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Editorial

Biotechnology is "the integration of natural sciences and engineering in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services".

Biotechnology, for more than a century, has acted as a vital buffer between people, pollution and the environment. The ever-increasing stresses we impose upon the world's ecosystems mean that this field is becoming ever more important. It may be that biotechnology will not only be able to help clean up the messes that we inflict on the Mother Earth, but also prevent them ever happening in the first place.

In the race for modern agriculture for increased yields the environment is methodically polluted with chemicals jeopardizing the very existence of the birds that form part of the natural habitation. Groundwater is tapped at greater depths in the earth leaving little scope for recharging with sporadic rainfall. The nature thus is forced to adopt some unusual counter measures to balance itself.

As the need is better appreciated to move towards less destructive patterns of economic activity, while maintaining improvement of social conditions in spite of increasing population, the role of biotechnology grows as a tool to make a major contribution to protect and remediate the environment. As we move into the next millennium this will become even more vitally important as populations, urbanisation and industrialisation continue to climb.

This bulletin on "Environmental Biotechnology" meticulously guest edited by Dr. S.F. D'Souza, Head, NA & BTD, BARC reviews the various areas of environmental biotechnology together with their related issues and implications. The overall aim is to provide balanced information and advance public debate.

G.A. Rama Rao

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From the Secretary's Desk

Dear Members,

I wish you a very happy new year.

We are just through with our Annual General Body meeting held at Amritsar during NUCAR-05. I would like to share with you some of the important developments and resolutions passed during the AGM pertaining to IANCAS Awards.

The age limit of Dr T. Datta Memorial Award has been increased to 45 years from the existing 35 years and will be valid from the year 2005. This was done to increase the level of competition through larger participation and number of applications. I may bring to your kind attention that many of the eligible candidates had a problem of choice i.e. whether to apply for Dr T.Datta Memorial Award or Dr H.J. Arnikar Best Thesis Award. Although the latter does not have age limit, the window period is only one year i.e. the candidate has to apply for the Award in the same year in which the degree has been obtained. This window period (for Dr H.J. Arnikar Best Thesis Award) has been increased to two years instead of the existing one year. This is expected to increase the number of applicants, and, also to take care of the year- to- year fluctuation in the quality of the award winning theses. A proper notification giving the details will be carried in our future issues as well as the web site.

Our web site (iancas.org) now carries the list of life-members. I would request all the members to visit the web site and suggest if any changes are to be made in their mailing addresses. This list is accessible to members only and for that you need to register to receive the user ID and password required to access the data base.

The current issue deals with environment and biotechnology. Biotechnology is rapidly replacing a number of technologies based on chemical synthesis to more eco-friendly, non-polluting green chemistry routes. We certainly foresee its usefulness in nuclear industry too. The current issue deals with some of these areas, and, I am sure, you will enjoy going through the articles. I take this opportunity to thank all the authors and the Guest Editor, Dr S.F. D'Souza, Head, Nuclear Agriculture & Biotechnology Division, BARC.

P.K. Pujari

Environmental Biotechnology

Guest Editor

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Focus

Dr. K.B. Sainis

A few weeks ago, I was in North Eastern Hill University, Shillong, on the occasion of the Silver Jubilee of their Biochemistry Department. While we were discussing the syllabus for the M.Sc. (Biochemistry) course, we were told that it is now compulsory to have a course or a paper on Environmental Biochemistry as per a Supreme Court order. This, in my opinion, is a clear indication of the ever increasing concern for environment which is being alarmingly threatened by incessant industrial activity, mounting pollution and other harmful human activities. This environment includes air, water, biota, as well as soil.

That the environmental pollutants must be reduced, nay, completely gotten rid of is agreed to by one and all. The focus of discussion and debate is on the methodologies or technologies to be adopted for the same. Biology has been heralded as the science of the 21st century given the astounding developments related to understanding of metabolic processes and their regulation, genetics and heredity, sequencing of human genome and those of several other organisms including pathogens, ability to modify the genetic constitution of microbes, animals and plants to confer specific properties etc. These developments, in turn, led to the recognition of the discipline of biotechnology which relies on harnessing the biological species and their constituents like enzymes and polysaccharides for a specific technological purpose, though it was being done for centuries. Environmental biotechnology relates to the use of biotechnological approaches to solve environmental problems. Such biotechnologies can be further distinguished into old and new types. The old ones include processes for portable water production, waste water and solid waste treatment, using microbes, natural pesticides etc. The newer technologies that have come to the fore are bio-pesticides, bio-leaching, bio-remediation, bio-sensors, bio-fuels etc.

Microbes have proved to be the handiest of tools when it comes to cleaning of environment. Different chemolithotrophs and anaerobes etc. have the capability of biotransforming the natural or synthetic waste. Photoautotrophs like cyanobacteria could be a good biofertilizers. There are also possibilities of converting waste into wealth as in biogas production. The ability of microbes to bind heavy metals and radionuclides has been demonstrated. Adsorption and uptake of heavy metals are properties which are also displayed by hairy root of some plants.

The ability to genetically modify microorganisms or plants to confer desired characteristics has provided the foundation of new or modern biotechnology. Accumulation of pollutants, in water bodies, atmosphere and soils etc. create a stressed environment which also prevails in industrial wastes or highly irrigated soils. Extremophilic organisms which can grow in such an environment and utilize the pollutants as nutrients are very useful for their remediation. The study of responses of microorganism to stresses such as osmotic, ionic, salinity, metal ions, organic solvents, pesticides and radiation would enable scientists to identify genes that control these responses. It is unlikely that such responses can be controlled by single genes. Therefore, approaches may be required to pyramid these genes in the transformants to be used in such extreme conditions. These would enhance the efficiency of microorganisms to handle complex waste and higher volumes. The approach has to be two pronged – the end of the pipe approach may help cleaning up the waste after it has been

created while the front of the pipe approach would employ technologies that would, in the first place, generate lesser pollutants or wastes.

Synthetic chemical pesticides were introduced more than 40 years ago. They appeared to yield miraculous results. Soon, the problems of hazard to human health surfaced. Worse still, some of the pests started developing resistance. Genetic engineering came to rescue with the development of Bacillus thuringiensis endotoxin based bio-pesticides. After its success in the control of pests of cotton, this gene may find application in transformation of soil microbes to control soil dwelling insects.

One of the major requirements for monitoring of environmental pollution is the availability of rapid, sensitive and inexpensive techniques. Enzyme linked immunosorbent assays (ELISA- dipstick methods) and bio-sensors are the recent biotechnological methods which would see larger application.

Our task includes restoration of depleting resources and conservation of existing resources of water, soil quality, fossil fuels etc. The "Nisargaruna" approach represents a slightly modified bioprocessing of kitchen and other biodegradable waste to generate methane as well as a very useful soil conditioner. Bio-diesel – primarily consisting of vegetable oils has proved to be an economic alternative which also has tremendous possibilities of augmenting rural income and means of livelihood for rural poor.

Chemotaxis and adhesion to a substratum are conducive to formation of biofilms. Such biofilms of both anaerobic and aerobic microbes have a tremendous potential in sewage and waste water treatment.

Bio-leaching or bio-mining of certain metals e.g. uranium would be of considerable interest to those in DAE. But the economic viability and practicability have to be satisfactorily demonstrated. Likewise, precipitation of heavy metals including radionuclides using genetically altered organisms or biosorption techniques may open new vistas in bio-remediation. Here too, a genetic engineering approach using a highly radioresistant microbe like Deinococcus radiodurans may prove very promising.

There will be many more applications of biotechnology in environmental protection and clean-up. This issue of IANCAS gives a glimpse of some of the biotechnologies which have either been assessed for last several decades or are on the anvil and are useful not only to address more common problems of solid waste management but also to industry specific issues of heavy metal mining and polution or radionuclide bio-remediation.

Guest Editorial

Dr. S.F. D'Souza

The awareness to pollution generating effects of human activities has increased dramatically during the past 2-3 decades. In response man has strived to minimize the damaging effects of his activities on the environment by developing technologies to clean up the pollution generated by other technologies and/or production technologies, which are cleaner and generate less pollution. Biotechnology is rapidly replacing a number of technologies based on chemical synthesis to more ecofriendly, non-polluting green chemistry routes. In addition to development of ecofriendly processing aids, biotechnology has given the possibility of producing a large number of ecofriendly products such as biofuels, biofertlisers, biopesticides, biosurfactants, bioplastics/biopolymers, and a host of other additives of biological origin which would considerably reduce the chemical stress on the environment and the living beings. Biotechnological approaches will also gain importance in various processes such as bioleaching of ores, bioremediation of environmental pollutants, production of plant secondary metabolites in bioreactor without their ecological destruction etc

Microorganisms excel at using organic substances natural or synthetic as source of nutrient and energy thereby playing a fundamental role in global recycling of matter. In turn such technologies have often helped in the conversion of a waste into wealth as in the case of biogas production. Microbes also have an affinity to bind heavy metals and radionuclides. These characteristics have been exploited in using them in the remediation of environmental pollutants. Scientists are looking for micro-organisms in hostile natural environments (for example very hot, cold, radioactive or oily places) to use the talents they have developed for industrial uses. Plants inherent capability to take up organic and inorganic nutrients can be exploited for remediation of sites contaminated with heavy metals, radionuclides and toxic organics. One of the important aspects in environmental pollution control is the real time monitoring of contaminants. In this direction highly specific biosensors based on biological recognition elements will gain importance. Many of the biotechnology applications described above can be varied or refined using genetic engineering.

The present issue addresses some of these topics. I hope that the readers will find the contents interesting and informative.

Biomining - A Promising Approach

Dr. A.K.Mathur *is presently working as an Incharge, Mineral Technology Laboratory, Atomic Minerals Directorate for Exploration and Research, Department of Atomic Energy, Hyderabad. He is a microbiologist by profession and is associated with isolation and identification of bacteria and bacterial leaching of uraniferrous ores from different parts of the country. He has also carried out studies on bioremediation of uranium and radium using different species of microorganisms from uranium effluents. He was Chief Research Investigator for a project from International Atomic Energy Agency (IAEA), Vienna, on "Bioremidiation of* Ra^{226} *from Mill Effluents". He was also associated in field tests on bacterial leaching studies on lean sulphide ores and plants tailings for recovery of copper at Malanjkhand, Balaghat district, Madhya Pradesh in association with scientists of Hindustan Copper Limited and pilot scale tests on the recovery of uranium at Domiasiat mine site, Meghalaya.*

Introduction

The enthusiasm of the microbiologists working on the development of the "biomining" techniques is matched by a need in the minerals industry to find alternatives to conventional methods of mining, ore processing and waste water treatments. The need arises from recent trends in the industry due to:

(a) Continued depletion of high grade mineral resources

(b) The resulting tendency for mining to be extended deeper underground

(c) The growing awareness of environmental problems

(d) Burning of sulphur rich fossil fuels

(e) The rising cost of high amount of energy required in the conventional recovery methods

Now a days bioleaching occupies an increasingly important place among the available mining technologies. It is a promising technology with an economical alternative for treating specific mineral ores. A number of large-scale bioleaching operations are located in developing countries which have significant mineral reserves and mining constitutes one of their main sources of income. Another advantage of adopting this technology may be of its simplicity and low capital cost requirements.

History

Hydrometallurgical extraction (leaching) of copper from ore and its precipitation from the resultant solutions with metallic iron (cementation process) is an ancient technology. The Chinese practiced a form of this technology as far back as 100 – 200 BC. Copper was important to ancients as a metal and as an ingredient of bronze, a copper alloy. Historical records indicate that copper ore leaching and cementation were also known in Europe and Asia, at least as far back as the second century. The technology was probably known to these civilizations much earlier whether from China is not known. As we now realize, leaching was probably the only way that the ancients used to extract copper from sulphide ores because smelting in those days was run in open hearth and was effective only with CuO and carbonates.

The practice of copper leaching and cementation was refined through the centuries and has continued to the present day. The Moors during their conquest of Spain appear to have instituted heap leaching at the Rio Tinto mines. By 1752, the Spanish had developed a process of copper leaching from partially roasted ore at Rio Tinto. In 1951 Colmer, Temple and Hinkle reported the involvement of bacteria in the oxidation of pyrite

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inclusions of bituminous coal and identified this bacteria as *Thiobacillus ferrooxidans*. Later L.C.Bryner, J.V.Beck and their students at Brigham Young university in Provo, Utah found the same bacteria, *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* in copper mine drainage from Kennecott's open pit mine in Bingham Canyon, Utah. M.P.Silverman and D.G.Lundgren in 1959 isolated this organism in pure form and cultured in laboratory on the artificial medium described as 9K by them. Demonstration of bioleaching of some other metal sulfides like MoS, ZnS, NiS and PbS followed later.

