

UNDERSTANDING MARINE MICROBES -TRENDS AND FUTURE DIRECTIONS

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Microbes which form an integral part of any ecosystem including the marine ecosystem are represented by groups such as viruses, bacteria, fungi, diatoms, algae, protozoa etc. These being fundamental blocks of an ecosystem their activities have very significant influence on the various chemical and biological vital processes in nature like nutrient regeneration, biodegradation of toxic pollutants, chemosynthesis in deep-seas etc. They exhibit tremendous versatility not only in surviving extreme environments but also in exhibiting invaluable commercial potential.

Many marine bacteria have shown a requirement for sodium, potassium and magnesium ions. Some of them also require chloride ions and ferric ion. Nearly 80 % of the organic matter produced in the top 100 to 300 meters of the ocean is metabolized before it sinks below the photic zone. Because of this there is little organic material reaching the bottom water layers. Because of this low organic matter concentration in the deeper levels, the heterotrophic bacteria in the pelagic and deep ocean are often oligotrophic and are inhibited by high organic concentrations. Many are psychrotrophic (grow at 0 °C and also above 20 °C) or psychrophilic (grow at 0 °C but not above 20 °C). Many of the deep sea microbes are capable of withstanding high pressures (barotrophs) and even some of them require high pressure for their normal growth (barophilic).

In the 1970s, a new community of life forms was discovered in the ocean. It was formed around hydrothermal vents on the ocean floor and obtained its energy from the chemoautotrophic metabolism of the sulphide-rich water issuing from the vents. In contrast to the deeps, unique microbial communities get themselves established in estuaries where the physical and chemical properties undergo rapid changes. (In fact the biomass of the epiphytic microbial (algae, diatoms and bacteria) communities is extremely large and may exceed the weight of the typical estuarine sea grasses. Further the range of conditions (pH and Eh) that exist in the estuary support a wide range of microbial groups (sulphur bacteria, iron bacteria, heterotrophs etc.) to grow and contribute to the nutrient cycling.)

(Globally much advancement has been made on all these aspects especially during the past two decades and the use of new and modern techniques have brought into light new group of organisms, better understanding of the microbial processes and exposed the potential of marine microbes for various industrial applications. For example use of analytical flow cytometry has shown the existence of prochlorophytes in oceanic waters contributing significantly to the primary production, an aspect which escaped in our calculations. Similarly immunological and nucleic acid probes increased our level of understanding in areas such as biodiversity, health of marine

animals and plants, microbial processes etc. As some of these organisms live in extreme conditions like salt pans, cold Antarctic waters, hot hydrothermal vents and deep seas, they have developed unique metabolism to cope up with these extremes and understanding of this uniqueness has given us the opportunity to look for new solutions for our growing needs. Considerable mycological and bacteriological studies have been carried out in India in coastal areas including estuaries. However, in the last decade many investigations were carried out in offshore locations. The studies so far conducted include microbial biogeochemical cycles (carbon, sulphur, nitrogen and phosphorous), diseases of marine plants and animals, biodegradation of crude oil and natural biopolymers, heterotrophic activities, free enzyme activities in sediments, microbial antagonism, metal-microbe interactions and microbiological pollution of coastal waters. Among the various groups of bacteria sulphate-reducing bacteria and bioluminescent bacteria are the ones which received considerable attention so far. Besides, some special groups such as thraustochytrids (fungus like protists) because of their ubiquitousness and physiological capabilities to utilize a wide variety of complex organic substrates attracted the attention of scientists. Very recently industries started looking at the marine microbes as new sources of novel products and processes especially for environmentally clean technologies e.g. biobleaching of paper pulp, decolourisation of paper mill effluent etc. Few attempts have also been made to understand the molecular biology of marine bacteria.

Even though there are certain on-going programmes on several aspects in this area of research, some important aspects are still missing. For example, in India, there is no serious programme to study the marine viruses and pathogenic bacteria. (More concerted efforts are necessary to systematically study the microbiological processes not only in the coastal areas in relation to ever increasing pollution but also in offshore locations where the role of microbes in trophic dynamics and biogeochemical cycles have not yet been fully understood. Recognizing the need by industries for a number of bioactive compounds for various applications more specific tools using modern molecular techniques have to be developed or applied. The microflora of our tropical seas offer great promise to develop highly flexible bioremediation methodologies to protect our deteriorating environment.)

INTRODUCTION

There was a tendency in oceanography about 50 years back to neglect the role of microbes in the economy of the sea. The reasons for this situation were attributed to

- (1) The lack of interaction between marine microbiologists and other type of oceanographers
- (2) Insufficient training of oceanographers in the field of microbiology
- (3) A tendency to underestimate the microbes' role-mainly because they cannot be seen with the naked eye. However, things have changed drastically especially during the recent past because of the introduction of new and advanced methodologies and instrumentation. We have now learned that marine environment differs from others in the relative importance of the microbes and the kind of community they form. This is due to conditions which are peculiar to the marine environment.

Exclusive of insects, few of which are marine, more than half of all known animal species live in the sea. Virtually all major animal phyla are represented by marine species and part of the phyla are exclusively marine. The plant kingdom is represented by more than 11,000 species—mostly phytoplankton, although several hundred species of sea weeds and other sedentary plants are known. The known species of marine viruses, bacteria, yeasts, filamentous fungi and microflagellates are numbered in hundreds only.

Marine microbiology encompasses the study of microbes that live in not only the surficial marine waters but also the abyssal depths from the coastal to the offshore regions, from the general oceanic to the specialized niches like the blue waters of the tropical coral reef ecosystems to the black smokers of hot thermal vents. Microbes have a large surface to volume ratio and this has an advantage for their mode of life. Like the terrestrial and freshwater counterparts these organisms cover all the nutritional mode from chemoorganic- to chemolitho- to photoorgano- and photolitho-trophy. The range of microorganisms inhabiting the marine world is a spectrum covering the strictly aerobic occupying niches at +800 mv to the strictly anaerobic occupying niches having -400 mv. Likewise we have organisms thriving at extremes of pressure i.e 1 atm in the surface to several hundreds of atm in the deep trenches. There are activities in both realms albeit in the latter it is several orders lower. The gamut of temperature optima also vary. There are those that thrive at near 0 °C to those multiply above 100 °C. Though the marine microbes can thrive in a broad range of Eh, light, temperature, many marine microbes have an absolute requirement for sodium, potassium and magnesium ions. Some also require chloride ions and ferric ions and grow very well above pH 7.0 or near pH 8.0. These are happy around 35‰ and pH 8.0 which is the average and conservative value for salinity and hydrogen ion concentration in sea water and the distribution of marine microbes revolves around these very characteristic parameters.

There are eight basic kinds of habitats for marine microorganisms (Fig.1):

- (i) neustonic (air-sea interface),
- (ii) planktonic (bacterioplankton - 0.2 to 2.0µm or picoplankton),
- (iii) nekton (large living animals),
- (iv) seston (animal produced organic debris),
- (v) epibiotic,
- (vi) endobiotic,
- (vii) pelagic (water borne habitats) and
- (viii) benthos (sea-sediment interface). Within these habitats, all the complex interactions of marine microorganisms ensue.

