGISA |
| 12 | Pacific Ocean Shelf Tracking | POST |
| 13 | Tagging of Pacific Pelagics | TOPP |

**DNA barcoding of marine microbes**

Assessment of biodiversity in the microbial world has always been a challenging task. Rapid and accurate identification of the microbes is frequently necessary to prevent the spread of diseases caused by microbes. Protists are eukaryotic microbes which have short generation time and asexual reproductive capability. An ecologically significant group of protists are the dinoflagellates which serve as primary producers, coral symbionts and cause red tides. DNA barcoding of marine environmental samples revealed massive dinoflagellate diversity

**DNA barcoding of seagrasses, mangroves and marine phytoplanktons**

Seagrasses are important submerged flowering plants that have very noticeable ecological influence on the coastal environment due to their nutrient recycling ability and high primary productivity. Besides this, they contain valuable secondary compounds like phenolic acids which are used in traditional medicines. Rosmarinic acid and zosteric acid obtained from seagrasses are widely used as an antioxidant and effective antifouling agent respectively. Although these marine plants have wide geographical distribution worldwide there is rapid decline in sea grass species and cover globally. It is reported that seagrasses are disappearing at the rate of 110 $km^2$ per year, since 1980 Hence, there is urgent need for assessment and conservation of seagrasses. Seagrasses perform both, sexual and asexual reproduction, but vegetative reproduction is more common and

sexual progenies are short lived. Species identification becomes difficult because the flower as a distinct morphological trait is often unavailable. In such a situation, DNA barcoding can serve as a useful identification tool. Different markers have been used for identification of seagrasses like nuclear ITS for *Halophila* , trnK introns and rbcL for *Zostera* , ITS1, 5.8S rDNA and ITS2 for*Halophila*. By using rbcL and matK sequences it was revealed that it is possible to develop DNA barcoding for seagrasses

Mangroves at the intersection of terrestrial, estuarine and near shore marine ecosystem have immense ecological and economic significance. The ecosystem services provided by mangrove forests are worth at least US$1.6 billion per year worldwide. This dynamic and unique ecosystem is increasingly threatened and depleted. The conservation of mangroves is of utmost importance in order to maintain the health of this fragile environment. Loss of evolutionary unique species in the mangrove ecosystem has been reported and DNA barcoding provided phylogenetic information for developing unified mangrove management plan worldwide The Sunderbans is the single largest block of tidal halophytic mangrove forest listed in the UNESCO world heritage list (http://whc.unesco.org/en/list). It is regarded as the world's largest natural nursery where a large number of marine and estuarine species come to breed and the juveniles stay back to exploit its rich natural resources. In a study conducted in the Sunderbans mangrove ecosystem, molecular methods based on *rbc*L subunit of RuBisCO enzyme were used for identification of phytoplankton groups lesser than 10 μm size

**DNA barcoding of marine algae**

Different species of red marine macro algae are often difficult to identify by using morphological techniques. Two molecular markers namely mitochondrial COI gene and UPA (Universal Plastid Amplicon) domain V of the 23S rRNA gene were used for identification of different species of red alga belonging to the family Kallymeniaceae. Results showed that COI was a more sensitive marker and led to the discovery of a new species *Euthora timburtonii* ( Clarkston and Saunders, 2010). A similar study was conducted involving inter tidal red macro algae in China with three molecular markers – COI, UPA and ITS (nuclear internal transcribed spacer). Although COI was effective to identify species but not all species gave successful amplicons due to lack of universal primers. UPA had effective universal primers but showed problems with closely related species, while ITS was the least effective.

Gracilariaceae is a red algal family which is commercially important for its use in biotechnology and microbiology research as a phycocolloid agar. *Gracilaria* species are difficult to identify morphologically and DNA barcoding holds promise in species level identification. Recently, a novel microalga was isolated and characterized from Indian Ocean which has biofuel potential. In this study 16S rRNA and 23S rRNA were used

as barcode. DNA barcoding can be useful as a rapid, sensitive and reliable method for monitoring programs of marine and coastal ecosystems for detecting Harmful Algal Bloom (HAB) species.

**DNA barcoding of marine zooplanktons**

Zooplanktons have great ecological significance and represent 15 animal groups (phyla). Therefore, DNA barcoding of zooplanktons is an important aspect of modern ecological studies. Census for Marine Zooplanktons (CMarZ) is devoted to the study of global zooplankton assemblages. The DNA Barcoding Centers of CMarZ are located in UConn (USA), Bremerhaven (Germany), ORI (Japan), Qingdao (China) and Goa (India). Fig. 1shows the five CMarZ barcoding centers of the world. Barcode analysis using COI gene involving 52 specimens of 14 species of chaetognaths could successfully discriminate different species of chaetognaths across the phylum. The average K2P distance within species was 0.0145. Among the marine zooplanktons the copepods are one of the most systematically complex and ecologically significant groups with more than 2500 species. Several studies have been conducted on this diverse group. The occurrence of cryptic species is widespread among the copepods which necessitates more DNA barcoding studies. Some important publications on DNA barcoding of marine copepods are shown in Table 4.