In the early 1960s uranium mine operators found that the mine waters were acidic in nature and also contained soluble uranium. *Thiobacillus ferrooxidans* was also found to be present in these mine waters. This confirmed the active catalytic role of this iron oxidiser in solubilisation of uranium from the ores. Bacterial leaching has earlier been commercially employed only for recovering copper and uranium. But now, this can also be considered for extraction of other metals sulphide ores at commercial level.

Since early 1960's, Denison Mines Ltd., Elliot Lake, Ontario with the support from CANMET (Canada Center for Mineral & Energy Technology) started leaching to extract uranium from its pyritic quartzites and quartz pebble conglomerates. The company applied bacterial leaching on low grade ores. A lot of study on morphology and physiology of *Thiobacillus ferrooxidans* has been carried out over the years and different strains have been identified. Other bacterial species of Lepto spirillum and thermophilic *Sulpholobus* sp. have also been studied and used for leaching of metals from sulphide ores.

Morphology and Nutrient Requirements of Thiobacillus sp.

The principal bacteria which play the most important role in solubilising sulfide minerals at moderate temperatures are species of the genus *Thiobacillus*. These are gram negative rods with rounded ends either polarly or non-flagellated. Most species are mesophilic, acid tolerant and acidophilic. Some grow best at pH 2 and may grow even at 0.5 pH. *Thiobacillus* are chemolithoautotrophs, which means that carbon dioxide is the only source of carbon and they derive their energy from chemical transformation of inorganic matter or ferrous iron available in the form of pyrite in ores. All *Thiobacilli* oxidize sulfur or sulfur compounds to sulphate or sulphuric acid.

Thiobacillus ferrooxidans

These are 0.8 to 1.2 μm in length and 0.4 μm in width, motile by polar flagellum, gram negative, chemoautotrophic and on agar with ferrous iron, forms colonies encrustated with ferric hydroxide. It can be cultured on 9K media (Silverman and Lundgren, 1959) in laboratory.

Composition of 9K medium (grams per litre) for culturing of Thiobacillus ferrooxidans

$(NH_4)_2SO_4$ – 3.0, KCl – 0.1, K_2HPO_4 – 0.5, $MgSO_4$ – 0.5, $Ca(NO_3)_2$ – 0.01 and $FeSO_4$ – 44.22

(a) $FeSO_4$ is dissolved in 300ml of distilled water and filtered through bacterial filter.

(b) The remaining salts are dissolved in 700 ml of distilled water and autoclaved separately for sterilization.

Both the solutions(a&b) are mixed and pH is adjusted to 3.5 with 0.1N H_2SO_4 solution.

Mechanism of metal sulphide leaching

Thiobacillus ferrooxidans can oxidise hydrogen sulphide, thiosulphate, polythionates or elemental sulphur. They produce hydrogen ions thereby lowering the pH of medium, often below pH 2, in some cases even below pH 1.

Chemical reactions during leaching

$$HS^- + 2O_2 \rightarrow SO_4^= + H^+ \quad (1)$$

$$S^0 + H_2O + ½\ O_2 \rightarrow SO_4^= + 2H^+ \quad (2)$$

In addition to the oxidation of sulfur and sulphur compounds *Thiobacillus ferrooxidans* is able to oxidise ferrous to ferric ion and so derive its energy from this exergonic reaction.

$$2Fe^{++} + 2H^+ + ½\ O_2 \rightarrow 2Fe^{+++} + H_2O \quad (3)$$

$$2Fe^{+++} + 6H_2O \rightarrow 2Fe(OH)_3 + 6H^+ \quad (4)$$

$$2Fe^{++} + 5H_2O + ½\ O_2 \rightarrow 2Fe(OH)_3 + 4H^+ \quad (5)$$

Owing to its ability to oxidise ferrous ion to ferric ion(ferric sulphate), which is the principal agent of ore leaching at moderate temperature.

$$FeS_2 + H_2O + 3½\ O_2 \rightarrow Fe^{++} + 2SO_4^{--} + 2H^+ \quad (6)$$

$$^*MS + 2O_2 \rightarrow M^{++} + SO_4^= \quad (7)$$

- Metal sulphide
- Metal

Oxidation of Nonferrous minerals to sulphates

(a) Nickle sulphide (Millerite)

$$NiS + 2H^+ + 5O_2 \rightarrow Ni^{++} + SO_4^{--} + H_2O \quad (8)$$

(b) Zinc sulphide (Sphalerite)

$$ZnS + 2.5\ O_2 + 2H^+ \rightarrow Zn^{++} + SO_4^{--} + H_2O \quad (9)$$

(c) Cupric sulphide (Chalcocite)

$$Cu_2S + 2.5O_2 + 2H^+ \rightarrow 2Cu^{++} + SO_4^{--} + H_2O \quad (10)$$

(d) Pyrite and chalcopyrite

$$FeS_2 + 3.5O_2 + H_2O \rightarrow FeSO_4 + H_2SO_4$$

$$FeSO_4 + 0.5O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O \quad (11)$$

$$CuFeS_2 + 2Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5FeSO_4 + 2S^0 \quad (12)$$

$$2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \quad (13)$$

(e) Uraninite

$$UO_2 + 2Fe_2(SO_4)_3 + 2H_2SO_4 \rightarrow H_4[UO_2(SO_4)_3] + FeSO_4 \quad (14)$$

During these oxidation reactions, sulphuric acid and ferrous sulphate are regenerated. Sulphuric acid generated maintains the acidic pH of the leaching solution. Further addition of sulphuric acid is avoided. Ferrous sulphate is required as the energy source for growth of *Thiobacillus ferrooxidans.*

Industrial Applications

Bacterial leaching of ores has been applied on a large scale for many years almost solely to leach copper and uranium ores. In the past few years this has been carried out in many countries like Canada, USA, Mexico, Australia, India, USSR, Turkey, Yugoslavia, Romania, Hungary, Spain and some other countries.

Dump/ Heap Leaching

The most common applied method is based on percolation of leach solutions on the big ore dumps set up on impermeable ground usually valleys. The ore size has to be such that leach liquor could percolate through the heap or dump and air may enter from the sides (Figs. 1 & 2).

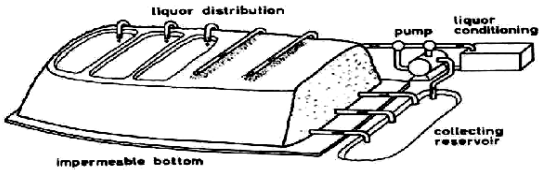

Fig. 1 Dump ore leaching

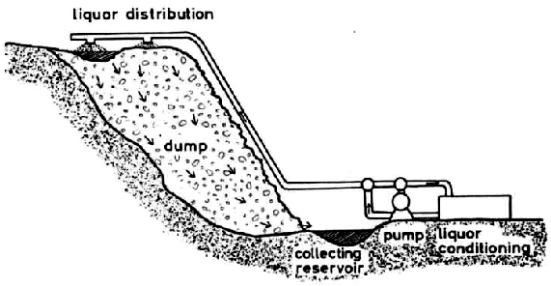

Fig. 2 Dump ore leaching on a slope

The leach liquor is sprayed on the top of the dumps/heaps by sprinklers or flooding of ponds. At the bottom liquor is collected in a reservoir from which it is pumped back on top of the dump after adjusting pH and nutrients in the liquor. The height of the dumps may range from 20 m to 200m and they may contain up to 10^9t of ore. The metals can be recovered from leach liquor using cementation or ion exchange process and the barren solution can be recycled. Copper industry has made extensive use of heap and dump leaching and produced about 25% of copper production by this source. In USA 200,000 to 250, 000 t of copper was produced annually by bacterial leaching.

Australian mines such as Giralambone and Nifty have operated extensive heap leach operations from chalcocite ores. Minera Pudahuel for the Lo Aguirre property in Chile developed thin layer leaching technique by adding sulphide ores to oxide ore dumps and is continuing the production of copper on large scale since 1988.

In the uranium industry there were several applications of biologically mediated heap leaching. At Dension Mines in Elliot lake, Ontario, an under ground heap leaching method was successfully developed, eventually contributing almost a quarter of mine production. Uranium was leached by acidic ferric solution produced by bacterial oxidation of pyrite present in the ore. The leach cycle took approximately 18 months to reach 70% recovery. About 800,000 kg of uranium alone was produced during 1987 and 1988.

Gold heap and dump leaching s are generally built on oxidized ore containing little or no sulphide mineralization. The majority of the bioleach work in the gold industry, in contrast to copper and uranium has been in tank bioleach of concentrates (Tank leaching).

Insitu Leaching

In this technique the ore is not moved from its geological setting with the advantage that excavating costs can be saved. But difficulties arise if the ore body is impermeable or if there are only a few channels through which the leach liquor would pass without percolating through the whole ore body. In such cases the ore body has to be cracked by explosions, because it is necessary to collect the leach liquor after it has passed through the ore body. Unsuitable siting may lead to escape of large quantities of leach solution under ground which may pollute the ground water.

Insitu leaching was developed in 1960 in the former Soviet Union as well as in central and eastern Europe. Commercial insitu leach uranium mining in U.S.A began in mid 1970. The first commercial insitu leaching was upgraded at Utah construction and mining corporation at their Shirley Basin mine at Wyoming (Figs. 3 & 4). Both acid and alkaline leach had been adopted. Other countries which have

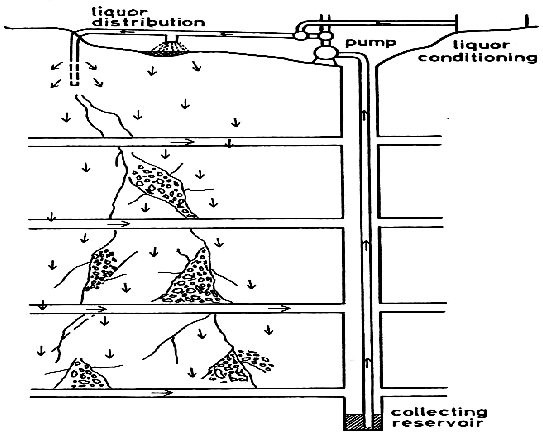

Fig. 3 In situ leaching in a mine

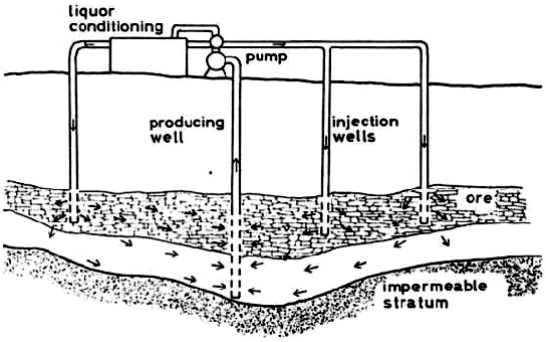

Fig. 4 In situ ore leaching from injection wells to producing wells

adopted this technique are Germany, Bulgaria, Ukraine, Uzbekistan and China.

The leaching solution (acid or alkali) containing oxidants that can mobilise uranium are injected into the ore zone below the water table. The leaching solutions are pumped up to the surface from production well where the uranium is recovered by ion-exchange method and further processed.

Thiobacillus ferrooxidans culture can be mixed with the acidic solutions in place of chemical oxidants to enhance the oxidation rates of the reactions which could be a useful tool for enhancing the uranium leachability. Experimental studies need to be carried out at laboratory stage before applying on industrial scale.

New Developments and Technology Suppliers

Tank Leaching

While biological heap leaching has been practiced for a long time, agitated tank bioleaching is more recent and largely been restricted to gold ores. Biox ® process from Gold fields, GFL, Mining services at their Fairview plant in South Africa was started in 1986, its first commercial scale operation to process 10 tonnes per day of arsenopyrite concentrate. Subsequently it was scaled upto 40 tpd and 1000 tpd (Ashanti Gold fields Sansu project in Ghana, 1995).

Other companies like Bach Tech Environmental (Australia) now based in Toranto (Canada) and Mintek (South Africa) have formed a joint venture to market this technology. The only commercial application of tank bioleach process in the North American Gold industry is at Tonkin springs in Neveda, U.S.A.