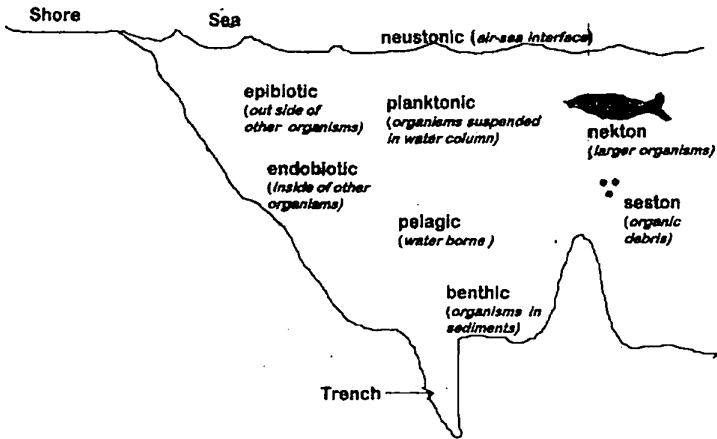


Figure 1. Basic habitats of marine micro-organisms.

1. EUBACTERIA

Throughout the photosynthetic zone the abundance of bacteria appears to be more closely related to the distribution of phytoplankton than to any other single ecological factor. The beneficial effect of plankton is attributed partly to the organic matter which they provide and partly to solid surfaces. Most of the bacteria are seen attached to suspended solids such as the bodies or remains of planktonic organisms. The bacterial biomass is high in coastal waters ($100\text{-}500\text{ mg/m}^3$) compared to off-shore waters ($50\text{-}200\text{ mg/m}^3$). In the open ocean the abundance of bacteria generally increases with depth from surface down to a depth of $25\text{-}50\text{ m}$ below which they decrease in numbers.

The rapid variations in physical and chemical properties in estuaries lead to the establishment of unique microbial communities. The biomass of epiphytic (on estuarine plants) microbial communities is extremely large and may sometimes exceed the weight of typical estuarine plants such as sea grasses. This epiphytic microbial community consist of algae, diatoms and bacteria. Because of high nutrient levels the biomass is also high. Because of the severe environmental fluctuations in temperature and salinity the microbial species diversity is not great as expected under those circumstances. It is however greater than the species diversity in plants and animals in the estuary since bacteria are capable of existing and growing over wider ranges of environmental conditions. As an example of this, consider the range in pH and Eh in an estuary, it is very large for both parameters, but many of the conditions are capable of supporting different groups of bacteria. Recent studies have shown that bacteria constitute up

to 50 % of marine pelagic biomass and in some cases even up to 90 % of benthic biomass. The total bacterial organic biomass in nearshore areas can range from 1 to 180 g m⁻³ y⁻¹.

Most of the earlier work and some substantial part of the current quantitative work also depended on the classical techniques of counting and isolating representative Colony Forming Units (CFU). Interestingly, it was also found out that a substantial part of the micro-organisms in the marine ecosystem did not grow in culture and that it can be as high as 99 % sometimes. Dr. Colwell and her associates¹ often showed that most of the bacteria go into a state of being viable but non-culturable state. A close scrutiny of the literature would show that most of the classical research in marine microbiology centered around this small fraction that is culturable. There is still a strong support to the argument that though this fraction is much smaller than the other much unaccounted fraction it is this percentage that could make important contribution. Besides, it is this portion of the population that lends itself to routine cultivation and therefore further studies. In fact, even the advanced research on the non-culturable fraction would depend on the cultivable isolates .

Similarly the occurrence of filterable bacteria i.e. those which are capable of passing through 0.4 µm, in seawater has also been reported on several occasions. Many could not be isolated but the largest single group belonged to the genus *Spirillum*. Other isolates were placed in the genera *Leucothrix*, *Flavobacterium*, *Cytophaga* and *Vibrio*.

1.1 Nutrient Cycling

For any ecosystem to be viable and functional, the necessary energy and nutrients must be generated mostly within the system on a continued basis. This is achieved by proper balancing of production and regeneration mechanisms by the system. The structural materials that are building blocks of biota must be returned to the general pool in the biosphere to be recycled again. It is remarkable to note that a number of organisms are themselves sustained in the process of degradation and decomposition, in coexistence of heterotrophy and autotrophy. These processes are often referred to as nutrient cycling and the microbes such as bacteria, fungi and even viruses are known to play the key roles.

The carbon cycle (Figs. 2 and 3) is synonymous with the energy or trophic cycle within the ecosystem and hence understanding of carbon flow is very crucial in describing the functions of that system. The photosynthetic organisms, both macro and micro, begin carbon cycle by fixing the atmospheric CO₂. The plant material formed in this process is attacked by animals that feed on it and followed by micro-organisms. This contributes to the formation of

- (1) particulate detritus and
- (2) Dissolved Organic Matter (DOM). Detritus in various stages of microbial colonization is transported laterally by currents and eventually settles on the bottom. Beneath the oxidized surface layer, the sediment is anaerobic and chemically reducing.

CARBON CYCLE (AEROBIC)

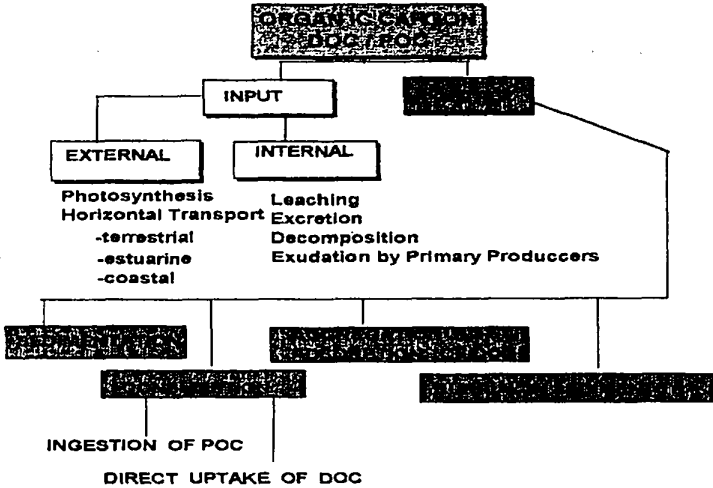


Figure 2. Aerobic carbon cycle.

CARBON CYCLE (ANAEROBIC)

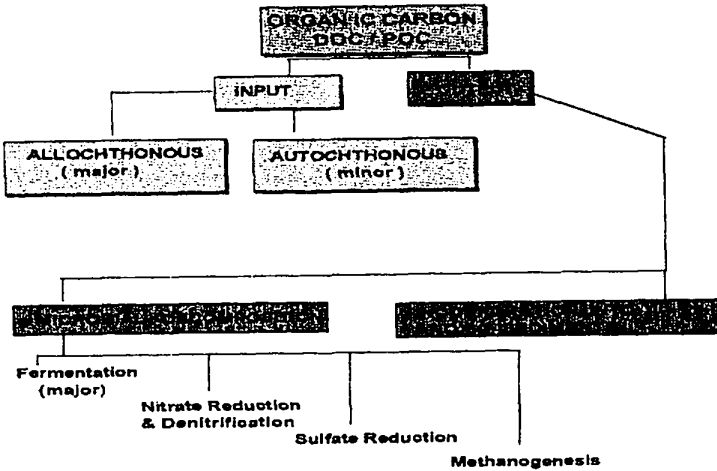


Figure 3. Anaerobic carbon cycle.