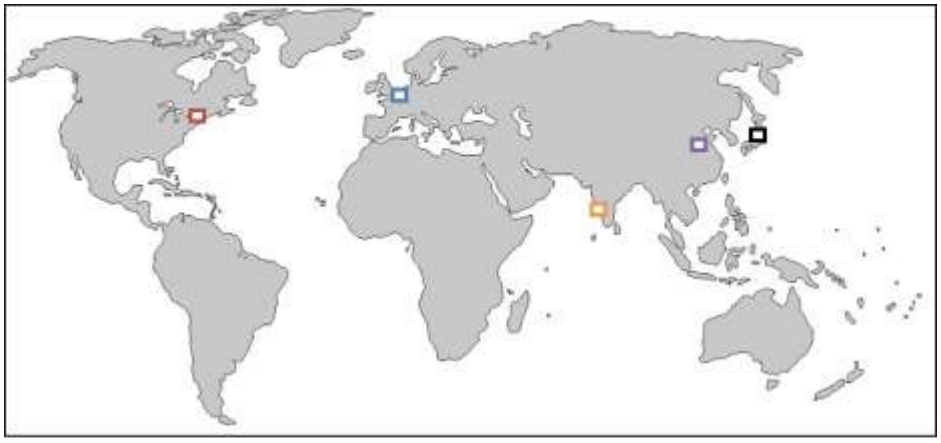


Figure 1. Five CMarZ barcoding centers of the world.

Since it is difficult to identify the different chaetognath species based on morphological characters, especially with those preserved in alcohol, DNA barcoding can be very effective to resolve this problem. A study was conducted with*Neocalanus* copepods involving four marker genes namely COI, 12S, nuclear ITS, and 28S. The results showed that although all the four markers could identify distinctly all the species but distinction of the form variants was only confirmed by the COI sequences. DNA sequence variation of a 575 base-pair region of

28S rDNA, from North and South Atlantic regions could accurately and reliably identify the three species of *Oithona,* an ecologically important copepod species.

**DNA barcoding of marine invertebrates**

The pteropods which belong to the phylum Mollusca and class Gastropoda are of unique research interest due to their vulnerability to ocean acidification. Barcoding of*Diacavolinia* pteropods indicated that the Atlantic specimens comprise a single monophyletic species and show probable species-level divergence between Atlantic and Pacific population. DNA barcoding comprising 227 species of Canadian marine mollusks indicated possible cases of overlooked species. DNA barcoding projects should be developed for megadiverse groups such as mollusks to facilitate species discovery and conservation. A study involving 315 specimens from around 60 venerid species showed that DNA barcoding can be very effective in species delimitation. Marine oysters are bivalves that have great economic significance. Identification of oysters largely based on phenotypic characters like shell morphology is problematic due to the taxonomic controversies. Shell morphology, used as a primary distinguishing feature is greatly affected by habitat. In such cases, molecular identification proves to be useful (Table 4).

Echinoderms are exclusively marine animals. DNA barcoding of 191 echinoderm species belonging to five classes was undertaken. Based on shallow intraspecific versus deep congeneric divergences 97.9% specimens were assigned to known species. Sponges have canal system inside the body and possess pharmaceutical properties. Sponge Barcoding Project, http://www.spongebarcoding.org is a global initiative. A DNA barcoding workflow capable of analyzing large sponge collections has been developed through this project. Nematodes are known for their role as indicator of anthropogenic stress in the marine ecosystems. In the nematodes, 18S gene was able to amplify across several taxa and showed identification success rate of 97%. Universal primers for diverse group of marine metazoan invertebrates are available. (Table 4).

**DNA barcoding of lower chordates**

Ascidians are filter-feeding marine urochordates which are regarded as model organisms used to study complex biological processes. They are used to study the transcriptional control of embryonic development, mechanism of metal accumulation, evolution of the immune system, conservation of gene regulatory networks in chordates, development of heart, etc. (Holland and Gibson-Brown, 2003, Trivedi et al., 2003, Satoh et al., 2003, Stolfi and Christiaen, 2012, Tolkin and Christiaen, 2012 and Razy-Krajka et al., 2014). The genome of an ascidian species *Ciona intestinalis* is the smallest of any experimentally manipulable chordate, as a consequence it is used in genome analysis studies. COI gene analysis of *Ciona* specimens from New Zealand revealed for the first time, the existence of solitary ascidian *Ciona savignyi* in the Southern Hemisphere (Smith et al., 2012). A

new ascidian species belonging to the genus *Diplosoma* has been revealed through DNA barcoding in the Ryukyu Archipelago of Japan ( Hirose and Hirose, 2009).

**DNA barcoding of marine fishes**

Marine fish is an important source of protein, vitamin D, vitamin $B_{12}$, iodine, selenium and omega-3 fatty acids. Marine fisheries sector has a very significant contribution in food security and economic welfare. Proper identification of fish species is important for management of fisheries and authentication of food products. DNA barcoding allows fast and efficient means of fish identification. Two main global barcoding initiatives for fish are FISH-BOL (http://www.fishbol.org) and SHARK-BOL (http://www.sharkbol.org). DNA barcoding is useful not only for the identification of whole fish but also for the identification of larvae, eggs, fillets, fins or other fragments of the body which are difficult to identify based on morphology. This molecular technique was used to identify shark fins that were confiscated from illegal fishers in Australia (Holmes et al., 2009). Demand for ornamental fish is rapidly increasing globally. COI gene analysis of 391 ornamental fish species from 8 coral reef locations revealed that most (98%) of these species belonged to distinct barcode clusters (Steinke et al., 2009a and Steinke et al., 2009b). Some important publications on DNA barcoding of marine fishes are depicted in Table 3.