New Developments

Biotechnology in mining is now going for a big leap forwards by using new bacterial cultures, mixed cultures of thermophiles which are active at more than 50^0C in slurries in agitated tanks. Early laboratory testing of processes using extreme thermophiles have indicated much higher recoveries and significant development work is underway at three seperate pilot operations to demonstrate three methods of thermophile leaching. Billiton Mineral Technology have published some positive results of their programme while treating copper and copper-nickle sulphides. Recoveries of about 94 to 99% have been achieved. Bachtech – Mintek companies have formed joint venture with Industries Penoles in Mexico to operate a pilot plant for their proprietary thermophile leaching technology. The third major development in the field is Pacific Ore Technology's Bioheap process is currently undergoing production-scale testing at Titan Resources's Radio Hill Operation in Australia.

The current panorama of bioleaching in developing countries is encouraging. In the coming years several new commercial – size bioleaching plants are expected to be installed.

Heap leaching will continue to be the choice of low grade ores and tailings specially treating low grade uranium deposits of India and tailings from mill, while tank bioleaching technology would be useful for leaching gold, copper and other base metal ores. Developing countries should increase their efforts in research and development in bioleaching technology as they have comparative advantage over conventional leaching technology.

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Biofilms

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Introduction

In most environments micro-organisms exist predominantly as multi-cellular surface attached communities called biofilms [1]. Biofilms invariably develop on all solid surfaces exposed to aquatic environments [2]. They are ubiquitous and perform important environmental functions such as primary productivity and nutrient cycling [3]. Biofilms mode of growth is the most favorable way of living for bacteria and it has been estimated that >90% of earths microbes live in biofilms [1]. Biofilms are composed of microorganisms (Fig. 1) such as bacteria, diatoms, cyanobacteria, fungi, microalgae, filamentous forms, protozoa and their excretory products such as extracellular polymeric substances (EPS). The biofilms are defined "as a consortium of biotic elements like bacteria, cyanobacteria and algae attached to a substratum by microbially produced extracellular polysaccharide matrix which entraps soluble and particulate matter, immobilizes extracellular enzymes and acts as a sink for nutrients and inorganic elements" [2].

Depending on the situation in which they are growing, biofilms can be beneficial, or detrimental. The economic importance of biofilms is very well registered as they affect a variety of materials, which

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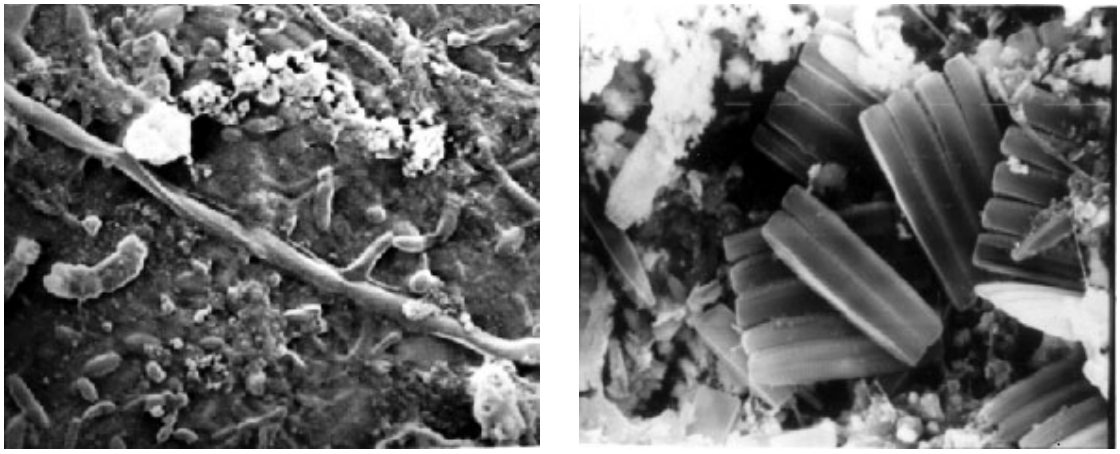

Fig. 1 SEM pictures (X 1725) showing a mix consortium of micro -organisms, diatoms and filamentous forms in a freshwater biofilm

includes, drinking water distribution lines, dairy pipelines, and power plant condenser tubes. They are used in remediation of pollutants and contaminants for environmental restoration as well as in sewage treatment plants. Biocatalysis of industrial processes, production of life saving drugs and biometallurgy and bioleaching are the other areas of importance where biofilms have a significant role [4]. Biofilms are also important in view of fouling and microbial corrosion, when the natural waters are used as source water for industrial cooling. Industrially, biofilms cause problems in cooling circuits, by reducing heat transfer efficiency which result in significant pressure drop in the condenser and impairing the performance of associated ancillary systems [5]. Biofilms are also implicated in several diseases such as cystic fibrosis and shunt infections.

Biofilm Formation

Biofilm formation starts whenever a clean surface is exposed to aquatic medium. Initially, an organic conditioning film is formed, which is influenced by ambient environment and properties of the substratum [6]. This layer of chemical concoction attracts the microbes by chemotaxis and helps in the initial settlement. During the adhesion process bacterial cells undergo phenotypic changes in response to the proximity of a surface. In the course of biofilm formation, sessile bacteria form microcolonies along with the copious exopolymeric matrix. The paradigm of biofilm development on a clean surface can be outlined in the following steps and schematically illustrated in Fig. 2 [7].

STEP I - REVERSIBLE ADHESION: This important step in biofilm formation begins with approach of microorganisms to the conditioned surface. This process may be active or passive, depending on whether the bacteria are motile or transported by the surrounding aqueous phase. During early phases of reversible cell adhesion, physical forces play a major role and therefore, physico-chemical properties of the substratum as well as cell surface are important criteria, which determine the adhesion rate. Desorption may occur during this stage as a result of the release of reversibly adsorbed cells due to fluid shear forces.

STEP II - IRREVERSIBLE ADHESION: This is the critical step in biofilm development. Bacterial surface appendages such as flagella, fimbrae and exopolysaccharide fibrils play important role in this process. Polymeric fibrils form a bridge between the bacterium and the surface, thereby irreversibly reinforcing the association. During this period, the attached cells begin to secrete additional polymers (extracellular polymeric substances [EPS]) which further cement the cells to the surface and stabilise the colony against fluctuations in the surrounding macro-environment.

STEP III - BIOFILM MATURATION: The growth of microbes on the surface leads to the formation of

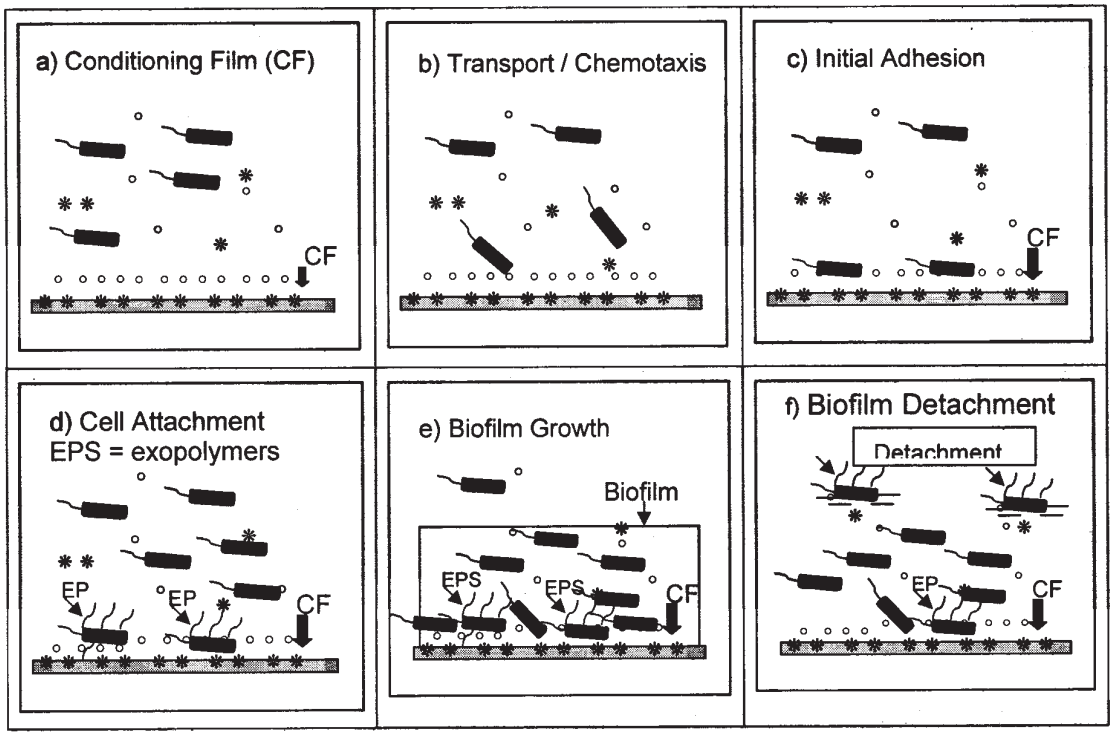

Fig. 2 Schematic illustration of sequence of events in biofilm formation.

microcolonies. Further development of the biofilm involves microbial cell-cell interactions between the primary biofilm formers and other microorganisms possessing different nutritional requirements. Biofilm could be considered as a "quasi tissue" with measurable rates of respiration and nutrient uptake [1]. Eventually, the biofilm reaches a plateau phase with a certain thickness and metabolic capacity. It is generally accepted that in natural environments, bacteria lead the adhesion on the new surfaces, which is then followed by diatoms, cyanobacteria, microalgae and other higher organisms [2]. In natural environment, this leads to the process of macrofouling, a common problem described especially in marine systems.

STEP IV - BIOFILM DETACHMENT AND DISPERSAL: In order to achieve propagation, the biofilm microorganisms detach, disperse and colonise new niches. Microbial growth in biofilms also brings with it the need to disperse and propagate [1]. Biofilm accumulation increases surface roughness and also provides shelter from shear forces and increases both the surface area and convective mass transport near the surface.

Biofilm formation is influenced by several factors like temperature, pH, ionic concentration, and flow of the bulk fluid, wettability of the substratum, hydrophobicity and surface charge of the bacterial cells. Biological factors such as inoculum age (in laboratory studies), physiological status, motility, nutrient conditions, type of surface proteins and extracellular polymeric secretions, compound the effect on biofilm development [4].

Biofilm Architecture

Biofilms are not a simple assemblage of microorganisms. A mature biofilm has a typical three-dimensional architecture. Recent advances in microscopy such as confocal laser scanning microscopy (CSLM) and applications of microelectrodes have brought in considerable insight in biofilm research. The latest paradigm depicts that the microcolony based growth leads to tall mushroom shaped structures formed by variety

of microbes embedded in EPS along with extensive water channels spanning from biofilm / water interface to the substratum. These channels and the fluid flow through them are analogue to primitive vascular system, which provides oxygen as well as nutrients to all sections of biofilm [8].

The spatial organisation of microbial cells in the biofilm is also subject to variations. The growth characteristics and metabolic activities of biofilm bring in structural heterogeneity. The flow rate of fluid affects the packing of cells in biofilms. Shear forces of water flow and expression of genes for production of enzymes like alginate lyase lead to controlled release of cell for colonisation of new surfaces. Biofilms shows very coordinated behavior. The architecture provides biofilm the well known resistance to biocides and antibiotics [7].

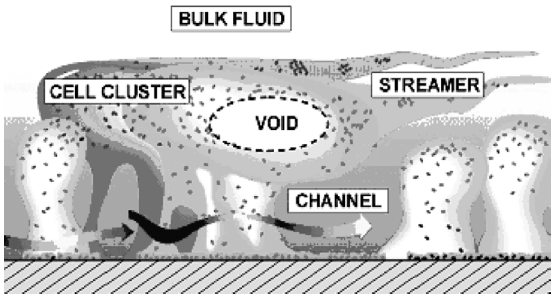

Fig. 3 Schematic of CSLM image of a biofilm

Biofilm Matrix (Extracellular Polymeric Substances)

Most of the microorganisms that colonise various surfaces produce polymers and form a gel matrix on the surface. The exopolymers have numerous effects on interfacial processes, like immobilising water at the biofilm substratum interface, trapping of metal species and corrosion products at the interface, and decreasing diffusion. Some of the exopolymers which are polyelectrolytes in nature bind metal ions and hence promote corrosion. The exopolymer gel is not a passive matrix but exhibits physical, chemical, and electrical responses to environmental stimuli. Biofilm matrix is a complex mixture of many components. Table 1 gives a typical composition of EPS matrix [9].