So the detritus not only undergoes aerobic decomposition in the water above the bottom but also anaerobic decomposition in the sediments. The initial stages of anaerobic decomposition of organic detritus is often fermentative giving rise to substances such as lactate and acetate. These tend to diffuse upwards into the aerobic zone, where they are oxidized by aerobic chemoheterotrophic bacteria or are used as hydrogen donors by phototrophic bacteria. In another method of decomposition the bacteria utilize sulphate, nitrate or carbon dioxide as hydrogen acceptors leading to the formation of hydrogen sulphide, ammonia or methane. Of these sulphate reduction is quantitatively important since sulphate is abundant in seawater. Hence, the anaerobic zone is characterized by the production of hydrogen sulphide which is finally precipitated as ferrous sulphide. The reduction of iron from ferric form to ferrous form leads to the liberation of adsorbed phosphates which is important in nutrient regeneration. Sulphides can be oxidized by white sulphur bacteria or by green and purple sulphur photosynthetic bacteria and the final product is elemental sulphur or sulphate. Hence, sulphates (and to a lesser extent nitrates) may be considered as alternatives to oxygen in mediating the oxidation of organic matter in sediments. It has been estimated that more than 50 % of the mineralisation of organic matter is catalyzed *via* the sulphur cycle.

The methane produced by the methanogenic bacteria in the sediments is often oxidized anaerobically by the sulphate reducing bacteria and the unoxidized methane escapes through water into the atmosphere. The CO_2 produced *via* methane oxidation can be converted back to methane, fixed photosynthetically, precipitated as insoluble carbonates or it may escape to the atmosphere. Cell material produced by the methane oxidizers and producers eventually reenters the fermentative processes. Sulphate which is the major ion in the sea water is known to inhibit methanogenesis. SRB also use hydrogen as a major electron source outcompeting methanogens. However, once sulphate is depleted in a particular niche, methanogens take over. It should be pointed out that both these groups are not mutually exclusive as once thought. In fact, they could co-exist under availability of excess competitive substrates or limited non-competitive substrates. Otherwise, spatially and temporally methanogenesis follows the sulphate reduction. The removal of hydrogen by SRB allows the otherwise thermodynamically unfavourable reaction (methane formation) to occur. Therefore, the anaerobic degradation tends to shift from the formation of hydrogen, CO_2 and mixed organic acids to the formation of acetate to CH_4 and CO_2 . Acetate is further converted to methane by other methanogens.

Nitrogen fixation in the marine environment occurs through the activity of blue-green algae, bacteria and yeasts. Free living nitrogen fixing bacteria belong to different families *viz.* Azotobacteriaceae, Enterobacteriaceae and Bacillaceae. In addition to this, several anaerobic bacteria such as sulphate

reducing bacteria, photosynthetic bacteria (Thiorhodaceae, Athirhodaceae and Chlorobacteriaceae) are known to fix atmospheric nitrogen. Nitrogen is known to undergo a number of transformations in nature which involve organic, inorganic and volatile compounds. Microbial mineralisation results in the degradation of polypeptides, aminoacids, nucleic acids and other organic compounds. The opposite process, nitrogen immobilization or assimilation, leads to biosynthesis of complex molecules of microbial protoplasm from ammonium and nitrate. Besides, ammonium and nitrate are also removed by plant cover. In addition to removal, through denitrification, nitrogen is directly removed from the system and is not immediately available to most macro- and micro-organisms because the end product of denitrification is free nitrogen. The free nitrogen again enters the cycle through nitrogen fixation and results in accumulation of fresh organic compounds.

1.2 Microbes as Food

Bacterial numbers vary from $0.1 \times 10^8 \text{ l}^{-1}$ in the deep sea to $50 \times 10^8 \text{ l}^{-1}$ in coastal waters of which 80-90 % may be planktonic. Since microbes form a large part of the biomass in the oceans, they are of greater importance in the trophic levels than to be merely assigned to the role of decomposers. Bacteria and allied organisms are known to colonize detrital particles making them a more nutritious substance. In addition, bacteria can utilize dissolved organic matter in the sea water more efficiently than any other biological forms because of their small size and higher surface to volume ratio. They can efficiently scavenge dilute dissolved organic compounds down to concentrations of μg per litre in the water. Oceanic water contains 0.4-2.0 mg l^{-1} Dissolved Organic Carbon (DOC). Estuarine water contains considerably higher quantities. DOC in seawater arises due to phenomena such as leaching from macrophytes and microalgae, 'sloppy feeding' of the herbivores, autolysis, excretion of animals and terrestrial input. It has been estimated that as high as 40 % of the photosynthetically produced carbon leaks into sea water as Dissolved Organic Carbon (DOC). Considering the high rate of primary production in coastal region ($100 \text{ g C m}^{-2} \text{ y}^{-1}$) and upwelling areas ($300 \text{ g C m}^{-2} \text{ y}^{-1}$), the leakage would have been a waste unless this DOC is converted into Particulate Organic Carbon (POC) which is possible by bacteria. Because of the ability of bacteria to colonize particles, various organisms such as cladocerans, sponges, appendicularians, ascidians, copepods, euphasids and molluscs are capable of utilizing bacteria as food. Researchers, using ^{14}C -labelled bacteria, have shown that bacteria may contribute even to the extent of 40 % of food for fishes and prawns.

2. ARCHAEA

As the name indicates these are an ancient group of microbes that have evolved little as compared to the bacteria of other group *viz.* the eubacteria.

In keeping with the early history these groups have found niches which are extreme in some way or the other. Thus they could be inhabiting the hot thermal vents or hypersaline salt marshes. These groups of microbes are smaller than their eubacterial counterparts but are diverse. In short, they are a class of unusual bacteria that phylogenetically are neither prokaryotes nor eukaryotes. They have some characteristics of prokaryotes such as absence of well defined nucleus and cell organelles and some characteristics that are unique to them such as composition of cell wall and type of membrane lipids. Accordingly the archaeobacteria are believed to represent third primary kingdom such that 3 lines of descent lead from a common ancestor to archaeobacteria, prokaryotes and eukaryotes respectively. Most of the archaeobacteria are anaerobes. The rRNA of these groups are as different from each other as they are from the Eukaryotes. The group Archaeae thus includes basically the extreme halophiles, thermophiles/thermoacidophiles and methanogens. Some of the other features that distinguishes them from the eubacteria are quite distinct.

2.1 Halophiles

Extreme halophiles are thought to be the most evolved of the Archaeobacteria. An extreme halophile is a microbe that requires 1.5 M (8.8 %) NaCl for growth and many require 3-4 M (17-23 %). Marine saltern is the common habitat of halophiles and the most common genera are *Halococcus* and *Halobacterium*. Sometimes, elemental sulphur acts as the electron acceptor as in the case of methanogenic and thermophilic microbes. Thus sulphur reduction is an universal trait among the archaeobacterium. *Halobacterium* requires a large amount of sodium and this cannot be replaced by potassium. These bacteria are generally pigmented as red or orange due to carotenoids called bacteriorubrans. Light mediated synthesis of ATP takes place in these micro-organisms. The halophiles are capable of both aerobic and anaerobic growth.

2.2 Thermophiles

Pyrodictium is a typical example which is quite an interesting organism in that it grows at a temperature optimum of 105°C. The growth occurs between 82 °C and 110 °C. Other inhabitants of such environments include *Thermococcus*, *Pyrococcus* and *Staphylothermus*. It has been consistently observed that the extreme thermophiles have a common metabolic theme i.e. the use of elemental sulphur as an electron acceptor for anaerobic growth. These microbes are unable to use sulphate as the electron acceptor.

In contrast, however, there is yet another microbe which is extremely thermophilic and is able to use sulphate as the electron donor and this organism is referred to as the *Archaeoglobus* from the hot sediments of

marine hydrothermal vents. The optimum growth temperature is 83 °C with a growth range from 64 °C to 92 °C. Another surprising feature of this microbe is that it combines traits of a methanogen and a sulphate reducer. It possesses the coenzyme F420 and methanopterin the key coenzymes involved in methane production. In fact, this bacteria produces minute amount of methane (mini methanogenesis). Molecular taxonomy using 16S rRNA show that this microbe could be a missing link between sulfate reducing bacteria and the methanogens.