Table 3.Some important publications on DNA barcoding of marine fishes.

| Serial No. | Topics | References |
|---|---|---|
| 1 | Red Sea fishes | Trivedi et al. (2014) |
| 2 | Mediterranean Sea and Cantabric Sea fishes | Ardura et al. (2013) |
| 3 | Caribbean and western central Atlantic fishes | Weigt et al., 2012 |
| 4 | Antarctic fishes | Dettai et al. (2011) |
| 5 | Arctic marine fishes | Mecklenburg et al. (2011) |
| 6 | Marine and brackish water fishes from Argentina | Mabragaña et al. (2011) |
| 7 | Marine fishes from Japan | (Zhang and Hanner, 2011) |
| 8 | Marine fishes of China | (Zhang, 2011) |
| 9 | European marine fishes | (Kochzius et al., 2010) |
| 10 | Marine fishes of India | (Lakra et al., 2010) |
| 11 | Campaign to barcode all fishes | (Ward et al., 2009) |
| 12 | Coral reef fishes | (Steinke et al., 2009a) |
| 13 | Indo-Pacific, Australian and South African Marine Fishes | (Zemlak et al.,. 2009) |

| Serial No. | Topics | References |
|---|---|---|
| 14 | North American marine fishes | (Steinke et al., 2009b) |
| 15 | Salmon and trout species from North America | (Rasmussen et al., 2009) |
| 16 | North-east Atlantic deep-water sharks | (Moura et al., 2008) |
| 17 | Sharks and rays of Australia | (Ward et al., 2008b) |
| 18 | Fish larvae in Great barrier Reef, Australia | (Pegg et al., 2006) |
| 19 | Marine fishes of Australia | (Ward et al., 2005) |

Table 4. Some important publications on DNA barcoding of marine invertebrates.

| Group | Article |
|---|---|
| Arthropoda (Copepoda) | DNA Barcoding of Marine Copepods: Assessment of Analytical Approaches to Species Identification |
| | DNA barcoding of Arctic Ocean holozooplankton for species identification and recognition. |
| | A "Rosetta Stone" for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (Northwest Atlantic Ocean) |
| | Zooplankton diversity analysis through single-gene sequencing of a community sample |
| | Comparison of molecular species identification for North Sea calanoid copepods (Crustacea) using proteome fingerprints and DNA sequences |
| | Comparison of morphological and molecular traits for species identification and taxonomic grouping of oncaeid copepods |
| | Morphological and molecular phylogenetic analysis of evolutionary lineages within *Clausocalanus* (Copepoda: Calanoida) |
| | Dissimilarity of species and forms of planktonic Neocalanus copepods using mitochondrial COI, 12S, nuclear ITS, and 28S gene sequences |
| | Speciation of two salinity associated size forms of *Oithona dissimilis* (Copepoda: Cyclopoida) in estuaries |
| | Evolution in the deep sea: Biological traits, ecology and phylogenetics of pelagic copepods |

# 2017

| Group | Article |
|---|---|
| | Morphological and genetic variation in the North Atlantic copepod, *Centropages typicus* |
| | Multi-Gene analysis reveals a lack of genetic divergence between *Calanus agulhensis* and *C. sinicus* (Copepoda; Calanoida) |
| | Comparative phylogeography and connectivity of sibling species of the marine copepod *Clausocalanus* (Calanoida) |
| Arthropoda (Amphipoda) | Probing marine *Gammarus* (Amphipoda) taxonomy with DNA barcodes |
| Metazoa | DNA Barcoding of marine Metazoa |
| | DNA Barcodes for Marine Biodiversity: Moving Fast Forward? |
| Mollusca (Gastropods) | Complete lack of mitochondrial divergence between two species of NE Atlantic marine intertidal gastropods |
| | Species diversity of planktonic gastropods (Pteropoda and Heteropoda) from six ocean basins based on DNA barcode analysis |
| | A new *Poecilogonous* species of sea slug (Opisthobranchia: Sacoglossa) from California: comparison with the planktotrophic congener *Alderiamodesta* Loven, 1844 |
| | Patterns of DNA Barcode Variation in Canadian Marine Molluscs |
| Mollusca (Bivalves) | Local scale DNA barcoding of bivalves (Mollusca): a case study |
| | Molecular phylogeny of oysters belonging to the genus*Crassostrea* through DNA barcoding |
| | Four genes, morphology and ecology: distinguishing a new species of *Acesta* (Mollusca; Bivalvia) from the Gulf of Mexico |
| | Phylogeny of venus clams (Bivalvia: Venerinae) as inferred from nuclear and mitochondrial gene sequences |
| Chaetognatha | Barcoding of arrow worms (Phylum Chaetognatha) from three oceans: genetic diversity and evolution within an enigmatic phylum |
| Platyhelminthes | DNA taxonomy of Swedish *Catenulida*(Platyhelminthes) and a phylogenetic framework for catenulid classification |
| Nemathelminthes | Disentangling taxonomy within the *Rhabditis* (*Pellioditis*) *marina*(Nematoda, |

| Group | Article |
|---|---|
| | Rhabditidae) species complex using molecular and morphological tools |
| | Development and evaluation of a DNA-barcoding approach for the rapid identification of nematodes |
| Annelida | *Grania*(Annelida: Clitellata: Enchytraeidae) of the Great Barrier Reef, Australia, including four new species and a re-description of *Grania trichaeta* Jamieson, 1977. |
| Porifera | Morphological description and DNA barcodes of shallow-water*Tetractinellida* (Porifera: Demospongiae) from Bocas del Toro, Panama, with description of a new species |
| Cnidaria | DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments |
| Bryozoa | Mating trials validate the use of DNA barcoding to reveal cryptic speciation of a marine bryozoan taxon |
| Echinodermata | DNA barcoding discriminates echinoderm species |
| | Genetic barcoding of commercial Bêche-de-mer species (Echinodermata: Holothuroidea) |

Most DNA barcodings are focused on animals and more effort is needed on the barcoding of plants and protists. One main reason may be the lack of universal barcode gene in plants that makes the situation comparatively tricky. Despite of some limitations, DNA barcoding approach can be used for survey of marine biodiversity and prioritizing conservation strategies. In conclusion it can be said that DNA barcoding can play a very significant role in assessment and conservation of biodiversity in the massive and diverse marine ecosystem.

**Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE**

17MBP105A
I MSC MICROBIOLOGY
MARINE MICROBIOLOGY

| Unit V | | | | | |
|---|---|---|---|---|---|
| Half life (t ½) is the time required to: | Change the amount of a drug in plasma by half during elimination | Metabolize a half of an introduced drug into the active metabolite | Absorb a half of an introduced drug | Bind a half of an introduced drug to plasma proteins | Change the amount of a drug in plasma by half during elimination |
| Aspirin is chemically | Sodium salicylate | Acetylsalicylic acid | Salicylamide | Sodium salicylamide | Acetylsalicylic acid |
| Which is the most appropriate to the term “receptor” | All types of ion channels modulated by a drug | Enzymes of oxidizing-reducing reactions activated by a drug | Active macromolecular components of a cell or an organism which a drug molecule has to combine with in order to elicit its specific effect | Carriers activated by a drug | Active macromolecular components of a cell or an organism which a drug molecule has to combine with in order to elicit its specific effect |
| What does “affinity” mean? | A measure of how tightly a drug binds to plasma proteins | A measure of how tightly a drug binds to a receptor | A measure of inhibiting potency of a drug | A measure of bioavailability of a drug | A measure of how tightly a drug binds to a receptor |

| | | | | | |
|---|---|---|---|---|---|
| A measure of bioavailability of a drug | A measure of how tightly a drug binds to a receptor | An agonist is a substance that: | Interacts with the receptor without producing any effect | Interacts with the receptor and initiates changes in cell function, producing various effects | Increases concentration of another substance to produce effect |
| An agonist is a substance that: | Interacts with the receptor without producing any effect | Interacts with the receptor and initiates changes in cell function, producing various effects | Increases concentration of another substance to produce effect | Interacts with plasma proteins and doesn't produce any effect | Interacts with the receptor and initiates changes in cell function, producing various effects |
| An antagonist is a substance that: | Binds to the receptors and initiates changes in cell function, producing maximal effect | Binds to the receptors and initiates changes in cell function, producing submaximal effect | Interacts with plasma proteins and doesn't produce any effect | Binds to the receptors without directly altering their functions | Binds to the receptors without directly altering their functions |
| A competitive antagonist is a substance that: | Interacts with receptors and produces sub maximal effect | Binds to the same receptor site and progressively inhibits the agonist response | Binds to the nonspecific sites of tissue | Binds to one receptor subtype as an agonist and to another as an antagonist | Binds to the same receptor site and progressively inhibits the agonist response |

| | | | | | |
|---|---|---|---|---|---|
| The Irreversible interaction of an antagonist with a receptor is due to: | Competitive Ionic bonds | Irreversible Hydrogen bonds | Agonist-Covalent bonds | Partial Weak bonds | Agonist-Covalent bonds |
| Tick the second messenger of G-protein-coupled (metabotropic) receptor: | Adenylyl cyclase | Sodium ions | Phospholipase C | cAMP | cAMP |
| What is the type of drug-to-drug interaction which is connected with processes of absorption, biotransformation, distribution and excretion? | Pharmacodynamic interaction | Physical and chemical interaction | Pharmaceutical interaction | Pharmacokinetic interaction | Pharmacokinetic interaction |
| Chloramphenicol is derived from | Streptomyces venezulae | Streptomyces griseus | Streptomyces kanamycin | Pencillin | Streptomyces griseus |

| | | | | | | |
|---|---|---|---|---|---|---|
| A hydrophilic medicinal agent has the following property: | Low ability to penetrate through the cell membrane lipids | Penetrate through membranes by means of endocytosis | Easy permeation through the blood-brain barrier | High reabsorption in renal tubules | | Low ability to penetrate through the cell membrane lipids |
| The feature of the sublingual route: | Pretty fast absorption | A drug is exposed to gastric secretion | A drug is exposed more prominent liver metabolism | A drug can be administrated in a variety of doses | | Pretty fast absorption |
| Pick out the parenteral route of medicinal agent administration: | Rectal | Oral | Sublingual | Inhalation | | Inhalation |
| Parenteral administration: | Cannot be used with unconsciousness patients | Generally results in a less accurate dosage than oral administration | Usually produces a more rapid response than oral administration | Is too slow for emergency use | | Usually produces a more rapid response than oral administration |
| Volume of | Concentra | Concentra | Therapeut | A daily | | Concentra |
| Biotransformation of the drugs is to render them: | Less ionized | More pharmacologically active | More lipid soluble | Less lipid soluble | | Less lipid soluble |

| | | | | | | |
|---|---|---|---|---|---|---|
| Tick the drug type for which microsomal oxidation is the most prominent: | Lipid soluble | Water soluble | Low molecular weight | High molecular weight | | Lipid soluble |
| Cell surface receptors are | C protein coupled receptors | G-protein coupled receptors | Protein A tyrosine kinases | Protein A B tyrosine kinase | | G-protein coupled receptors |
| The receptor serves as | Recognition molecule | Non recognition molecule | Target sites | Active sites | | Recognition molecule |
| Which one of the following not bound to membrane? | Tyrosine linked receptors | Steroid receptors | ion channel linked receptors | G-protein coupled receptors | | steroid receptors |
| When the person remains well only when he is taking the drug is termed as the State of | psychic dependence | physical dependence | withdrawal syndrome | Non Psychic dependence | | physical dependence |

| | | | | | | |
|---|---|---|---|---|---|---|
| If the abusing drug is withdrawn the person develops | Abstinence | physical dependence | Tolerance | psychic dependence | | Abstinence |
| If a greaster dose of the drug is required to elicit the normal pharmachological Effect the state is known as | dependence | abstinence | tolerance | intoleranc e | | tolerance |
| Th 1 cells | enhance CMI | enhance humoral immunity | inhibit CMI | inhibit humoral immunity | | enhance CMI |
| If the drug | | | psychic | drug | | pharmaco |
| A repeated injection of egg albumin in such an animalcauses a violent reaction Called | cytotoxic type reaction | cell mediated reaction | immune complex mediated reaction | anaphylaxis | | Anaphylaxis |
| A state | | | psychic | drug | | |
| Best example of psychic dependence is | cigarette smoking | barbiturates | sulphonamides | salicylates | | cigarette smoking |