Table 1

Component	% of matrix
Water	Up to 95%
Microbial Cells	2-5% (Many species)
Polysaccharides (homo and hetero)	1-2% (Neutral and polyanionic)
Proteins (extracellular and from lysis)	<1-2% (Many, including enzymes)
DNA and RNA	<1-2% (From lysed cells)
Ions	bound and free

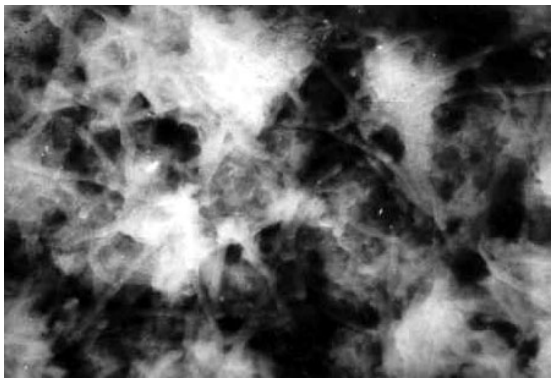

Fig. 4 SEM picture (X 1725) showing exopolymer matrix of Pseudomonas sp biofilm

EPS vary greatly in their composition and hence in their physical and chemical properties. Majority of them are polyanionic because of the presence of uronic acids and pyruate ketals, although few of them may be polycationic or neutral [9]. They contain functional groups that bind metal ions from the aqueous phase Bacterial EPS are acetylated and these groups strongly inhibit the EPS-cation (metal ions) interactions. EPS is essential for irreversible attachment of bacteria [7].

Proteins are very important part of the EPS matrix; they interact with EPS directly or may act as enzymes. Enzymes play important role in development and dispersion of biofilm bacteria. Glycosyltransferases in oral biofilms can synthesize levans and dextrans. Esterases can remove the acetyl group from the EPS, thereby changing matrix

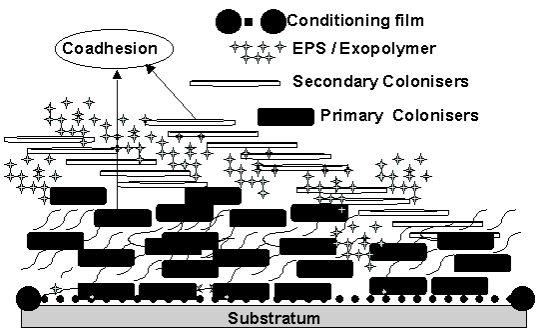

Fig. 5 Schematic illustration of Coaggregation among two bacterial types

structures. Autolysins can lyse specific cells in the biofilms [9]. EPS lyases detach the cells from the surface and help in dispersion. Nucleic acids in the biofilm matrix are presumed to be derived from cell lysis, although some bacteria, e.g. Pseudomonas aeruginosa has been known to produce substantial amounts of extracellular DNA. Extracellular DNA has been found to be important in the initial phase of biofilm development [10].

Role of Co-aggregation

The first organisms to attach on a new surface are called primary colonizers or primary biofilm formers. They attach to the surface by specific or non-specific adhesion mediated by physico-chemical interaction with the conditioning film present on substratum. The attachment of secondary colonizers is believed to be through co-aggregation in two ways. First is the direct specific attachment of secondary colonizers from the bulk and second is the prior coaggregation of cells of different genera in bulk followed by the attachment of the complex to the biofilm. This attachment is called co-adhesion (a phenomenon by which two genetically different bacteria get attached together). The co-adhered communities then grow to make complex multispecies biofilms. In dental plaques Streptococcus plays the role of primary colonizer and in freshwater biofilms this role is believed to be played by Micrococcus sp. Co-aggregation was first reported in dental plaque biofilms. It was found to be mediated though a group of proteins called adhesins on one cell and relevant saccharide receptors on other cells. Now this phenomena is well established in biofilms in many other environments [11].

Quorum Sensing in Biofilms

Intercellular signaling in bacteria has been well established. Several groups of molecules are found to be involved in this process viz amino acids, cAMP, short peptides, cyclic dipeptides (CDPs), butanolides, fatty acid derivatives and acylated homoserine lactones (AHLs) [12]. Recently, a novel type of universal autoinducer has been isolated which contains boron [13]. Signaling molecules are involved in many phenotypic expressions like swarming, EPS production, exoproteases, catalase and superoxide dimutase, bioluminescence, pigment production, biofilm differentiation and virulence factor production [12]. Gram positive bacteria mostly use short peptides for quorum sensing. Signal

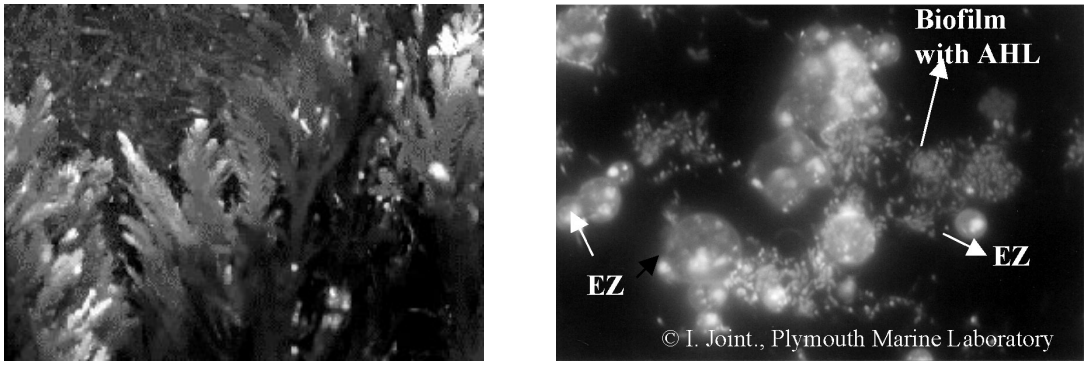

Fig. 6 (a) Benthic algal species Delisea pulchra, which inhibits biofilm formation, (b) Enteromorpha zoospores (EZ) colonizing surfaces with biofilms releasing AHLs signals (image produced with permission).

processing either involves a two component system where signal transduction takes place across the cytoplasmic membrane or the signal molecule is internalized. AHLs have been studied as gram negative quorum sensing molecules. Pseudomonas and Vibrio sp. were the role models. Variation in acyl chain on the basic homoserine lactone generates four groups of molecules; group 1 has unsubstituted acyl chain, group 2 has hydroxyl group at 3' position, group 3 has oxygen with ester linkage at 3' position and group 4 has an unsaturated bond in acyl chain [12]. Although most of these molecules are freely diffusible, some, like N-(3-oxododecanoyl) homoserine lactone, are actively taken inside the cell. These molecules are to be involved in interaction of bacteria with higher organisms. Higher organisms respond to these molecules in different ways, like furanones are antagonist of AHLs produced by a benthic marine alga Delisea pulchra which prevent biofilm formation on its leaves. Enteromorpha zoospores sense AHLs signals and use them as cues to attach to the surface as the AHLs produced by stable and old biofilms are indicator of stability of surface [14].

Biofilms and Environmental Biotechnology

Of late biofilm technology is showing tremendous potential in biotechnological applications. Biofilms have contributed immensely in environmental biotechnology. The activated sludge process and trickling filters are typical examples. Both aerobic and anaerobic biofilms have been used either on carrier materials or in carrier less form for treatment of waste water, particularly in sewage treatment. Bioreactors are designed and operated to promote biofilm growth for effective treatment of environmental wastes such as sewage, industrial effluents, waste gases and contaminated groundwater. Several types of reactors are used in treatment processes, like rotating biological reactors, fluidized bed reactors or fixed bed reactors in aerobic conditions and packed bed reactors, downflow stationary fixed film reactors in anaerobic conditions. These reactors are operated either in continuous or semi-continuous or batch or sequential batch mode as per the requirement of the system [15].

Recently, aerobic microbial granules have attracted tremendous research interest in environmental biotechnology particularly the treatment of both industrial and municipal wastewater. Various studies highlight the importance of biodegradation and biosorption capabilities of aerobic granules for treating organic or metal or mixed waste in laboratory scale. However, for better application of these granules (Fig. 7) in remediation technology, the nature and type of microorganisms and the mechanism of granulation need to be understood. Bioenhancement of aerobic granules, enriching the mixed culture granules with desired catabolic activity is quite promising area of research [16]. Natural or augmented biofilms attached to the soil particles help in degrading recalcitrant contaminants in soil, released accidentally or otherwise. Genetically engineered strains are used for developing biofilms along with the natural microbial population of contaminated site in controlled environment. This process helps in transferring the required genes by horizontal gene transfer (Fig. 8) to the native population, which in turn enhances the bioremediation capabilities [17].

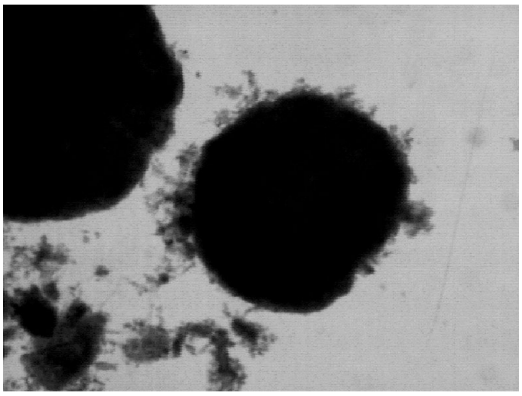

Fig. 7 Aerobic granule in sequential batch reactor for degradation of organics (unpublished data)

In nature, biofilms attached to the plant roots of some crops help cycle nutrients to and from the plant, resulting in increased agricultural productivity. Biofilms can also be used to produce a wide variety of biochemicals that are then purified and used, including medicines, food additives, or chemical additives for cleaning products.

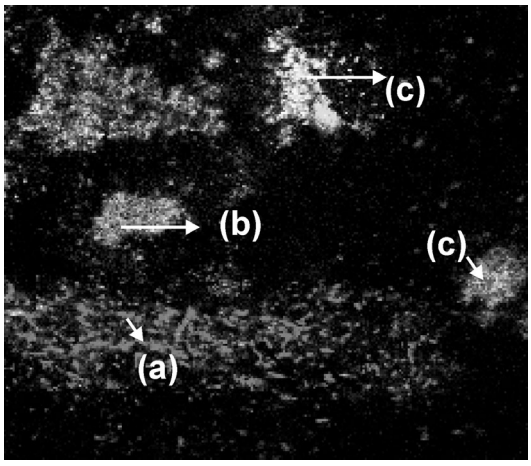

Fig. 8 Confocal image showing plasmid-mediated gene transfer in a biofilm. Pseudomonas putida (a = red), carrying a DsRed-encoded pJP4 plasmid transferred the plasmid to a gfp-expressing recipient bacterium (Pseudomonas fluorescence, b = green). The resultant transconjugants appear yellow (c) due to co-localisation of the green and red fluorescent proteins.

Industrial and mining activities have led to large-scale contamination of our environment with toxic heavy metals and radionuclides. Although chemical approaches are available for metal remediation, they are expensive to apply and lack the specificity required to treat target metals against a background of competing ions. Such approaches are not cost-effective remediation technologies for large-scale subsurface contamination. The bioremediation route, which is environment friendly, is emerging as one of the several alternative technologies for removing pollutants from the environment, restoring contaminated sites and preventing further pollution. Bioremediation is now a technically viable remedial option based on good science and engineering. Successful, cost-effective bioremediation programs are dependent on several factors which include contaminant signature, sensor development, microbial ecology, and other factors.