A methanogenic bacterium *Methanopyrus* capable of growth at 100 °C has also been recorded. It is capable of methanogenesis from H₂ and CO₂ at this temperature with the generation time of less than one hour. The cell have large amounts of glycolytic derivative cyclic 2,3, diphosphoglycerate, dissolved in the cytoplasm which is supposed to act as an thermostabilizing agent of enzymes inside the cell. Thus it has been possible to explain the biogenic origin of hydrocarbon in hot thermal sediments. Besides, the habitat of these bacteria the water remains as liquid at 2000 m and 350 °C suggesting that growth of thermophilic methanogens is possible at perhaps even higher temperature.

2.3 Methanogens

Methanogens are strict anaerobes and are found to occur where O₂, NO₃⁻, Fe³⁺, SO₄²⁻ are limiting. In pure cultures methanogens are sensitive but in nature the activity of other organisms protects them from the toxic effect of O₂. In environment rich in SO₄²⁻ the end product is H₂S and from carbon the end product is CO₂. If SO₄²⁻ is limiting CH₄ replaces H₂S. The ratio CH₄:H₂S i.e. acetoclastic vs. sulphidogenic degradation indicates whether methanogenesis predominates. Sulphidogenesis predominates in estuarine, marine and hypersaline environment. Beneath the regions of high productivity, methanogenesis dominate. Even in surface layers where sulphate is in excess some methanogenesis may occur from methylated compounds such as trimethylamine and dimethyl sulphide. Trimethylamine is often found in marine sediments where it is formed from betaine glycine. This compound is a osmoprotectant compound produced by marine plants and bacteria for osmotic balance between cytoplasm and seawater. Methanogens convert it to CH₄, CO₂ and NH₃. Thus it is a non-competitive substrate and many of these forms belong to Methanosarcinae.

Ramamurthy and others² have shown that in the Indian mangrove sediments of Pitchavaram, both sulphate reduction and methanogenesis occurred simultaneously in the same region provided there was enough of non competitive substrates methanol and methylamine (substrates not used by SRB) or an abundance of competitive substrates like H₂/CO₂/CH₃COOH). Mohan Raju and others³ have shown that the distribution of these forms in the Indian mangroves are dependent on the environmental

factors like pH, salinity and Eh. Methyltrophic methanogens which exclusively use methylamines, methanol and dimethyl sulphide have been reported from Indian waters⁴ and elsewhere.⁵

3. FUNGI

The role of fungi in terrestrial and freshwater ecosystems is well known when compared with the information available on the marine environment. Although fungi occur commonly in the sea as parasites of living organisms and on dead organic matter, their importance in the processes in the sea has still not been sufficiently evaluated.⁶

There is no proven instance of mycelial fungi growing planktonically in the sea. However, the unicellular fungi, yeasts are common in the water column although much lower in number than bacteria. Information on the number and role of yeasts in the sea is meager and this area needs more detailed investigations.

The marine, monocentric, biflagellate fungi—the thraustochytrids⁷ are extremely common in the sea and can be isolated from a wide variety of substrates. However, the role that these organisms play in the heterotrophic marine processes is poorly understood and has been much less investigated than that of bacteria and even filamentous fungi.^{8&9}

A great amount of work has been carried out on fungi in wood submerged in the sea, dead tissues of sea grasses, mangrove leaves and mangrove seedlings. There are several studies which give evidence on the role of fungi in carbon and nitrogen immobilization in coastal marine plant litter systems.

The role of fungi in the sea might be greater than hitherto recognized, since niches suitable for growth of different groups of fungi are plentiful.⁶ Numerous situations in the sea have not been adequately analysed for the fungi.

4. VIRUSES

The concentration of virus like particles in seawater are typically in excess of 10^7 ml^{-1} as estimated by both electron microscopy and fluorescence microscopy.¹⁰ The indigenous marine viruses are known to infect a number of important groups of marine organisms including bacteria, cyanobacteria, microalgae and protozoan grazers. About 20 % of marine heterotrophic bacteria are infected by viruses and 10-20 % of bacterial community is lysed daily by viruses. Some of the experiments conducted suggest that 2-3 % of primary productivity is lost to viral infection.¹¹ Even though phages are abundant in sea water contacts with the host cells are important for infectivity. It has been demonstrated that 1 % of the contacts would result in infection, indicating most of the cells are probably resistant to the most abundant phages.¹²

Viruses which infect cyanobacteria occur at even higher concentrations and 106 infectious units ml⁻¹ can be found in coastal waters infecting especially a single strain of *Synechococcus* (Texas area).¹³ Viruses in waters generally get adsorbed to solids which protect them from inactivation by biological, chemical and physical factors. Marine sediments, for example, can adsorb more than 99 % of poliovirus suspension (containing 108 plaque forming units per ml) and may contain 10-10,000 times the concentration of viruses in the overlying water.¹⁴ This sediment bound viruses can also be taken up by shellfish, thus allowing their bioaccumulation in marine life near sewage outfalls.¹⁵ Furthermore, sediment-associated viruses are known to maintain their infectivity¹⁶ for a long time. Due to considerable variability in enumerating enteric viruses, results are generally expressed on presence/absence basis.

Our knowledge on marine viruses is still very much limited. Even though bacteriophages for many marine bacteria were reported, till 1989, there was no authentic report of any bacteriophage for Sulphate Reducing Bacteria (SRB).¹⁷

An increased awareness of the key role that viruses may play in ocean processes, many laboratories started their new programmes on marine viruses in recent times. Broadly they may be classified as :

- Determining the effect that viruses can have on the primary productivity and phytoplankton population dynamics;
- Isolating and characterizing novel viral pathogens from the marine environment;
- Developing molecular approaches for enumeration and identification of viral pathogens;
- Determining the temporal and spatial distribution of specific viruses; and
- Identifying the rates of and the mechanisms responsible for viral decay in the marine environment.

5. MICROBES AND BIOGEOCHEMISTRY

5.1 Ferromanganese Nodules

Ferromanganese in the form of nodules and crusts occur to varying extent on the seabed of the world's oceans. These nodules are reported to contain Mn (9.5-42.3 %), Fe (2.47-19.64 %), Ni (0.13-1.57 %), Cu (0.07-1.28 %) and Co (0.16-0.48 %). Detailed investigations carried out for the last two decades clearly indicate that nodules and deep sea sediments harbour bacteria. Some are able to catalyze Mn (II) oxidation and others are able to catalyze Mn (IV) reduction. The Mn (II) oxidizing bacteria, in the process of generating Mn (IV) oxides in the form of nodules, promote the removal

of other cations such as Ni, Cu and Co from sea water and deposit them in the nodule. The enzymatic nature of these reactions has been demonstrated. Bacteriological investigations of the ferromanganese nodules collected from the Indian Ocean indicate that the microbial populations associated with the nodules are in general, comparable to those of the Pacific and Atlantic Oceans.¹⁸ Members of *Vibrio*, Enterobacteriaceae, *Bacillus*, *Micrococcus*, *Staphylococcus*, *Arthrobacter* and coryneforms were found associated with the nodules.

5.2 Magnetite Formation

The ability of magnetotactic bacteria to orient and navigate along geomagnetic field lines is due to the controlled intracellular deposition of the iron oxide mineral, magnetite (Fe_3O_4). Microbiological formation of magnetite can occur in the presence of molecular oxygen in *Magnetospirillum magnetotacticum* which can use oxygen or nitrate as the terminal electron acceptor.