| | | | | | | |
|---|---|---|---|---|---|---|
| The state when the person seeks drugs purely for psychological pleasure is | drug dependence | physical dependence | psychic dependence | pathological equilibrium | | psychic dependence |
| bstances like lead can remain deposited in bones without producing toxic effects Which is called | passive immunization | additive effect | antagonism | synergism | | passive immunization |
| Inflammatory reactions initiated by mononuclear lymphocytes and not by Antibody alone are called | type I hypersensitivity | type II hypersensitivity | delayed hypersensitivity | type III hypersensitivity | | delayed hypersensitivity |

| | | | | | |
|---|---|---|---|---|---|
| Methadone is | agonist of opioid receptors | antagonist of opioid receptors | agonist of μ receptors | antagonist of μ receptors | agonist of opioid receptors |
| Opioids used for abusing are by themselves | CNS stimulants | CNS depressants | CVS stimulants | CVS depressants | CNS depressants |
| The drug naltrexone is | agonist of opioid receptors | antagonist of opioid receptors | agonist of μ receptors | antagonist of μ receptors | antagonist of opioid receptors |
| The drugs used to treat abusing of opioids is | Ibu brufen | methadone | Diclofenac | Analgesic | Methadone |
| If the opioid abusers are doctors,nurses and other health workers The choice of drug used for treatment is | methadone | methadyl acetate | naltrexone | pethidine | Pethidine |
| Amphetamine is an | antifatigue agent | fatigue agent | nausea inducer | heroin | antifatigue agent |

| | | | | | |
|---|---|---|---|---|---|
| Polydrug abuse common in USA is | cocaine and heroin | heroin and ampheta mine | ampheta mine and cocaine | nicotine | cocaine and heroin |
| The half life of cocaine is | 2 hrs | 3 hrs | 15 hrs | 1hr | 1hr |
| Drug used for the treatment of acute cocaine overdose is | Naproxen | ampheta mine | diazepam | Ibu brufen | diazepam |
| The mechanis m of action of labetalol used for acute cocaine overdose is | blocking of $Ca^{2+}$ channel | blocking of α and β receptor | blocking of $K^{+}$ channel | blocking of $P^{+}$ channel | blocking of α and β receptor |
| The drug of choice for CNS complicati ons due to acute cocaine overdose is | labetalol | nifedipine | diazepam | sulphona mides | diazepam |
| The craving of cocaine is reduced by | labetalol | nifedipine | desiprami ne | diazepam | Desiprami ne |
| Cryopreser | Heating in | Freezing in | Drying in li | Steaming in liquid nitrogen | Freezing in liquid nitrog |

| | | | | | |
|---|---|---|---|---|---|
| In Cryopres | -72°C | -86°C | -196°C | -96°C | -196°C |
| The stabiliz | Glycerol | Phenol | Terpenol | Lysol | Glycerol |
| _____ | Glycerol | Phenol | Terpenol | Lysol | Glycerol |
| Lyophilizat | Freeze etch | Freeze dryi | Freeze sha | Freeze liquid nitrogen | Freeze drying |
| In Lyophiliz | -196°C | -86°C | -70°C | -96°C | -70°C |
| The metab | dry dehydr | vacuum de | Spray dehy | Dry heat dehydration | vacuum dehydration |
| Lyophilized | 1°C | 8°C | 7°C | 4°C | 4°C |
| Which | Water | lipid | ionsoluble | Non | lipid |
| Science that deals with drug | Pharmacy | pharmaco gnosy | pharmaco dynamics | pharmacol ogy | Pharmacol ogy |
| Inhibition of MAO causses an | decrease in the deaminati on of noradrena lin | increasse in the deaminati on of dopamine | increase in the deaminati on of noradrena lin | decrease in the deaminati on of dopamine | decrease in the deaminati on of noradrena lin |
| Systemic clearance (CLs) is related with: | Only the concentra tion of substance s in plasma | Only the eliminatio n rate constant | Volume of distributio n, half life and eliminatio n rate constant | Bioavailab ility and half life | Volume of distributio n, half life and eliminatio n rate constant |
| Eliminatio n rate constant (Kelim) is defined by the following parameter : | Rate of absorptio n | Maximal concentra tion of a substance in plasma | Highest single dose | Half life (t ½) | Half life (t ½) |

| | | | | | | |
|---|---|---|---|---|---|---|
| The time required to kill 90% of the microorganisms in a sample at a specific temperature is the | decimal reduction time | thermal death point | F value | D value | | decimal reduction time |
| Which of the following is best used for long term storage of microbial samples when carried out properly? | Storage in a freezer at -10°C | Storage in a freezer at ultra low temperatures (-70°C) | Storage in a refrigerator on an agar slant | Storage on a petri plate at room temperature | | Storage in a freezer at ultra low temperatures (-70°C) |
| Which of the antibiotic is not used as a food preservative ? | Pimaricin | Nisin | Tylosin | β-lactam antibiotic | | Nisin |
| Which antibiotic has a beta-lactam ring? | Cephalosporin | Penicillin | Tetracycline | Streptomycin | | Penicillin |

| | | | | | | |
|---|---|---|---|---|---|---|
| In eukaryotic cells, ribosomes are | 70S | 60S | 80S | Not specific | | 80S |
| Porins are located in | the outer membrane of gram-negative bacteria | the peptidoglycan layer of gram-positive bacteria | the cytoplasmic membrane of both gram-negative and gram-positive bacteria | the periplasmic space of gram-negative bacteria | | the outer membrane of gram-negative bacteria |
| For most bacteria, the optimum pH for growth lies between | 6.5-7.5 | 3.5-4.5 | 4.5-5.5 | 5.5-6.5 | | 6.5-7.5 |
| Suspension cultures consist of cells and cell aggregates, growing dispersed in | liquid medium | solid nutrient medium | solid or liquid medium | semisolid media | | liquid medium |