Survey for bacterial and extremophile populations that are capable of surviving in hazardous waste contaminated environments resulted in the discovery of various bacterial species

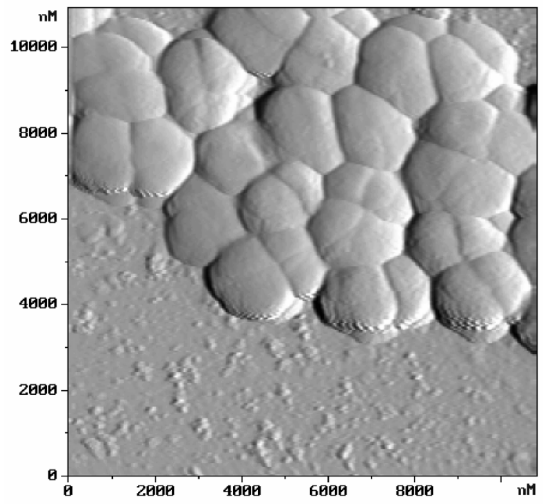

Fig. 9 AFM image of Deinococcus biofilm on metal surface

having promise in bioremediation application. Deinococcus radiodurans has remarkable resistance to ionizing radiation, this bacterium is being developed using genetic approaches for the treatment of both metals and organic contaminants. Recent studies have also shown that D. radiodurans is able to reduce Cr(VI) directly, and U(VI) and Tc(VII) using the electron shuttle anthraquinone-2,6-disulfonate. The mercury detoxifying gene as already been incorporated in D. radiodurans and field trials are being carried out successfully. Fig. 9 is an atomic force microscopy image of Deinococcus radiodurans biofilm formed on silicon wafer. D. radiodurans biofilms have immense potential in surface decontamination. Demonstration of radionuclide solid phase capture in response to microbial induced mineral precipitation, suggests that considerable potential exists to adapt this novel biotechnological concept to the development of new clean-up strategies for aquatic and terrestrial sites [18].

Significant advances have been made in understanding the roles of microorganisms in biofilms, and in the application of biofilm processes for the remediation of metals and radionuclides. Complete genome sequences for several environmentally relevant microorganisms, will surely prove useful for determining the precise

mechanisms of environmentally relevant metal–microbe interactions. Genomic approaches are set to revolutionise many aspects of biology and will undoubtedly make an impact in the arena of environmental biotechnology.

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Phytoremediation

Dr. Susan Eapen *joined BARC in 1972 after completing the 15th Batch training school in Biology and Radio Biology. Since then, she has carried out extensive work on improvement of crop plants using biotechnological approaches and has developed the first transgenic pulse crop. She has also developed genetically transformed hairy roots from hyperaccumulator plants and used them for rhizofiltration of radionuclides and heavy metals. Currently she is heading Plant Biotechnology and Secondary products Section in Nuclear Agriculture & Biotechnology Division. She has about 100 research publications in journals of National and International repute.*

Dr. Stanislaus F. D'Souza *is the Head of the Nuclear Agriculture and Biotechnology Division of BARC. He obtained his Ph.D. in Biochemistry from the University of Bombay. Post Doctoral Research Associate at University of Texas Health Science Centre and Veterans Administration, Dallas, Texas, USA (1981-82). His major research interests are in the field of Enzyme and Microbial Biotechnology with special reference to immobilized cells for use in bioprocessing, biosensors and bioremediation. He has to his credit about 150 scientific papers and invited reviews in International/National journals/books. He is a member of the editorial board of scientific journals and member/expert at National Scientific committees including Member of the DBT-Task Force on "Biotechnological approaches for Food and Nutrition Security', and Vice President of 'Biosensor Society of India'. Member of the board of studies and Research advisory committees and has guided a number of Ph.D and M.Sc Theses. He is the recipient of the AMI-LOUIS PASTEUR AWARD for his significant contributions to the field of Microbiology and has been honoured as a Fellow of the National Academy of Sciences, Fellow of the Association of Food Scientists & Technologists and Fellow of the Maharashtra Academy of Science.*

Introduction

Rapid industrialization and increased anthropogenic activities have enhanced the level of environmental contaminants, which need effective methods of remediation. Bioremediation, which uses materials of microbial and plant origin, has been recognized as an alternate method of environmental clean up compared to conventional methods. Plants have the inherent ability to absorb and translocate essential and non-essential elements and organic chemicals from the soil through roots.

Phytoremediation, the use of green plants to clean up environmental pollution by removal, containment and rendering them harmless has received a lot of attention in the last decade because of its environmentally non-destructive, aesthetically pleasing qualities and low cost. Phytoremediation can be used for removal of toxic metals including radioactive elements and organic compounds from soil and water. Elemental pollutants are immutable by any biological process and can be contained, while organic pollutants can be degraded and also their mineralization can be achieved. Bioremediation of radionuclides, heavy metals and organic waste has been a major recent activity [1-4]. Recently several indigenous plants have been tested for remediation of metals, radionuclides and organic pollutants.

Phytoremediation is a cost effective plant based approach, which utilizes the remarkable

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ability of plants to concentrate metals and radionuclides and degrade/mineralize organic pollutants from the environment. A better understanding of the physiological and molecular basis of metal uptake and accumulation by hyperaccumulator plants and degradation of organic pollutants will result in improvement of phytoremediation strategies for cleaning up the environment. Development of transgenic plants tailored for phytoremediation will result in enhanced environmental clean up, thus supporting biodiversity and preservation of vital ecosystems.

Phytoremediation is an umbrella term, which include the following subsets

(a) Phytoextraction: Use of high biomass metal accumulating plants to remove and concentrate metals/organic pollutants in harvestable plant parts such as shoots, which are harvested using conventional agricultural methods.

(b) Rhizofiltration: Use of plant roots to adsorb/absorb pollutants – mainly metals from water and aqueous waste streams.

(c) Phytodegradation: The use of plants and associated microorganisms to degrade organic pollutants.

(d) Phytostabilization: The use of plants to reduce the bioavailability of pollutants in the environment by stabilizing them in soils.

(e) Phytovolatilization: The use of plants to volatilize volatile metals through foliage (e.g. mercury, selenium).

(f) Removal of pollutants from air.

The potential of certain species of plants called as "hyperaccumulators' to accumulate high concentrations of metals in the above ground parts has been recognized for potential exploitation for phytoremediation.

Hyperaccumulators of Metals and Radionuclides

The term "hyperaccumulator" is used for plants which accumulate very high concentrations of metal in the above ground tissue when grown in their natural habitat. Currently, the accepted concentration of metals in shoots of hyperaccumulators is 0.1% for nickel, cobalt, copper and lead and 0.1% for cadmium. Some of the plants belonging to Brassicaceae such as *Alyssium* species, *Thlaspi* species and *Brassica juncea*; Violaceae such as *Viola calaminaria*, Leguminosae such as *Astragalus racemosus* are known to take up high concentrations of heavy metals [5]. Plants belonging to Chenopodiaceae, such as beet (*Beta vulgaris*) quinoa *[Chenopodium quinoa]*, Russian Thistle *[Salsola kali]* and Amaranthaceae such as Red root pigweed *[Amaranthus retroflexus]* are known to take up ^{137}Cs, while *Atriplex* genus are high ^{90}Sr accumulators. Indian mustard *[Brassica juncea]* and sunflower *[Helianthus annuus]* are found to be good accumulators of uranium.

Uptake and transport vary depending on the metals and plant species used. Plant roots normally have large surfaces and are known to cover several miles in the soil. Some of the metal accumulating plants release metal chelating compounds into the rhizosphere, which include organic acids such as citric acid, malic acid and oxalic acid, which will chelate the metal and decrease the rhizosphere pH thus enabling easy uptake of metals Metals are first taken into the apoplast of roots. Some metals are transported across the cell membrane, while some metals remain adsorbed to the cell wall components. Root surfaces also have high affinity chemical receptors. Metal transporters help in the transport of metals such as Zn and Fe from root symplast into the xylem apoplast. In case of *Alyssium* species, histidine is known to chelate nickel and help in translocation, while organic acid chelators help in xylem translocation of other metals. Uptake of metal ions from xylem apoplast into shoot symplast is mediated by metal transporters in the shoot cell membranes. Once the metals have reached the shoot cells, they are stored in cellular locations such as vacuoles. Metal transporters and metal binding proteins play a major role in sequestration. Metallothioneins[MTs] help in binding of metals such as Ag, Cd, Co, Cu, Hg and Ni. Phytochelatins are a group of metal binding proteins in plants, which may complex the metals and help in storage in vacuoles.

Organic compounds after uptake by roots can be translocated to aerial plant parts. They may undergo partial or complete degradation and can be volatilized. Most of the organic compounds appear to undergo some degree of transformation in plant

cells before being sequestered in vacuoles or bound to cellular structures such as lignin. Pesticides and herbicide phytoremediation have been extensively studied. Metabolism of other xenobiotics like TCE, TNT, GTN, polyaromatic hydrocarbons such as PAHs, TCB has also been reported. Most of these compounds are metabolized, but only a few xenobiotics are mineralized. The green liver concept [6] deals with the similarity of plant enzymes comparable to those of human liver. Transformation, conjugation and compartmentalization determine the fate of xenobiotics in the plants. The xenobiotics can be oxidized by microsomal cytochrome P450. Other enzymes involved in xenobiotic metabolism in plants are peroxygenases, peroxidases, glutathione. S-transferases, carboxylasesterases, O-glucosyltransferases and N-malonyltransferases.

Phytoremediation of Radionuclides – Bench and Pilot Scale Studies

A drastic reduction was found in ^{137}Cs in solution in which sunflower plants were grown hydroponically [7]. Studies in a pond in the vicinity of Chernobyl, Ukraine showed that sunflower plants grown hydroponically in the pond could take up 90% of ^{137}Cs [from 80 Bq/L ^{137}Cs] in 12 days. Based on further experiments it was estimated that 55 Kg of dry sunflower biomass could remove the entire radioactivity in the pond in the Chernobyl having 9.2 x 10^6 Bq ^{137}Cs and 1.4 x 10^8 Bq ^{90}Sr. In experiments conducted at Brookhaven National Laboratory [BNL], NY, *Amaranthus retroflexus* was shown to accumulate high concentrations of ^{137}Cs. Indian mustard and corn were also shown to remove high amount of ^{137}Cs in field trials at BNL. Studies at Idaho National and Environmental Laboratory showed that *Kochia scoparia* could remove ^{137}Cs from soil. Our experiments in collaboration with WMD, BARC has shown that some of the indigenous plants could remove ^{90}Sr and ^{137}Cs from solutions within a short period of exposure. For phytoremediation of uranium, the addition of citric acid could increase U uptake in *Brassica* and *Amaranthus* species [8]. A commercial scale pilot rhizofiltration system set up at Ashtabula site containing waste water with U contamination resulted in 95% U being removed in 24 h. Studies have shown that hydroponically grown sunflower could significantly reduce Sr concentrations within 48 h. Plants such as *Salsola kali* and Atriplex are known to accumulate ^{90}Sr. Studies by Phytotech Inc and International Institute of Cell Biology, Kiev showed that sunflower plants could effectively remove ^{90}Sr from ponds at Chernobyl with bioaccumulation concentration of 600 for both shoots and roots. Uptake of Pu is known to vary with plant species, age and soil characteristics. Tritium phytoremediation project using trees effectively reduced tritium concentration in waste discharges at Argonne National Laboratory Site at Illinois, U.S.

Phytoremediation of Organic Pollutants – Practical Approaches

Hybrid poplar plants were found to have remarkable ability to take up and degrade halogenated organic solvents in studies at University of Washington. *Myriophyllum aquaticum* was successfully used for remediation of soils contaminated with TNT, TCE and PCP [9]. The US Army Environmental Centre has used phytoremediation to clean up groundwater containing residues of explosives [TNT, RDX, HMX and DNT]. A pilot scale study using plant lagoon system at Georgia Institute of Technology indicated that TNT remediation matched laboratory study predictions, with 85.4 to 99.7% TNT being removed. Treatment of soil contaminated with PAH was removed by phytoremediation using alfalfa, switch grass and little blue stem grass. Within 6 months, 57% reduction in PAH was observed. Petroleum hydrocarbons were also used for remediation studies in numerous industrial facilities using plants.