Thus magnetite would not be expected to form under reducing conditions. However in September, 1993, Sakaguchi and others from Japan¹⁹ have reported the isolation and pure culture of dissimilatory sulphate reducing bacterium which can synthesize intracellular magnetite particles. This bacterium is also capable of extracellular iron sulphide precipitation. This isolate illustrates the wider metabolic diversity of magnetic bacteria and suggests the presence of novel mechanism of magnetic biomineralization. The discovery of such bacteria may also explain why large quantities of magnetite have been observed in sulphate-rich oil-bearing sedimentary deposits.

6. ANIMAL-MICROBE INTERACTIONS

A good number of investigations have been carried out on the animal-microbe interactions. These interactions can essentially be divided into two major categories i.e. harmful and beneficial. A wide range of viruses, bacteria, fungi and protozoa have at various times been associated with the diseases or parasitic conditions in marine vertebrates and invertebrates. The pathology of fin-fish and shell-fish (prawns) in farmed conditions has received our attention in the past.

In aquaculture especially in prawn farming, disease is a major factor responsible for losses. Outbreaks of diseases are usually encountered in early developmental stages (larval and postlarval) in hatcheries. One of the viruses *Monodon baculovirus* was identified using transmission electron microscopy.²⁰ Presumed Yellow-head disease and Hepatopancreatic Parvovirus-like virus have also been detected in the postlarval and juvenile *P. monodon* and *P. indicus*. IHHNV and Reo-like viruses have also been identified. The larval and postlarval stages of prawns are also susceptible to bacterial,

fungus, protozoan and metazoan parasites. The following bacteria viz. *Vibrio harveyi*, *V. anguillarum*, *V. parahaemolyticus*, *Pseudomonas* sp., *Aeromonas hydrophila*, *Leucothrix* (filamentous bacteria), protozoans such as *Zoothamnium* and fungus (*Lagenidium* sp.) were isolated and identified from the Indian penaeid prawns (*P. indicus* and *P. monodon*). *Vibrio* species have been reported to cause serious diseases of larval and postlarval stages of *Penaeus monodon*. Mortalities of *P. monodon* and *P. merguensis* larvae associated with luminescence have been observed in hatcheries in many countries including India.²¹ Most of these *Vibrio* species are part of the autochthonous flora of marine waters and therefore the source of infection is suspected to be near-shore seawater.

Researches on coral reef pathology gained momentum in the last decade because of the recognition of sudden deaths of corals in Caribbean, Atlantic, Red sea and South-East Asian waters. The reason probably lies in the complicated interactions between the environmental factors, microbes and the polyps themselves. The sudden and rapid outbreak of coral "bleaching" which occurred in the Caribbean in 1987 took many by surprise. Coral polyps started losing their symbiotic photosynthetic zooxanthelle with such rapidity that the waters become cloudy with them. Consequently, corals died and became white. No definite cause is known.

Another common coral disease also called as the "black band disease" caused by a cyanobacterium *Phormidium corallyticum* was noticed recently in Indian waters.²² This disease is known to be extremely contagious and lethal. Many massive corals in coastal waters exhibit necrotic patches and the causal agent is a dark mycelial fungus *Sclerobasidium* sp. Both these diseases have been noticed in Andaman and Lakshadweep islands. More systematic and in depth investigations are necessary to understand the disease physiology.

7. SPECIAL ASSOCIATIONS

7.1 Bioluminescent Bacteria and Fish

Another group of marine bacteria which received world wide attention is the bioluminescent bacteria. Light organ association of luminous bacteria with many marine fishes and squids is reported to be species specific. In that these animals maintain a homogeneous and pure culture of a single species of luminescent bacterium in their light glands. Several species of fishes in as many as 20 families have so far been reported to harbour either *Photobacterium phosphoreum*, *P. leiognathi*, *Vibrio fisheri* or non-cultivable bacterial symbionts as their light organ symbionts. Their bioluminescence being extremely sensitive to the toxicants, has been employed in bioassays for detecting nano or picomolar concentrations of impurities in pharmaceuticals and in the food industry. A detailed study was undertaken in India to find out how bacterial bioluminescence is affected in coastal

environs which receive various types of effluents.²³ They are useful as biomarkers in the assessment of environmental health.

7.2 Nitrogen Fixing Bacteria and Shipworms

It has been reported that adult shipworms, wood-boring bivalves of the family Teredinidae, can grow on a diet of wood alone, unsupplemented by filter feeding. There was considerable controversy regarding the origin of cellulases and the source of much needed nitrogen. However, in 1983 researchers isolated a novel bacterium in pure culture from the gland of *Deshayes* in six species of teredinid bivalves.²⁴ It is the first bacterium known to both digest cellulose and fix nitrogen and it is a participant in a unique symbiotic relation with shipworms. They appear to be unique, fitting into none of the known genera of bacteria.

7.3 Bacterial Associations in Hydrothermal Vent Organisms

Another spectacular discovery on animal-microbe interaction was made in late seventies.⁸ At a time when many are looking to outer space for new forms of life, a self contained community of unusual creatures has been discovered deep within the ocean. These animals have been found living at depths of 2500 to 2700 m, in an area just over 380 km from the Galapagos Islands. The filter feeding animals—limpets, serpulid worms, enormous clams and mussels, to name a few—are living in and around hot water vents in what is known as the Galapagos Rift area, an active zone of sea-floor spreading. These recently discovered animal colonies, however, are using as their ultimate food source, the products of chemical synthesis—that is, the upwelling of minerals (mostly sulphur compounds) from the earth's molten interior that support a population of bacteria subsisting on hydrogen sulphide and carbon dioxide. The most striking feature of some of these animals is the absence of a digestive system and the presence of specialised organs loaded with symbiotic bacteria. A number of reports have been published ever since this interesting observation was made.

7.4 Microbially Produced Bioactive Compounds in Animals

Another finding which has changed our concept is the origin of bioactive compounds in marine animals and plants. For many years chemists have been describing new or novel compounds from the marine organisms. Researchers have been trying to answer the question of what are the basic reasons for the existence of these chemicals in the organisms.

Even though many of these compounds were routinely isolated and characterised by researchers, nobody thought that microbes associated with animals could be the actual producers and not the animal themselves. Slowly the evidences started accumulating. In 1972, Frincke and Faulkner²⁵ isolated

an antibiotic (Mimosamycin) from the sponge, *Reniera* sp., and later it was found that the antibiotic was actually produced by *Streptomyces lavendule* associated with the sponge. In 1987, Karuso²⁶ isolated from the same sponge, 'Renierone' which has a striking similarity to 'Mimocin' produced by *Streptomyces lavendule*. Further another compound, 'Renieromycin', isolated from the same sponge also showed similarity to 'Saframycin' produced by the streptomycete. Later, Stierie and others²⁷ obtained 'Diketopiperazines' from another sponge (*Tedania ignis*) and also from *Micrococcus* sp. associated with the sponge. More significant discovery in the recent past was that bacteria isolated from the puffer fish produced 'Tetrodotoxin' which was originally believed to be produced by the fish itself.²⁸ In India, the survey conducted by Vanaja Kumar²⁹ indicated that nearly 75 % of the streptomycete isolates obtained from marine molluscs produced a variety of antibiotics. Even the omega-3 fatty acids which are known to be essential for the brain development in children, controlling cholesterol build up and diabetic in human beings, found abundantly in fish oils and marine fishes, are in fact produced by marine bacteria. The evidences for such animal-microbe interactions in the production of bioactive substances are slowly but steadily increasing.