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**Reg. No. : ----------**
**[17MBP105A]**

**KARPAGAM UNIVERSITY**
**KARPAGAM ACADEMY OF HIGHER EDUCATION**
(Deemed University Established Under Section 3 of UGC Act 1956)
Eachanari post, Coimbatore – 641 021, Tamil Nadu, India
**DEPARTMENT OF MICROBIOLOGY**
**FIRST INTERNAL TEST, AUGUST 2017**
**FIRST SEMESTER**
**MARINE MICROBIOLOGY**

**Time: 2 hours** **Maximum: 50 marks**
**Class: I MSc. MB**

**PART-A (Answer all the questions) 20 x 1 = 20 marks**

1. Attachment of small particles or molecules to a larger particle by electric charge is called as
   A. Adsorption B. absorption
   C. fixation D. attachment
2. _____ is derived from an environment other than that in which it is found.
   A. autothonous B. Allochthonous
   C. hetrothonous D. xenothonous
3. _____are organism which grows at high pressure rather than at atmospheric pressure.
   A. Barophile B.halophile
   C.thermophile D.neutrophil
4. TVC means _____
   A. Total Viable Counts B.Total NonViable Counts
   C.Time Variable Counts D. Time nonVariable Counts
5. _____ is Mass of living matter present.
   A.biogroup B. Biomass
   C. biodiverse D. bioaccumulation
6. Particulate (organic) material which is only partly disintegrated is called as_____
   A. demeris B. divergent
   C. detritus D. debris
7. An organism which grows preferentially in high salinities.
   A.Halophile B. Barophile
   C.Chemophile D.Divergephile
8. Living together of two organisms with mutual advantage and without losing their identity is called as
   A. Antagonism B. Commensalism
   C. Symbiosis D. Mutualism
9. Which were the investigators lived at the same time?
   A. Koch and Pasteur B. Darwin and Woese
   C. Leeuenhoek & Ricketts D. Berg and Hooke
10. Which of the following bacteria lack a cell wall and are therefore resistant to penicillin?
   A.Cyanobacteria B.Mycoplasmas
   C.Bdellovibrios D.Spirochetes
11. Chemotaxis is a phenomenon of swimming
   A. away of bacteria B. towards bacteria
   C. away or towards of bacteria in presence of chemical compound D. Not swimming
12. The structure responsible for motility of bacteria is
   A. pilli B. flagella
   C. sheath D. capsules

13. DNA to RNA is called as

A. replication B. biosynthesis

C.translation, D. transcription

14. _____ group of bacteria grows in high pressure

A. Halophiles B. Basophiles

C. thermophiles D. psychrophiles

15. _____ group of bacteria grows in high temperature

A. Halophiles B. Basophiles

C. thermophiles D. psychrophiles

16. The group of gram positive bacteria having low G+C contents are called as

A. cyanobacteria B. Nanobacteria

C. Firmicutes, D. Actinobacteria

17. BGA expanded as

A. Blue Green Algae B. Blue Grown Algae

C. Blue non Grown Algae D. Brown Green Algae

18. Bacteria are _____organisms

A. Obligate B. single celled

C. multicellular D. seen by naked eye.

19. Who is father of Marine Microbiology?

A.Leewenhoek B. Zobell

C. Edward Jenner D. Louis Pasteur

20. Prokayotic ribosomes are made up of _____ subunits

A.two B. three

C.five D. ten

**PART-B (Answer all the questions) 3x 2= 6 marks**

21. What are Extremophiles
22. Give a brief note on Marine environment
23. Explain the application of 16s RNA

**PART-C (Answer all the questions) 3x 8 = 24 marks**

24. A. Explain in short about the sampling of benthic marine organisms.

(Or)

B. Write in detail about enumeration of marine bacteria

25. A. Explain in short about the sampling of deep marine organisms.

(Or)

B. Write in detail about International and national collection centres.

26. A. Give a detailed note on Marine ecosystem

(Or)