Genetic Engineering of Plants

Genetically engineered plant tailored for phytoremediation can be developed by introduction of genes from plant, bacterial and animal sources, which can enhance metal accumulation [10] or degradation of organics. Development of transgenic plants with specific proteins or proteins for binding with pollutants, increasing the activity of enzymes such as peroxidases, laccases, oxygenases, nitroreductase, nitrilase etc., introduction of genes for secretion of organic acids such as citric acid, malic acid and oxalic acid and other compounds such as phenolics, flavanoids or coumarins that induce rhizospheric bacteria to degrade xenobiotics and

Table 1 - Selected examples of transgenic plants for phytoremediation

Gene transferred	Origin	Target plant species	Effect
MT2 gene	Human	Tobacco, oil seed rape	Cd tolerance
MT-1 gene	Mouse	Tobacco	Cd tolerance
MTA gene	Pea	A. thaliana	Cd accumulation
γ-Glutamylcysteine synthetase	E.Coli	Indian mustard	Cd tolerance
Glutathione synthetase	Rice	Indian mustard	Cd tolerance
Cysteine synthase	Rice	Tobacco	Cd tolerance
Nt CBP4	Tobacco	Tobacco	Ni tolerance Pb accumulation
Glutathione-S-transferase,	Tobacco	Arabidopsis	Al, Cu, Na tolerant
Citrate synthase	Bacteria		Al tolerance
Zn transporters ZAT(At MTPI)	Arabidopsis	Arabidopsis	Zn accumulation
Arsenate reductase γ-glutamylcysteine Synthase	Bacteria	Indian mustard	As tolerance
Selenocystine methyl transferase	A. bisculatus	A. thaliana	Resistance to selenite
YCF1	Yeast	Arabidopsis	Cd and Pb tolerance
Phytochelatin synthase (Ta PCS)	Wheat	Nicotiana glauca	Pb accumulation
Cytochrome p4502E1	Mammals	Tobacco	Metabolism of TCE

introduction of genes for degradation of organic pollutants are some of the strategies which can be employed for improving phytoremediation.

Transfer of metallothionein genes as in case of human MT-2 gene to tobacco and oil seed rape resulted in plants with increased Cd tolerance [11]. Transfer of a pea MT gene to *Arabidopsis thaliana* enhanced Cu accumulation [12]. Enhanced Cd tolerance was also obtained by transfer of two genes γ – glutamyl cysteine, synthetase and glutathione synthase [13, 14]. Transfer of citrate synthetase gene resulted in secretion of citric acid by roots with enhanced Al tolerance [15]. Introduction of *mer A* and *mer B* genes resulted in transgenic plants which could volatilize mercury [16]. Transgenic *Arabidopsis* plants with arsenic reductase and γ – glutamyl cysteine synthase genes resulted in higher uptake of arsenate. Some selected examples of transgenic plants developed for phytoremediation are shown in Table 1. Enhancing the uptake sites, altering uptake specificity of metals increasing number of high affinity binding sites, altering rate of transport into organelles and creating artificial metal sinks in shoots are some of the strategies which may yield fruitful results for phytoremediation.

Transgenic plants expressing PETN reductase could grow in presence of chemicals used for explosives such as GTN and TNT and could denitrify the explosives by enzyme activity. Mammalian Cyt P 450 gene when transferred to plants, the plants could oxidize a wide range of organic pollutants.

Phytoremediation : Prospects and Limitations

The main advantages of phytoremediation are that it is far less disruptive to environment, lack of requirement for huge disposal sites, high public acceptance, avoidance of excavation, lack of noise and frequent worker activity and its potential versatility to treat a diverse range of hazardous materials. The disadvantages of phytoremediation include limitation of growth of plants under extreme conditions, possibility of contaminants being released back into the environment by litter fall and

enhancement in solubility of some contaminants resulting in pollutant migration. Some of the other limitations are that phytoremediation may not be capable of 100% reduction in the pollutant, unfamiliarity of the technology by the regulators and long time period required for cleaning up sites. Besides, soil phytoremediation is limited in applicability to only surface soils.

In the last few years, several commercial companies on phytoremediation have sprang up in US and Europe and include Phytotech (USA), Phytoworks (USA), Thomas Consultants (USA), Bio-Planta (Germany), Consulagri (Italy) and OEEL (UK) etc. Phytotech Inc for example has used *Brassica* species to remove lead from soil and sunflower to remove uranium and cesium from aqueous waste streams. There have been more than two dozen field tests of phytoremediation. The concept of manipulating plant genes that regulate toxic metal uptake or degrade/ mineralize organic pollutants is cutting edge research. Transgenic mustard over-expressing phytochelatins have been studied in greenhouse in Leadville, Colorado and the plants were shown to accumulate significant levels of Cd and Zn.

Phytoremediation is a "Green Technology" which is appealing to the "Conservation movement" as an alternate environment friendly technology compared to chemical and physical technologies which are environmentally destructive. Phytoremediation's ability to make further strides will depend on how quickly the regulators become convinced of the efficiency of the technology. Phytoremediation is an emerging technology for contaminated sites, but it is not a panacea for all waste problems. The technology has been demonstrated, but not yet commercially exploited. Search for yet undiscovered hyperaccumulator plants and understanding the mechanism of uptake, translocation and sequestration in hyperaccumulators plants will pave the way to develop efficient plants which will clean up the environment. Proteome and DNA array technology may be applied for searching for candidate genes/proteins for phytoremediation. Utilization of the remarkable potential of green plants to accumulate elements and compounds from the environment and perform novel biological transformation may be a novel way of cleaning up of the environment of toxic elemental and organic pollutants for sustainable development.

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Radioresistant Microbes for Bioremediation of Nuclear Waste

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Introduction

Nuclear waste, either generated through chemical processing in nuclear industry or stored as nuclear arms stockpiles, is an exciting subject. On one hand it is a cause of serious concern for the nuclear industry and environmentalists interested in its safe disposal and proper containment. Conversion of large volumes of liquid waste to a desirable small volume of solid mass for disposal is a difficult task for nuclear scientists and engineers. The material thus generated (organic resins or a non-biodegradable glassy matrix) is very persistent in nature, being non-biodegradable, and needs to be stored over a very long period of time posing a serious environmental threat. On the other hand, the nuclear waste offers a goldmine for the recovery of precious metals (such as uranium or cesium) or other heavy metals (mercury, arsenic, cadmium or lead) which are otherwise serious environmental pollutants. The concentration of metals such as U or Cs in such waste is very low and therefore refractory to the chemical methods available for their precipitation. Bioprocesses, involving use of microbes or materials thereof, have been shown to work at low concentrations [1]. However, use of such technology, broadly described as "bioremediation" of nuclear waste, is limited because the nuclear waste is often ridden with *toxic organics, heavy metals* and *radioactivity*, all of which severely inhibit the survival and metabolic

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activities of living organisms. Ionising radiations, in particular at very high doses, cause severe DNA damage and mutations and are largely lethal. Organisms that can tolerate one or the other (but not all) of these detrimental factors are known. Constructing organisms (mostly microbes) that can survive in the inherently non-conducive environments typical of radioactive waste and facilitate bioremediation is a challenging task.

Why Radioresistant Bacteria are appropriate for Bioremediation ?

As mentioned earlier, it is possible to recover metals from low concentrations in the waste, using biological agents, either through bioaccumulation, biosorption, or bioprecipitation [1,2]. Bioaccumulation is the intracellular accumulation of metals which depends on metabolic activities of organisms and therefore, requires live cells. Recovery of accumulated metals from this process is not so simple. Biosorption refers to the adsorption of metals onto the cell surface of live or dead cells or to the surface of other biomolecules, wherefrom the metal can be recovered more easily. Bioprecipitation, on the other hand, is an enzyme mediated process, involving the generation of precipitant ligands, like inorganic phosphate, which in turn interacts with soluble metal ion in the vicinity and form insoluble metal phosphate on the cell surface. The whole process can be carried out by using either live or even dead cells having the enzyme in question located close to the cell surface (periplasm). For the purpose of recovery of metals from radioactive waste, bioprecipitation on the surface of dead cells offers the most attractive opportunity. However, in order that the bioremediation of radionuclides/metals from the nuclear waste is sustained over a long duration *in situ*, it is desirable to use live cells which can continuously produce either more cells for biosorption and bioaccumulation or more enzyme for bioprecipitation, right in the radioactive waste. It is in this context that radioresistant microbes stand out as the obvious choice for bioremediation of highly radioactive waste *in situ*.

Extreme Radioresistance of Deinococcus Radiodurans

Deinocccus radiodurans is an orange-red colored, gram-positive, non-pathogenic bacterium credited with a very high level of radioresistance (>15 kGy of ^{60}Co γ-rays) [3]. Discovered in rradiated meat cans in the 1950's, this bacterium is also known to survive severe desiccation stress. Following exposure to γ-rays, the DNA of the bacterium is extensively degraded but within few hours the entire chromosome is stitched back, piece by piece, without any detectable mutation and the organism revives fully [4]. The key to survive such extensive DNA damage obviously lies in the ability of *D. radiodurans* to repair damaged DNA, but the underlying mechanisms for the same are not very clear. United States Department of Energy (US-DOE) sponsored the sequencing of the genome of this organism in the hope to find the genetic basis for its survival under radiation stress. While analysis revealed a great deal of redundancy and multiplicity of several DNA repair genes, almost all the genes have functional homologues in other bacterial systems. Comparative study of genomes of *D. radiodurans* and 3 other radioresistant bacteria [5] have not described a shared group of uncharacterized novel genes that might account for the phenomenal DNA repair capabilities. In fact, the DNA repair systems identified in *D. radiodurans* appear to be less complex and diverse than those known in some of the radiosensitive bacteria. Mechanisms other than DNA repair, such as a novel protein recycling phenomenon during post-irradiation recovery [6], have also been implicated in the radiation survival of this organism.

Of course, the DNA repair genes of *D. radiodurans* play an extremely important role in its radioresistance - single gene mutations in *recA* or *polA* result in severe loss of radioresistance. But when these genes are transferred to radiosensitive organisms such as *E. coli*, they offer no protection against the radiation damage, clearly indicating that they play a context-specific role [3, 4]. Functions of over a thousand genes (nearly one-third of *Deinococcus* geneome) in this microbe are not known and some of them are predicted to play a role in post-irradiation recovery [7]. However, disruption of some of these novel genes has little effect on

survival after radiation. Viewed in this context, the current belief is that *Deinococcus* and other radioresistant bacteria may use relatively conventional DNA repair systems but with much higher efficiency aided by their metabolic make-up [8]. Perhaps a large contingent of enzymatic and non-enzymatic antioxidant defenses allow the DNA repair to function with superior efficiency in this bacterium [9,10]. Irrespective of the mechanisms involved, the extreme radioresistance of *D. radioduarns* is an asset for its biotechnological exploitation for bioremediation of nuclear waste.

Engineering *Deinococcus* for Bioremediation of Radioactive Waste

Enormous volumes of hazardous nuclear waste, generated from the nuclear weapons program in the early part of 20th century in the U. S. A. and stored at several sites, have reportedly leaked and contaminated the nearby soil and ground water. The reported radiation levels in some waste sites were as high as 10 mCi/dm^3 and contained radionuclides (^{235}U, ^{239}Pu, ^{99}Tc, ^{137}Cs and ^{90}Sr), metals such as chromium, lead and mercury along with a myriad of toxic organics (eg., toluene, trichloroethylene and others) [11,12]. The nuclear contamination of the environment is believed to be even more graver in the former Soviet Union [13]. Conventional physico-chemical methods for disposal of such waste are prohibitively expensive and unsafe. The Department of Energy (DOE) in the U.S.A. is exploring the possibility of employing genetically modified radiation resistant *Deinococcus radiodurans* [14] for *in situ* bioremediation of voluminous liquid waste which, if successful, undoubtedly emerges as a relatively inexpensive and viable technology [15]. Deinococci are quite ubiquitous, nonpathogenic and can easily be cultured [3]. That is how in recent times, a great impetus and emphasis were accorded to studies on genomics, proteomics and the fundamental biology of *D. radiodurans* [16,17]

Research aimed at developing *D. radiodurans* for bioremediation began in 1997 with the demonstration that it can grow in the presence of ionizing radiation dose of 60 Gy/h, comparable to the most radioactive US-DOE waste storage sites. Since then the bacterium has been tested with different cloned bioremediating genes (such as for detoxification of metals and organic solvents) that can be expressed during its growth at this radiation dose.

Metal detoxification using this microbe was first attempted for mercury. Metal resistance gene products often possesse the capability to transform metals into less toxic lower oxidation state. Mercury is the most prevalent heavy metal contaminant in DOE wastes and a series of genetic vectors that encode resistance to this metal have been constructed and tested in *D. radiodurans*, for example the *merA* locus from *Escherichia coli* strain BL308 [18]. The *merA* encodes a mercuric ion reductase which reduces highly toxic, thiol reactive mercuric ion, Hg(II) to much less toxic and nearly inert elemental and volatile Hg(0). Four different *D. radiodurans* expression systems were developed and used to regulate *merA* expression by varying the gene dosage between 1 to 150 copies per cell. *D. radiodurans* strains expressing *MerA* protein during growth at 60 Gy/h were, firstly, resistant to ionic Hg(II) at 30-50 μM well above the highest concentration reported for mercury contaminated DOE waste sites (10 μM) and secondly able to reduce it to non toxic-form [19].