8. MICROBIOLOGICAL POLLUTION

While little is known about the disease-causing microbes (pathogens) in our marine environment, we know that there are atleast two groups of significance: (i) those introduced from animal and human wastes and (ii) indigenous pathogens. Both groups contain opportunistic pathogens, which only present a health problem under certain conditions i.e. susceptible individuals or high densities in the presence of increased organic wastes.

Of the introduced pathogens, salmonellae and shigella probably survive the longest in marine waters. The causative agent of cholera, *Vibrio cholerae* O1 have been reported in the marine environment in few countries. The non-O1 strains are also common in sea water in addition to the endemic vibrios (*V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* and *V. mimicus*). Very low densities of *Pseudomonas aeruginosa* and *Staphylococcus aureus* have also been reported in marine environment. In India, since 1990 the coastal waters are monitored for the densities of various major pathogenic bacteria under the Coastal Monitoring And Prediction System (COMAPS) programme. Even though we have mostly quantitative data, we now know that our marine environment is having many of these pathogens. However, in the absence of any epidemiological data, it is very difficult to assess the impact of these pathogens.

The current knowledge indicates that traditional bacterial indicators do not reliably reflect the presence or absence of enteric pathogens in seawaters or sediments. More serious research programmes are required on the

usefulness of new index organisms such as *Clostridium perfringens* and key enteric viruses, the significance of underestimation of faecal bacteria due to viable but non-culturable forms and the prevalence of parasitic protozoa and native pathogens in marine environment.

9. COMMERCIAL APPLICATIONS OF MARINE MICROBES

9.1 Enzymes

Enzymes produced by marine bacteria are important in biotechnology due to their range of unusual properties. Some are salt-resistant, a characteristic that is often advantageous in industrial processes (detergents and industrial cleaning applications such as cleaning in reverse-osmosis membranes). Another research has demonstrated the presence in algae of unique haloperoxidases (enzymes catalyzing the incorporation of halogen into metabolites). These enzymes could become valuable products, because halogenation is an important process in chemical industries. Japanese researchers have developed methods to induce marine alga to produce large amounts of the enzyme superoxide dismutase, which is used in enormous quantities for a range of medical, cosmetic and food applications. Several fungi isolated from the coastal areas of India have been reported to produce lactase and other lignocellulose modifying enzymes.³⁰

An unusual group of marine micro-organisms from which enzymes have been isolated are the hyper thermophilic archaea (previously called Archaeobacteria) which can grow over 100 °C and therefore require enzyme systems that are stable at high temperatures. Archaea are typically found in extreme environments such as hot springs, animal guts, hydrothermal vents, sewage sludge digesters and hypersaline habitats. Thermophilic enzymes offer distinct advantages in research and industrial processes. Thermostable DNA-modifying enzymes, such as polymerases, ligases and restriction endonucleases, already have important research and industrial applications. Hot springs in Yellowstone National Park provided the first archaeon (*Thermus aquaticus*) from which thermostable DNA polymerases were isolated. These novel enzymes (Taq polymerases) became the basis for the Polymerase Chain Reaction (PCR), a useful technique for studying genetic material. In 1989, thermostable DNA polymerase was designated "Molecule of the Year" by Science magazine. Comparable enzymes continue to be discovered. Expanded study of enzymes from thermophilic marine microorganisms will contribute to the understanding of mechanisms of enzyme thermostability and should enable the identification of enzymes suitable for industrial applications as well as modification of enzymes to enhance thermostability.

9.2 Antibiotics

Interest on marine antagonistic micro-organisms grew substantially in the eighties. A low molecular weight (< 200 Daltons) inhibitory compound was isolated from a *Alteromonas* sp. associated with sea weeds. Japanese workers isolated another low molecular weight (1500-1700 Daltons) peptides, termed 'Marinostatins' from *Alteromonas*. 'Aplasmomycins' isolated from a marine strain of *Streptomyces griseus* not only showed pronounced activity against mycobacteria but also against *Plasmodium berghei* (causal agent of malaria). Marine bacteria also produce medically important enzyme inhibitors. Protease inhibitor have been isolated from *Alteromonas* sp. Several such interesting antibiotics including the brominated ones and other bioactive compounds (anitumour and anticancer)³¹ have been isolated from marine microbes. There are very few reports dealing with the production of antiviral compounds by micro-organisms.

9.3 Biopolymers and Biosurfactants

Biopolymers and surfactants have tremendous application value in food and petroleum industries. The microbial colonisation of a surface appears to depend on the ability of microbes to adhere to the surfaces. The adhesion is frequently attributed to the polysaccharides or lectin-like polymers found on microbial surfaces. It is possible to isolate a number of micro-organisms capable of producing such polysaccharides not only from the submerged surfaces but also from sea water and sediments. Once potential strains are isolated the product yield can be increased with recombinant DNA techniques. A marine isolate of *Acinetobacter* was found to produce a surfactant which was later commercialised ('EMULSAN'). This product was successfully marketed as a chelating agent for oil tankers and oil storage tanks. Another property of this compound is its ability to complex with uranium which may find application in recovery of uranium from waste material. These polymers are also useful in Enhanced Oil Recovery (EOR).

9.4 Bioremediation

9.4.1 Degradation of Petroleum Hydrocarbons

Marine micro-organisms have a marked ability to degrade complex molecules, including naphthalene, pesticides, wood pulp and petroleum. An interesting paper under the title 'Self-cleaning of the Gulf'³² was published in *Nature*. They found out that consortium of microbes comprising essentially cyanobacteria and oil-degrading bacteria was responsible for the degradation of crude oil released on the beaches of Kuwait during the 1990 Gulf War. The presence of such large numbers of oil-degrading bacteria immobilised within the mats suggests that oil degradation is being stimulated by nitrogen leaching from the cyanobacteria. In addition, the cyanobacteria also produce

oxygen which is used for oil degradation by the bacteria. Such environmentally safe methods, as practised by nature, have to be developed for combating pollution in the marine environment. In an attempt to develop such methods, marine microbial cultures (mainly bacteria and yeasts) capable of fixing atmospheric nitrogen, solubilizing insoluble phosphates and degrading crude oil were successfully combined to form a consortium in India.³³

The disposal of xenobiotic halogenated hydrocarbons, inorganics and heavy metals into the marine environment have become a serious problem in recent times. Fortunately, many of the marine bacteria are capable of removing them. Laboratory scale experiments have shown that some bacteria are capable of degrading even Polychlorinated Biphenyls (PCB), a highly toxic chemical that is strongly resistant to breakdown.

Anaerobic degradation of aromatic hydrocarbon has long been disputed because no activation reactions other than a direct attack with molecular oxygen seemed conceivable. Recently, a Sulphate Reducing Bacterium (SRB) has been isolated that completely oxidizes long chain saturated hydrocarbons such as hexadecane to CO₂ under strictly anoxic conditions.³⁴ In another report researchers informed that they have isolated a marine strain of SRB for the first time which has been shown to degrade an aromatic hydrocarbon (toluene).³⁵ The activation reaction of toluene in the newly isolated SRB remains unknown. This particular strain has been given a new name *Desulfobacula lotuolica* (gen. nov. et sp. nov.) as its characters do not fit with the existing ones. These observations clearly show that even among SRBs we may come across new strains with novel properties. What is necessary is to subject the marine anaerobic bacteria, especially SRBs to novel hypotheses. We may possibly find answers to many of the unresolved questions so far.