B. Discuss about the life at extreme environments

**Reg. No. : ----------**

**[17MBP105A]**

# KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act 1956)

Eachanari post, Coimbatore – 641 021, Tamil Nadu, India

**M.Sc. II Internal Test, October 2017**

**DEPARTMENT OF MICROBIOLOGY**

**FIRST SEMESTER**

**MARINE MICROBIOLOGY**

**Time: 2 hours** **Maximum: 50 marks**

**Date/Session:** **Class: I MSc MB**

**PART-A** **20 x 1 = 20 marks**

1. An organism which grows preferentially in high salinities are called as.....
   A. Halophile B. Barophile
   C.Chemophile D. Divergephile
2. Living together of two organisms with mutual advantage and without losing their identity is called as ....
   A. Antagonism B. Commensalism
   C. Symbiosis D. Mutualism
3. Which were the investigators lived at the same time?
   A. Koch and Pasteur B. Darwin and Woese
   C. Leeuenhoek and Ricketts D. Berg and Hooke
4. The unifying feature of the archaea that distinguishes them from the bacteria is.....
   A. habitats which are extreme environments with regard to acidity
   B. absence of a nuclear membrane temperature
   C. presence of a cell wall containing a characteristic outer membrane
   D. cytoplasmic ribosomes that are 70S
5. Organisms can synthesize ATP by oxidative phosphorylation when they.....
   A. ferment
   B. oxidize glucose to pyruvate
   C. pass electrons from the oxidation of chlorophyll through an electron transport system
   D. pass electrons to oxygen through an electron transport system containing cytochromes
6. How many molecules of carbon dioxide will be given off during ten turns of the Krebs cycle?
   A.10 B.20
   C.30 D.40
7. In glycolysis, ATP is created by.....
   A. photophosphorylation B. chemiosmotic mechanism
   C. substrate level phosphorylation D. the pentose phosphate pathway
8. In cellular metabolism, O2 is used.....
   A. to provide electrons for photophosphorylation
   B. in glycolysis
   C. as a terminal electron acceptor
   D. in the Krebs cycle
9. The concept of putting microbes to help clean up the environment is called as .....
   A. pasteurization B. bioremediation
   C. fermentation D. biolistics
10. If a canning procedure is not properly followed, which type of microbe is most likely to grow in the canned food?
   A. Obligate Aerobe B. Acidophile
   C. Mesophile D. Obligate Anaerobe
11. The cell walls of many gram positive bacteria can be easily destroyed by the enzyme known as.....
   A. lipase B. lysozyme
   C. pectinase D. Peroxidise

12. Bacteria reproduce by _____ mechanism
    A. fission B. own
    C.fusion D. Direct
13. Bacteria are sensitive to _____
    A. Interleukins B. Interferons
    C. Antibiotics D. Antitumours
14. _____media is used for cultivation of marine bacteria
    A. Nutrient agar B. ZMA
    C. EMB agar D. MHA
15. Swimming towards a chemical of bacteria is termed as.....
    A. positive chemotaxis B. negative chemotaxis
    C. phototaxis D. magnetotaxis
16. _____ group of bacteria grows in high pressure
    A. Halophiles B. Barophiles
    C. thermophiles D. psychrophiles
17. The group of gram positive bacteria having high G+C contents are called as.....
    A. cyanobacteria B. Nanobacteria
    C. Firmicutes D. Actinobacteria
18. A musty or muddy odor of the fish is attributed to.....
    A. the growth of *Streptomyces species* in the mud at the bottom of the body of water
    B. the mud at the bottom of the body of water
    C. the growth of *Pseudomonas species* in the mud at the bottom of the body of water
    D. the mud at the water
19. The predominant kind of bacteria causing spoilage in fish at chilling temperature is.....
    A. species of *Pseudomonas* B. *Micrococcus*
    C. *Bacillus* D. *E. coli*
20. Preservation of foods by using salts and sugars works by.....
    A. raising pH B. lowering osmotic pressure
    C. creating a hypertonic environment D. creating a hypotonic environment

**Part B** **(3x2= 6 marks)**

21. Describe about Biodegradation
22. How molecular methods help in marine microbiology research?
23. What is aquatic pollution? Add a note on oil spill removal.

**Part C** **(3x8 = 24 marks)**

23. A. Explain the marine nutrient cycles.

(Or)

B. Give a brief note on marine ecosystem.

24. A. Explain in detail about the microbial bioremediation.

(Or)

B. Give the detailed structure microbial pigments.

25. A. How the whole microbe genome is sequenced? Give an idea about it.

(Or)

B. Explain in detail about the aquaculture.

**Reg. No. : ----------**

**[16MBP105A]**

**KARPAGAM UNIVESITY**

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

**M.Sc. DEGREE EXAMINATION, NOVEMBER 2016**

**FIRST SEMESTER**

**MICROBIOLOGY**

**MARINE MICROBIOLOGY**

**Time: 3 hours** **Maximum: 60 marks**

## PART-A

**Multiple Choice Questions No.1 to 20 (Online Exam) 20 x 1 = 20 marks**

1. _____ is Mass of living matter present.
   A.biogroup, B. Biomass, C. biodiverse D. bioaccumulation
2. Particulate (organic) material which is only partly disintegrated is called as_____
   A. demeris, B. divergent, C. detritus, D. debris
3. An organism which grows preferentially in high salinities.
   A.Halophile B. Barophile, C.Chemophile D.Divergephile
4. Living together of two organisms with mutual advantage and without losing their identity is called as
   A. Antagonism B. Commensalism, C. Symbiosis, D. Mutualism
5. Which were the investigators lived at the same time?
   A. Koch and Pasteur B. Darwin and Woese C. Van Leeuenhoek and Ricketts
   D. Berg and Hooke
6. The unifying feature of the archaea that distinguishes them from the bacteria is
   A.habitats which are extreme environments with regard to acidity
   B.absence of a nuclear membrane temperature C. presence of a cell wall containing a characteristic outer membrane D. cytoplasmic ribosomes that are 70S
7. Which instrument is used for sterilization above 100° C
   A. Flame, B. Autoclave, C. Filters, D. Desiccators
8. _____ is the last first phase in growth curve
   A. Log, B. Lag, C. stationary, D. death
9. RNA to DNA is called as
   A. replication B. biosynthesis, C. translation, D. reverse transcription
10. The common word for bacteria which are curved in shape is
    A. cocci B. bacilli C. spirilla D. comma
11. Single or clusters of flagella at both poles is known as
    A. monotrichous B. peritrichous C. amphitrichous D. none of these
12. Which of the following bacterial genera (that produces endospore) have medical importance?
    A. Proteus B. Bacillus C. Salmonella D. *E.coli*
13. Swimming towards a chemical of bacteria is termed as
    A. positive chemotaxis B. negative chemotaxis C. phototaxis D. magnetotaxis
14. _____ group of bacteria grows in high pressure
    A. Halophiles, B. Barophiles, C. thermophiles, D. psychrophiles
15. The group of gram positive bacteria having high G+C contents are called as
    A. cyanobacteria B. Nanobacteria, C. Firmicutes, D. Actinobacteria
16. Which of the following articles cannot be sterilized in an autoclave?
    A. Gloves B. Culture media C. Dressing material D. sugars
17. Which of the following disinfectants act by disrupting microbial membranes?
    A. Cationic detergents B. Halogens C. Heavy metals D. Aldehydes
18. Which of the following is best to sterilize heat labile solutions?
    A. Dry heat B. Autoclave C. Membrane filtration D. Pasteurization
19. All the following are considered eukaryotes except

A. archaea B. fungi C. protozoa D. humans

20. _____are organism which grows at high pressure rather than at atmospheric pressure.