Since then, a variety of metal reduction genes have been tested in *D.* to ascertain whether they confer resistance to common metallic waste constituents as well as their ability to transform them. These include cytC3 from *Desulfovibrio vulgaris* for U(VI) [20]; *czc* from *Ralstonia eutrophus* CH34 for Cd(II), Zn(II) and Co(II) and *Bacillus thuringiensis* for Cr(VI) [21]. *D. radiodurans* itself possesses some metal remediating capabilities which may be expanded and used. Anaerobic cultures of this bacterium were found to reduce U (VI) and Tc(VII) in the presence of humic acids and Cr(VI) in the absence of humic acids [22]. Using its whole genome sequence, it may be possible to enhance these functions by genetic engineering. *Shewanella oneidensis* strain MR-1, a radiation sensitive bacterium, is known to be highly effective in reducing soluble U(VI), Cr(VI) and Tc(VII). Genome of this organism could contribute to the array of metal reducing genes targeted for expression in *D. radiodurans* for bioremediation purpose [15].

Another approach for metal remediation that is currently drawing attention is the bioprecipitation of metals mediated by inorganic phosphate. The precipitant ligand (Pi) is produced by a phosphatase enzyme localized in periplasm on the cell surface. The Pi, in turn, interacts with the metals like uranium in the near vicinity and forms insoluble metal phosphate which gets deposited on the cell surface. A genetically engineered *E. coli* expressing multiple copies of *phoN*, a gene for non-specific acid phosphatase from a local isolate of *Salmonella typhi*, has been constructed in the Molecular Biology Division at BARC. When used in low level radioactive waste, dead cells of this strain generated localized high concentrations of inorganic phosphate at the cell surface and precipitated actinides like $^{233/238}U$ from ammonium diuranate supernatants, generated during reprocessing of spent fuel rods. With time, due to loading with uranium phosphate, cells become heavy and sink to the bottom of the container and can be separated easily for recovering uranium. We have also cloned and expressed this gene successfully in *D. radiodurans* strain R1 and U bioprecipitation capabilities of this transgenic strain are currently under investigation. *Deinococcus* clones, expressing *phoN* or any other promising phosphatase gene, are likely to be useful for immobilizing ^{235}U from the radioactive waste sites such as those of DOE in the USA.

As mentioned earlier, several organic solvents co-exist with heavy metals in the nuclear waste and are generally quite inhibitory to the growth of microbes. Biorecovery of metals would be feasible only if the organisms being used can also handle the organics resident in the nuclear waste. *Deinococcus* per se can not grow with the organics present in the mixed DOE wastes (benzene, toluene, ethylbenzene and xylene - collectively called BTEX). However, BTEX are known to be utilized by a radiosensitive bacterium, *Pseudomonas putida*, as growth substrates. Trichloroethylene (TCE), a cocontaminant, can also be oxidized along with other compounds like toluene. This is due to broad specificity of oxygenases involved in toluene catabolic pathway.

Since *Pseudomonas* can not survive in DOE mixed radioactive waste sites due to high radiation, the toluene diooxygenase genes (*todC1C2BA*) of this bacterium were cloned and expressed in *D. radiodurans* [23]. The transformed strain was able to oxidize toluene, chlorobenzene and 3,4-dichloro-1-butene during chronic irradiation. Toluene was catabolized to 3-methylcatechol by this recombinant *Deinococcus* strain. Other *Pseudomonas* catabolic genes that convert 3-methylcatechol to pyruvate were also introduced into *todC1C2BA* containing D. radiodurans which yielded a strain that mineralised toluene and related compounds [24]. Brim et al have subsequently combined the *mer* gene of *E. coli* and the *tod* operon of *Pseudomonas* in a single *D. radiodurans* strain which not only metabolized toluene and chlorobenzene but also resisted and reduced toxic ionic mercury in the nuclear waste to volatile elemental mercury, during chronic exposure to 60 Gy/h radiation *in situ* [19].

Tributyl phosphate (TBP) is the major organic solvent used in DAE for reprocessing of spent fuel rods to recover actinides like uranium and plutonium. Recently, a TBP-biodegrading bacterium was isolated in the Molecular Biology Division at BARC from TBP waste stored at RSMS site of Waste Management Division, BARC. It can grow on TBP and releases inorganic phosphate. Transfer of this capability to a radioresistant bacterium like *Deinococcus* can provide a technology that can simultaneously degrade TBP and also precipitate uranium in nuclear waste *in situ*.

Present limitations of bioremediation using *Deinococcus*

Notwithstanding its impressive inherent radioresistance, use of *Deinococcus* for bioremediation does have a few limitations. In the order of increasing importance they can be listed as (i) sensitivity to heavy metals particularly actinides (ii) sensitivity to organic solvents, and (iii) poor radioresistance under nutrient deficiency. Actinide and metal toxicity of *D. radiodurans* was recently investigated [24]. Growth inhibitory concentrations of metals for this organism were found to range from 1.8 μM for Cd(II) to 32 mM for Fe(III) while for Pu(IV), U(VI) and Np(V) they were 5.2 mM, 2.5 mM and 2.1 mM respectively. Although the study concluded that actinide toxicity will not impede bioremediation using this microbe, it may be

necessary to engineer *Deinococcus* to handle each metal's toxicity individually as has been done for mercury. In a similar way, tolerance to each organic solvent will have to be genetically incorporated in this strain depending on the nature of the mixed nuclear waste.

A more serious concern is the relatively poor radioresistance of this microbe under nutrient deprivation. Although successful expression of several genes for remediating both inorganic and organic compounds in *D. radiodurans* strain R1 under chronic irradiation was achieved under optimal (nutrient-rich) growth conditions, these transgenic strains often failed to survive in nutrient-poor radioactive environments, that otherwise support their luxuriant growth when radiation was absent [25]. This phenotypic reversal of transgenic strains from radiation resistance to sensitivity is of concern as it jeopardizes the suitability of *D. radiodurans* as a bioremediation host in radioactive waste sites. In nutrient restricted conditions, DNA repair is seriously limited by this organism's metabolic capabilities and not by any nutritionally induced defect in the repair process. This information has helped the identification of amino acids and vitamins that restore growth of *D. radiodurans* in nutritionally restricted radioactive environments [25]. Correction of these defects, by genetic engineering, will be necessary to facilitate their effective use in bioremediation.

At times the conditions for bioremediation may be more demanding than what has been described earlier. *Deinococcus* is rather sensitive to high temperature and salt. To practice bioremediation in saline environment or at elevated temperature one may, therefore, look for other viable alternatives. Recently, *Deinococcus geothermalis*, a moderately thermophilic and radiation resistant bacterium, was engineered with *mer* operon of *E. coli* and *todC1C2BA* of *P. putida* for possible bioremediation of mixed radioactive waste at temperatures as high as 55^0C. Additionally, this thermophilic radiophile was found to be capable of reducing Fe(III)-nitrilotriacetic acid, U(VI), and Cr(VI) [26].

Future Prospects

Although *D. radiodurans* strain R1 has a very impressive radioresistance and desiccation tolerance, better strains of radioresistant bacteria for bioremediation continue to be discovered. There are several deinococcal isolates which are even more radiation resistant than *D. radiodurans* R1. One of them was isolated from Bombay Duck *(Harpodon nehereus)* at BARC more than 30 years ago, and was identified as *Micrococcus radiophilus* (renamed subsequently as *D. radiophilus*) [27,28]. Some bacteria isolated from Hanford nuclear waste site in the U. S. A. and resisting ionizing radiation dose of ~20 kGy were identified as *Deinococcus sp* [29]. A bacterial isolate from an aquifer contaminated with arsenic in West Bengal, India, was recently identified as *Deinococcus indicus* [30]. Of late, three new species of *Deinococcus* have been isolated from continental Antarctica. These are UV resistant, psychrophilic and tolerate salt (upto 10% NaCl) and were identified as *D. frigens, D. saxicola* and *D. marmoris*, respectively [31]. A program to screen for more radioresistant bacteria is in operation in the Food Technology Division at BARC and has yielded some useful bacterial isolates. An expansion of microbial capabilities to withstand radiation, heavy metal stress and organic solvents, as also salinity and temperature is most desirable for the success of bioremediation technology.

Poor radioresistance of *D. radiodurans* during nutrient starvation remains a major concern. A recent study has shown that *D. geothermalis* has much better growth capabilities than *D. radiodurans* in minimal growth media. This strain matches the radioresisatnce of *D. radiodurans* and can be used in preference to the latter [26]. A unicellular cyanobacterium *Chroococcidiopsis* has also shown resistance to 5-15 kGy doses of ionizing radiation [32]. Recent studies in the Molecular Biology Division at BARC have revealed an equally impressive radioresistance exhibited by photosynthetic nitrogen-fixing cyanobacteria, such as *Anabaena* strains. These autotrophs which are resistant to high temperature, salinity, desiccation and several heavy metals perhaps represent the organisms of the future waiting to be engineered for bioremediation purpose.

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Bioreactor for Production of Bioactive Compounds

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Introduction

In view of the growing world population, increasing anthropogenic activity and rapidly eroding natural ecosystems, the natural habitats for a large number of valuable plants are rapidly dwindling. Over 80% of about 30,000 known natural products are of plant origin. The continuous production of the desired bioactive compounds for pharmaceutical industries requires sustained input of plant materials from nature. There has been considerable interest in investigating the potential of plant cell and organ cultures for the large scale production of bioactive compounds as an alternative to traditional agricultural based production. Large-scale cultivation of plant cell and organ cultures under defined parameters retains the biosynthetic capability with reduced production cost.

Bioactive compounds currently extracted from natural plants are used as food additives, pigments, dyes, fine chemicals, insecticides, cosmetics and perfumes. Table 1 and 2 depict the important milestone in bioreactor technology for cultivation of cell and organ under controlled nutritional and environmental conditions [1-4]. Suspension cultures of *Catharanthus roseus* cultivated in 100 L bioreactor equipped with a helical impeller and with controlled on-line defined parameters, yielded 32 kg biomass on 16 days of cultivation, whereas naturally grown plants produced 3.2 kg biomass in one-year cultivation period. Organ cultures such as multiple shoots and hairy root cultures can be propagated in bioreactors to enhance yield. Successful mass propagation of *Stevia rebaudiana* multiple shoots was achieved in 500 L bioreactor and harvested 64.6 kg of shoots from an initial inoculum of 460 g, which showed 140-fold increase [5]. Similarly, a 10 L jar bioreactor was used for the production of potato tubers, which could be used as seed tubers and transferred directly to the field [6]. Hairy root cultures of various plants have been cultured in bioreactors, which provide a continuous source of bioactive compounds [7,8]. Optimizations of parameters in bioreactor for cell and organ cultures are known to enhance productivity.

India is a rich source of important medicinal plants. A concise list of some economically important plant derived bioactive compounds; their sources and therapeutic value are presented in a Table 3. Currently, pharmaceutical industries are extracting most of these compounds from naturally grown plants followed by purification. Mass cultivation of medicinal plant species under natural conditions may not be possible due to environmental, ecological and variable climatic conditions. Many more compounds are being added to this list every year, indicating the attention that is being given to this area all over the world (Table 4).

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Table 1 - Milestones in bioreactor technology for cultivation of Cell suspension cultures

Bioreactor type	Maximum capacity (l)	Plant origin	Reference
SGC	10	*Lolium, Paul' scarlet Rose, Ilex, Ginkgo*	Tulecke and Nickell (1959)
STR	30, 134	*D. carota*	Byrne and Koch (1962)
STR	15,000	*N. tabacum*	Noguchi et al., (1977)
STR	20,000	*N. tabacum*	Azechi et al., (1983)
STR	750	*L. erythrorhizon*	Curtin (1983)
STR	85	*C. roseus*	Smart and Fowler (1984b)
STR	450	*C. blumei*	Ulbrich et al., (1985)
STR	2000	*P. ginseng*	Ushiyama (1986)
STR	5000	*C. roseus*	Schiel and Berlin (1987)
ALB	300	*D. lanata*	Reinhard et al., (1989)
STR	75000	*E. purpurea*	Rittershaus et al., (1989)
ALB	20	*C. roseus*	Fulzele et al., (1994)
ALB	300	*P. somniferum*	Park et al., (1992)
Wilson-type	-	*T. cuspidata*	Pestchanker et al., (1996)
STR	2	*S. medusa*	Huang et al., (2001)
ALB	20	*T. wallichiana*	Navia et al., (2002)

SGC = Spared glass carboy; STR = Stirred tank reactor; ALB = Air lift bioreactor

Bioreactors

The conventional *in-vitro* environment in shake flasks is characterised by relatively high humidity, constant temperature, low photosynthetic photon flux density, large diurnal fluctuation in CO_2 concentrations, high concentration of sugar, salts, growth regulating substances in the culture medium and accumulation of toxic substances. These conditions often cause low rates of transpiration, photosynthesis, water and CO_2 uptake but a high rate of dark respiration, all of which result in poor growth of cell and organ cultures.