9.4.2 Effluent Treatment in Industries

One of the major problems industries face is the treatment of effluents. For example, in paper industry, the lignin rich effluent is hard to treat and cause severe environmental problems. At NIO, we have isolated few marine fungi from the mangrove swamps which degraded the lignin, there by making the effluent safe for disposal. In addition, several strains were also isolated which may be useful for bio-pulping and bio-bleaching.³⁶ This finding clearly shows the possibility of developing a totally chlorine free paper manufacturing process. This strain produces lignin peroxidases which are active at alkaline pH unlike the conventional one that act at acidic pH. Similar interesting and novel enzymes with possible industrial applications can be isolated from marine microbes.

10. RECENT TRENDS IN NEW METHODOLOGIES

10.1 Flow Cytometry

Analytical flow cytometry is a research technique borrowed from the medical field and is now used routinely for characterizing, enumerating and sorting of cells or other particles from heterogeneous assemblages. Phytoplankton may be cytometrically characterized³⁷ by the presence of chlorophyll-*a* autofluorescence with an emission maximum at 680 m. Bacteria have been distinguished from algae by using ethidium bromide.

Prochlorophytes are prokaryotic phototrophs that have both chlorophyll *a* and *b* but no phycobilins. Thus they resemble both cyanobacteria because of their chlorophyll '*a*' and prokaryotic nature and green plant chloroplast because of their chlorophyll '*b*' instead of phycobilins. Prochloron is the first prochlorophyte discovered and is found in nature as a symbiont in marine invertebrates (ascidians).

10.2 Molecular Biology Tools

Appropriate methods for the identification of different bacterial species and groups is a basic requirement for all bacteriological work. A common problem in the study of bacteria in nature is that only a small fraction of the observed bacteria can be successfully enriched and cultured. It has in recent years been possible to compare different bacteria's DNA sequences, and by that recognize species-specific sequences.³⁸ It has been found that the 16S *r*RNA sequence by its conservative structure is excellent for phylogenetic determinations. The sequencing of 16S *r*RNA in combination with the use of PCR amplification of the 16S *r*RNA non-cultivable bacteria or bacteria in very low concentrations can be estimated. Through cultivation and DNA sequencing it has been possible to identify the presence of important groups of bacteria in natural samples.

Fluorescently labeled *r*RNA-targeted nucleic acid probes today allow *in situ* identification of individual microbial cells in their natural habitats. Fluorescently labeled *r*RNA-targeted oligonucleotide probes are perfect tools for many areas of microbial ecology since they can monitor specific populations in environmental samples based on constant genotypic features and not on the variable phenotypic features like morphology. In the case of immobilized communities like biofilms, exact spatial distributions of microorganisms can be analyzed on a μm scale. Recent technical improvements have increased the number of potential applications considerably. Better fluorescent dyes enable today identification of routinely more than 50 % of cells even in oligotrophic aquatic samples in which the visualization of small cells with low numbers of ribosomes had been problematic. This compares favourably with the usually less than 1 % of microorganisms which can be characterized based on cultivation-dependent methods.

CONCLUSIONS

Even though marine microbiology, as a separate discipline of science, is hardly 50 years old, the pace of development has been very impressive. Thanks to advancement in technologies and methodologies which have really changed our concepts in many areas of basic science. Now we know that microbes are not only abundant in the sea but also they are responsible for various fundamental processes. Considering the new discoveries we have seen in the recent past, we can expect many more interesting and new things in the future.

It is also time to decide our priorities so that our goals are well set and we are benefited out of this research. Even though we have made great strides in understanding the microbes of the seas around India³⁹ several areas have not received our attention till date. Considering our future needs we may list the priorities as follows:

- (1) Microbiological pollution of seas has received considerable attention in the past several years through the COMAPS project funded by the Department of Ocean Development, Government of India. With increasing human activities (industries, aquaculture, sewage disposal etc.) in our coastal areas, it is highly essential to understand the qualitative and quantitative nature of the microbes in our coastal waters. More importance should be given to pathogenic bacteria and viruses. We have neglected the research on marine viruses so far and this has created a big gap in our knowledge. A nationally co-ordinated effort is required to collect and collate monitoring and epidemiological data, including the impact of toxic discharges, for the setting of appropriate Indian standards for primary contact, secondary contact and fisheries waters. Role of viruses in affecting the primary production in our waters should be seriously investigated as it would help us to make a realistic assessment of the production. Further it is time to start new programmes to assess the waste assimilation capacity of coastal waters and the role of microbes in it.
- (2) Both sulphate reducing bacteria and methanogenic forms are important geochemical agents responsible for the turnover of C, N and S. The effects of processes mediated by these forms have global implications not restricted to the marine but also the overlying atmosphere and stratosphere. Methane exerts a strong influence over the earth's climate and chemistry of the troposphere and stratosphere. Methane is a major regulator of OH radicals and is a primary sink for chlorine atoms which take part in ozone destroying chain mechanism. It has also been pointed out that surface ocean waters are typically super saturated with dissolved methane relative to atmospheric equilibrium.

Because anaerobic methanogenic bacteria are strict anaerobes, and the surface ocean waters are highly oxygenated, the observation of methane super saturation has been termed as the "oceanic methane paradox". Though methanogenic bacteria have been isolated from oceanic particulate matter faecal pellets and zooplankton, no data are available on *in situ* rates of methane formation in the microenvironments.⁴⁰ It has been pointed out elsewhere that certain strains of SRB like *D. desulphuricans* could generate H₂S in the process of coupling the oxidation of hydrocarbons to sulphate by ion reduction. Besides, these bio-geochemical processes, both these groups are involved either directly or indirectly in the conversion of toxic metal to insoluble precipitates (metal sulphides). In short what the aerobic micro-organisms' activity puts the metals into solution, the anaerobes' activity tends to trap them as insoluble metal complexes in a sulphate reducing environment. Besides both these organisms have an important role to play in the cathodic corrosion. SRB is also involved in the anodic corrosion. In brief, the study of these organisms in the marine environment would help us to understand them and the processes they mediate better. This would also mean that possibilities would be explored to convert what is generally harmful process into a useful one. We have been working on these organisms especially the SRB over a decade and a half⁴¹ and it would be highly pertinent to pursue the process oriented studies to exploit them better and to be able to mitigate problems arising out of their excess activities (for example off-shore oil wells).

- (3) The animal-microbe interaction studies deserve our attention with a view to find out new molecules for pharmaceutical and other industrial applications. Marine microbes have been identified as sources for newer biopolymers, surfactants, enzymes, antibiotics, anticancer compounds and Polyunsaturated Fatty Acids (PUFA).⁴² The animal-microbe interactions especially in deep-sea animal communities may yield new clues to our demands. So far no major programme has been initiated in the field of deep-sea microbiology. The major reason for low interest by researchers is due to lack of infrastructural facilities such as hyperbaric chambers for cultivation of these organisms, high pressure *in situ* sampling devices etc. in national laboratories.
- (4) The microflora of our seas offer great promise to develop novel highly flexible bioremediation methodologies to protect our deteriorating environment. Effluent treatment in any industry especially in those having harmful effluents is a serious problem. Millions of rupees are being invested to treat these effluents but with limited success. The future industries look for environment friendly technologies so that least damage is done to the environment

One success story in this direction is the isolation of a novel group of enzymes isolated from marine fungi for biobleaching, biopulping and decolourisation effluent in paper mills at the National Institute of Oceanography.³⁶ This has triggered the interests of many industries to look for novel organisms in the sea. In another nationally coordinated project financially supported by the Department of Biotechnology microbial consortia having nitrogen fixers, phosphate solubilizers and oil degraders were developed for combating oil pollution in the sea. So there is a need to initiate research programmes to harness the potential of marine microbes in this area.