A. Barophile B.halophile C.thermophile D.neutrophil

**Part B (5x6 = 30 marks)**

21. A. Explain in short about the pelagic marine microbes (Or)
    B. Write in detail about marine sampling by dreges.
22. A. Give a detailed note on marine natural products. (Or)
    B. What are extremophiles? Explain about them and their environment
23. A. Explain the marine nutrient cycles. (Or)
    B. Give a brief note on marine ecosystem.
24. A. Explain in detail about the autotrophic generation of ATP. (Or)
    B. Give the detailed structure about C4 pathway.
25. A. How the whole microbe genome is sequenced? Give an idea about it. (Or)
    B. Explain in detail about the vermicomposting.

**Part C (1x10 = 10 marks)**

26. Explain in detail about the sampling in marine environments highlighting on samples that can be collected, where they can be collected and equipment utilized.

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**Reg. No. : ----------**
**[16MBP105A]**

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**FIRST SEMESTER**
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**MARINE MICROBIOLOGY**

**Time: 3 hours** **Maximum: 60 marks**

## PART-A

**Multiple Choice Questions No.1 to 20 (Online Exam) 20 x 1 = 20 marks**

1. Attachment of small particles or molecules to a larger particle by electric charge is called as
A. Adsorption, B. absorption, C. fixation, D. attachment.
2. _____ is derived from an environment other than that in which it is found.
A. autothonous, B. Allochthonous C. hetrothonous, D. xenothonous
3. _____are organism which grows at high pressure rather than at atmospheric pressure.
A. Barophile B.halophile C.thermophile D.neutrophil
4. TVC means _____
A. Total Viable Counts, B.Total NonViable Counts, C.Time Variable Counts D. Time nonVariable Counts
5. _____ is Mass of living matter present.
A.biogroup, B. Biomass, C. biodiverse D. bioaccumulation
6. Particulate (organic) material which is only partly disintegrated is called as_____
A. demeris, B. divergent, C. detritus, D. debris
7. An organism which grows preferentially in high salinities.
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A. Antagonism B. Commensalism, C. Symbiosis, D. Mutualism
9. Which were the investigators lived at the same time?
A. Koch and Pasteur B. Darwin and Woese C. Van Leeuenhoek and Ricketts D. Berg and Hooke
10. The unifying feature of the archaea that distinguishes them from the bacteria is
A.habitats which are extreme environments with regard to acidity B.absence of a nuclear membrane temperature C. presence of a cell wall containing a characteristic outer membrane D. cytoplasmic ribosomes that are 70S
11. Organisms can synthesize ATP by oxidative phosphorylation when they
A. ferment B.oxidize glucose to pyruvate C.pass electrons from the oxidation of chlorophyll through an electron transport system D.pass electrons to oxygen through an electron transport system containing cytochromes
12. How many molecules of carbon dioxide will be given off during ten turns of the Krebs cycle?
A.10 B.20 C.30 D.40
13. In cellular metabolism, O2 is used
A. to provide electrons for photophosphorylation B.in glycolysis C. as a terminal electron acceptor D.in the Krebs cycle
14. In glycolysis, ATP is created by
A. photophosphorylation B. the chemiosmotic mechanism C. substrate level phosphorylation D. the pentose phosphate pathway
15. The concept of putting microbes to help clean up the environment is called
A. pasteurization B. bioremediation C. fermentation D. biolistics
16. If a canning procedure is not properly followed, which type of microbe is most likely to grow in the canned food?
A. Obligate Aerobe B. Acidophile C. Mesophile D. Obligate Anaerobe
17. Some cyanobacteria produce potent neurotoxins that, if ingested, will kill humans. These cyanobacteria are most likely to contaminate

A.water rich in organic carbon wastes but poor in phosphate B.water that are anoxic C. water rich in phosphate wastes but poor in organic carbon D. water rich in chlorine

18. A musty or muddy odor of the fish is attributed to

A. the growth of *Streptomyces species* in the mud at the bottom of the body of water

B. the mud at the bottom of the body of water C. the growth of *Pseudomonas species* in the mud at the bottom of the body of water D. the mud at the water

19. The predominant kind of bacteria causing spoilage in fish at chilling temperature is

A. species of *Pseudomonas* B. *Micrococcus* C. *Bacillus* D. *E. coli*

20. Preservation of foods by using salts and sugars works by

A. raising pH B. lowering osmotic pressure C. creating a hypertonic environment D. creating a hypotonic environment

**Part B (5x6 = 30 marks)**

21. A. Explain in short about the sampling of benthic marine organisms. (Or)
    B. Write in detail about enumeration of marine bacteria
22. A. Give a detailed note on RAPD. (Or)
    B. What is RFLP? How it helps in marine studies.
23. A. What is aquatic pollution? Add a note on oil spill removal. (Or)
    B. Give a brief note on marine nutrients
24. A. Explain in detail about the photosynthetic pigments (Or)
    B. Give the detailed structure about Calvin cycle.
25. A. Give a detailed note on SCP (Or)
    B. Explain in detail about the mushroom cultivation?

**Part C (1x10 = 10 marks)**

26. Explain the methods of isolation and identification of microbes from seafood samples.