- (5) Describing and understanding the identities, physiologies and behaviour of marine organisms are difficult because of the inaccessibility of much of the world's oceans. The development of new molecular tools and approaches, however, is enabling the study of marine organisms on an unprecedented scale in the recent times. Micro-organisms that cannot be cultured in the laboratory can now be characterised directly with molecular probes. The activities of microbial communities can be evaluated by screening for genes with specific functions or by analyzing for the products of gene expression. Such molecular approaches are rapidly leading to new understanding of marine processes and the role that micro-organisms play in maintaining a balance within the critical ecosystem. New methodologies including molecular biology techniques and instrumentation such as analytical flow cytometry and electron microscopy have to be used to assess various microbial processes in our waters. Only very recently DNA probes have been used to estimate the potential of microbial communities in nitrate reduction in the Arabian Sea. Similar molecular probes, especially the functional probes have to be developed for our needs locally.

Marine organisms can provide the basis for development of bio-sensors, bio-indicators and diagnostic devices for medicine, aquaculture and environmental monitoring. Bioremediation shows great promise for addressing problems in marine environments and in aquaculture. It appears as though we can find solutions in marine environment for each and every problem we face in this planet. If we go through the literature carefully, we find the challenges are growing steadily for which we may have to develop new strategies. The field is advancing so fast that we need additional manpower, advanced facilities and increased funding. India with its vast coast line and ever increasing population and its associated demands, should make use of this vast potential by proper planning, investment and management strategies.

REFERENCES

1. A. Huq, J. Hasan, L. Loomis and R.R. Colwell, (1994). *Ocean Technology* (Eds. Sushilkumar *et al*). Publication and Information Directorate, New Delhi, pp. 960.
2. T. Ramamurthy, R. Mohanraju and R. Natarajan, (1990). *Indian J. Mar. Sci.*, **19**, pp. 269.
3. R. Mohanraju, T. Ramamurthy and R. Natarajan, (1992). *Mahasagar*, **25**, pp. 111.
4. P.C. Kadam, D.R. Ranade, L. Mandelco and D.R. Boone, (1994). *Int. J. Syst. Bacteriol.*, **44**, pp. 603.
5. R.S. Oremland and D.R. Boone, (1994). *Int. J. Syst. Bacteriol.*, **44**, pp. 573.
6. S. Raghukumar, (1990). *Indian Acad. Sci. (Plant Sci.)*, **100**, pp.129.
7. S.T. Moss, (1986). *The Biology of Marine Fungi* (Ed. S T Moss) Cambridge University Press, Cambridge, pp. 105.
8. E.B. Jones, (Ed.), (1976). *Recent Advances in Aquatic Mycology*, Elek Sciences, London, pp. 1.
9. J. Kohlmeyer and E. Kohlmeyer, (Eds.), (1979). *Marine Mycology: The Higher Fungi*. Academic Press, New York, pp. 1.
10. J.H. Paul, S.C. Jiang and J.B. Rose, (1991). *Appl. Environ. Microbiol.*, **57**, pp. 2197.
11. C.A. Suttle, A.M. Chan and M.T. Cottrell, (1990). *Nature*, **347**, pp. 467.
12. J.B. Waterbury and F.W. Valois, (1993). *Appl. Environ. Microbiol.*, **59**, pp. 3093.
13. C.A. Suttle and A.M. Chan, (1994). *Appl. Environ. Microbiol.*, **60**, pp. 3167.
14. G.E. Schaiberger, T.D. Edmond and C.P. Gerba, (1982). *Water Res.*, **16**, pp. 1425.
15. G. Lewis, M.W. Loutit and F.J. Austin, (1986). *New Zealand J. Freshwat Res.*, **20**, pp. 431.
16. D.H. Taylor, A.R. Bellamy and A.T. Wilson, (1982). *Water Res.*, **14**, pp. 339.
17. K. Kamimura and M. Araki, (1989). *Appl. Environ. Microbiol.*, **55**, pp. 645.
18. D. Chandramohan, P.A. Loka Bharathi, Shanta Nair and S.G.P. Matondkar, (1987). *Geomicrobiol J.*, **5**, pp. 17.
19. T. Sakguchi, J.G. Burgess and T. Matsunaga, (1993). *Nature*, **365**, pp. 47.
20. P. Ramasamy, G.P. Brennan and P. Jayakumar, (1995). *Aquaculture*, **130**, pp. 129.
21. I. Karunasagar, G.R. Malathi and I. Karunasagar, (1994). *Aquaculture*, **128**, pp. 203.
22. C. Raghukumar and S. Raghukumar, (1991). *Mar Ecol.*, **12**, pp. 251.
23. N. Ramaiah and D. Chandramohan, (1993). *Mar Pollut Bull.*, **26**, pp. 190.
24. J.B. Waterbury and R. Turner, (1983). *Science*, **221**, pp. 1401.
25. J.M. Frincke and D.J. Faulkner, (1982). *J. Amer. chem. Soc.*, **104**, pp. 265.
26. P. Karuso, (1987). *Bioorganic Marine Chemistry*, Vol. **1** (Ed. P J Scheuer) Springer Verlag Berlin, pp. 45.
27. A.A. Stierle, J.H. Cardellina and F.L. Singleton, (1988). *Experientia*, **44**, pp.1021.
28. K. Kogure, H.K. Do and U. Simidu, (1989). *Proc First Intern Mar Biotech Conf Japan*, pp. 11.
29. Vanaja Kumar, (1979). *Studies on actinomycetes associated with molluscs from Portonovo coastal waters*, Ph.D. Thesis, Annamalai Univ. Annamalai nagar India.
30. C. Raghukumar, S. Raghukumar, A. Chinnaraj, D. Chandramohan, T.M. DeSouza and C.A. Reddy, (1994). *Bot Mar.*, **37**, pp. 515.

31. N. Selvakumar, (1979). *Studies on L-Asparaginase (anti-leukaemic agent) from coastal areas of Portonovo, S.India* (1979) Ph D Thesis Annamalai Univ. Annamalaiagar, India.
32. N. Sorkhoh, B. Al-Hasan, S. Radwan and T. Hoepner, (1992). *Nature*, **359**, pp. 109.
33. J.J. David, R. Gupta, C. Mohandas, S.Nair, P.A. Loka Bharathi and D. Chandramohan, (1995). *Proc Second Intern Oil Spill Res. and Development Forum* Vol.1, M.O.London, pp.14.
34. P. Rueter, R.Rabus, H. Wilkes, F. Aeckersberg, F.A. Rainey, H.N. Jannash and F. Widdel, (1994). *Nature*, **372**, pp. 455.
35. R. Rabus, R. Nordham, W. Ludwig and F. Widdel, (1993). *Appl. Environ. Microbiol.*, **59**, pp. 1444.
36. C. Raghukumar, D. Chandramohan, Michel Jr. and C.A. Reddy, (1996). *Biotech Lett.*, **18**, pp. 105.
37. P.H. Burkhill, (1987). *Cytometry* (Suppl. 1), pp. 643.
38. F.O. Gluckner, R. Amman, A. Alfreider, J. Pernthaler, R. Psenner, K. Trebesius and K. Schleifer, (1996). *Syst. Appl. Microbiol.*, **19**, pp. 403.
39. D. Chandramohan, (1997). *J. Mar. Biotechnol.*, **5**, pp. 73.
40. D.M. Karl and B.D. Tilbrook, (1994). *Nature*, **368**, pp. 732.
41. P.A. Loka Bharathi and D. Chandramohan, (1990). *Bull Mar Sci.*, **47**, pp. 622.
42. P. Bajpai and P. Bajpai, (1991). *J. Biotechnol.*, **30**, pp. 161.