Bioreactors provide nutritional and closely controlled environment for optimum growth of plant cell and organ cultures in which cells perform biochemical transformation to produce bioactive compounds. Bioreactors have several advantages for mass cultivation of plant cells. i) It gives better control for scale up of cell suspension cultures under defined parameters; ii) Constant regulation of physical and chemical parameters at various stages of operation is possible iii) Handling of culture such as inoculation or harvest is easy and saves time iv) Nutrient uptake is enhanced by submerged culture conditions which stimulate multiplication rate and higher yield v) Large number of plantlets are easily produced and can be scaled-up under microenvironment.

Controlled physical and chemical parameters in bioreactors promote growth and development, reduces morphological and physiological disorders and encourage more rapid and vigorous plant growth and development during acclimatization stage [3,9,10].

Important Parameters in Bioreactor for Growth and Production of Bioactive Compounds

Light

To control photo-morphgenesis, different types of light emitting diodes can be used to emit

Table 2 - Milestones in bioreactor technology for cultivation of organ cultures

Bioreactor type	Maximum capacity (l)	Plant origin	Reference
Roo culture STR	-	*N. tabacum*	Wilson et al., (1987)
STR	-	*H. muticus*	Flores et al., (1987)
TBR, ALB	1	*D. carota*	Kondo et al., (1989)
STR	14	*D. stramonium*	Hilton and Rhodes (1990)
STR	1.2	*P. ginseng*	Yoshikawa et al., (1993)
STR	-	*C. roseus*	Nuutila et al., (1994)
STR	2	*S. chirata*	Keil et al., (2000)
Balloon Type Bubble bioreactor	5, 20 and 500	*P. ginseng*	Sung et al., (2000)
BCR	15	*H. muticus*	Bordonaro and Curtis (2000)
Multiple shoot culture JB	-	*D. purpurea*	Hagimori et al., (1984)
JB	2	*A. annua*	Park et al., (1989)
JB	1	*A. annua*	Fulzele et al., (1991)
STR	500	*S. rebaudina*	Akita et al., (1994)
STR	500	*A. belladonna*	Kawamura et al., (1996)
Inner Loop Mist Bioreactor	-	*A. annua*	Liu et al., (1998)
Roller Bioreactor	-	*Stevia rebaudiana*	Bondarev and Nosov., (2002)
Mist reactor	-	*D. caryophyllus*	Correll et al., (2001)

STR = Stirred tank reactor; ALB = Air lift bioreactor; TBR = Turbine blade reactor; JB = Jar bioreactor.

blue, red or far-red light at a low cost. Spectral distribution of light from various light sources varies significantly. The application of different light emitting diodes for growing cell and organ cultures in bioreactor is a practical alternative to lighting systems [11]. White fluorescent light has been used as the primary source in micropropagation since the spectrum generally matches the requirement of *in-vitro* cultures and they give a relatively uniform horizontal distribution of photosynthetic photon flux density over the entire culture shelf. The CO_2 concentration in the bioreactor during the photoperiod has been shown to be low [12]. Light irradiation exhibited significant influence on the production of anthocynin by suspended cultures of *Perilla frutescens* cells in a 2.6 L bioreactor [13]. Low light conditions (105 $^{\mu}E\ m^{-1}\ s^{-1}$) increased biomass growth rate when cell suspension cultures of Chenopodium rubrum cultivated in a 20 L air lift bioreactor [13]. Anthocynin production from *Viccinum pahalae* cell cultures increased by using mercury lamps on an average of 240 $\mu m^{-1}\ s^{-I}$ PPF at the inner surface. [14]. Biomass of hairy roots of *Panax ginseng* was the highest in the cultures grown under dark or red light while ginsenoside accumulation was optimum in the cultures grown under fluorescent light [15].

Oxygen

Oxygen levels in bioreactor are largely in the gas phase, in the air bubbles inside the medium as well as in the dissolved oxygen medium. Air is released through a sparger located at the base of bioreactor. Plant cells have a lower metabolic activity rate than microbial cells and a slow doubling

Table 3 -Economically important plant derived bioactive compounds

Plant origin	Natural products	Therapeutic use
	Pharmaceuticals	
Catharanthus roseus	Vincristin, Vinblastine	Anti-cancer
Taxus baccata	Taxol	Anti-cancer
Nothapodyts foetida	Camptothecin	Anti-cancer
Ophioriza pumila	Camptothecin	Anti-cancer
Artemisia annua	Artemisinin	Anti-malarial
Digitalis lanata	Digoxin	Cardio tonic
Atropa belladonna	Atropin	Anti-cholinergic
Cinchona ledgeriana	Quinine	Anti-malarial
	Agrochemicals	
Junikerus viriginiana	Cederene	Repellent
Nicotina tabaccum	Nicotine	Insecticide
Chrysanthramum species	Pyrethrins, Pyretheroids	Insecticide
	Pigments	
Morinda citrofolia	Anthraquinonens	Red
Lithospermum erythrorhizon	Shikonin	Red
	Flavors	
Capsicum frutesion	Capsicin	Chilli
Crocus salium	Crocin, Picrocin	Saffron
Glycyrrhiza glabra	Glycyrrhizin	Liquorice

Table 4 - Bioactive compounds obtained from plant cell and organ cultures

Alkaloids	Flavors	Pigments
Allergens	Furanocoumarins	Peptides
Anthraquinones	Hormones	Perfumes
Aromas	Insecticides	Phenols
Benzoquinones	Latex	Proteins
Cardiac glycosides	Lipids	Steroids
Chalcones	Napthaquinones	Sugars
Dianthrones	Nucleic acids	Tannins
Enzymes	Nucleotides	Terpenoids
Flavonoids	Opiates	Vitamins

time and therefore require a lower O_2 supply. It was also reported that high aeration rates reduce the biomass growth of cultured cells [16]. The level of oxygen in bioreactor can be regulated by agitation or agitation through aeration, gas flow and air bubble size. Growth of *Euphorbia pulcherrim*a cell suspension was inhibited when the level of oxygen was dropped below 10% and cell number increased when the O_2 level increased to 80%. High aeration was found to inhibit cell growth in cell suspension

cultured in airlift bioreactor. Reducing O_2 level affected cell aeration of carrot embryogenic tissue.

Temperature

It is one of the important parameters in bioreactor for cultivation of cell and organ cultures. The control of temperature can be easily manipulated by a heating element in the vessel or by circulating water in an enveloping jacket outside the vessel. Temperature effects were studied on potato tuber formation in an airlift bioreactor [6]. It was observed that larger size of tubers developed at 25°C than at 17°C. Low temperature in 10 L bioreactor maintains stable high taxol production of 27 mg/L, which is 11 fold higher than elicited asynchronous cultures [15]. Optimal temperature of 20°C influenced the biomass accumulation and ginsenoside production in a large scale bioreactor by hairy root cultures of *Panax ginseng*.

Carbon Dioxide

The contribution of CO_2 supply during the proliferation and multiplication stage in culture medium supplemented with sucrose is debatable. The requirement of CO_2 for cell and organ cultures was not related to photosynthesis but for metabolic pathway involved in amino acid biosynthesis. High aeration rates rather than excessive oxygen levels inhibit growth due to depletion of CO_2. Carbon dioxide enrichment in bioreactor cultures of *Brodiacea* cluster did not affect biomass growth [17]. High CO_2 levels increased production of *Cyclamen persicum* pro-embryogenic masses.

Agitation and Aeration

The high speed agitation required for oxygenation and mixing generates a shear stress detrimental for most plant cells. The high rates of aeration tend to trip gaseous metabolites like CO_2 and ethylene (C_2H_4) out of the culture media thus reducing the capacity for plant cell and organ cultures [18]. Plant cell cultures require ~0.1 – 4.0 mmol O_2 l^{-1} h^{-1} about a 10^{th} of oxygen required by microbes. Air lift reactors and special designed impeller with more surface area can be used at much slower speed for agitation to provide adequate mixing and sufficient oxygenation for cell cultures. An agitation speed of 100 rpm was sufficient to mix cultures broth of *Podophyllum hexandrum* and increased podophyllotoxin production in bioreactor [19].

Large-scale Cultivation of Cell Cultures in Bioreactor andPproduction of Bioactive Compounds

The application of bioreactor system for large-scale cultivation of plant cells for the production of valuable bioactive compounds is an active field, whose application holds great promise for pharmaceutical industry. Large-scale cultivation of plant cells increases the biomass production much more than whole plants grown in the field. Culture cycles of cell suspensions would be a question of weeks as for example in case of shikonin plant, which usually is not harvested before the age of five years. Successful production of shikonin by cell cultures of *Lithospermum erythrorhizon* was the first commercial process using plant cell cultures.

Plant cells in liquid suspensions offer a unique combination of physical and chemical environments that must be accommodated in large-scale bioreactor process. Some of the well-known drawbacks of the cell suspension cultures include the instability of the productive cell lines, the slowness of the cell growth and limited knowledge about the secondary metabolite pathways. There are indications that sufficient oxygen supply and proper mixing in airlift bioreactors may not be suitable for high density (≥300 to 350 g/l fresh biomass) plant cell suspension cultures. Secondly, the problem of well-known shear sensitivity and rapid setting characteristics of plant cell aggregates and cell floating tendencies of the cell cultures have to be solved when bioreactors for plant cell cultures are designed.

Plant cells are 10 to 100 times larger than bacterial and fungal cells. Cultured plant cells range from 20-40 μm in diameter and from 100-200 μm in length and embrace vacuoles up to 95% or more of cell volume. These features have led to the belief that plant cells are shear sensitive. Also, the oxygen requirement of plant cells is low when compared to microbial cells. Such special features of cultured plant cells necessitate to design and develop a bioreactor, which could provide proper defined parameters throughout the cultivation period without forming dead zones and will not damage the cells during cultivation period.

Fig. 1 Scale-up of Catheranthus roseus cell cultures in 20 L airlift bioreactor

Fig. 2 Mass propogation of multiple shoot cultures of Catharanthus roseus in a 1 L bell-jar bioreactor

Large-scale high density *C. roseus* cell suspension cultures were grown in 100 L bioreactor (working capacity 75 L) equipped with helix impeller. The final yield of biomass was 32 Kg fresh weights in 16 days of cultivation. The higher and more efficient upward suspension pumping capacity at low shear rate of the helix impeller ensured sufficient and more uniform mixing of the delicate plant cell suspension and their better growth performance. Bioreactor based system for mass production of flavonoids from cultured plant cells have also been described for a few species [4,14]. Airlift bioreactor with controlled aeration increased the yield of ajmalicine by cell suspension cultures of *C. roseus*. Figure 1 depicts the large-scale cultivation of cell suspension cultures of *C. roseus* in a 20 L air lift bioreactor.

Bioreactor System for Multiple Shoot Cultures to Enhance Bioactive Compound Production

Multiple shoot cultures are complex shape compared to roots and are more shear sensitive. Although an increase in pO_2 (dissolved oxygen) stimulates growth and proliferation of shoots, oxygen required to maintain their metabolic activities in a bioreactor is minimum. This may be the reason that shoots can be successfully cultivated using different types of bioreactors. A 500 L bioreactor was used to cultivate *Stevia rebaudiana* shoots and achieved 64.6 Kg FW of shoots after 4 weeks of cultures [5]. This indicates that nutrient and oxygen transfer were sufficient in every area of the bioreactor. Shoots quality depends on their position in the bioreactor. Air bubbles injured shoot cultures just above the sparger and acclimatization of such shoots was more difficult [5]. Light sources influence shoot morphology and quality. It was reported that light strength influences the metabolic content [5]. Preventing continuous submersion in a liquid medium can also stimulate the growth of shoots. The most important factor for growth and secondary product stimulation is to enhance oxygen supply. It is reported that shoots of several plants were efficiently propagated in a nutrient mist medium. Multiple shoots of *Artemisia annua* were propagated in a bioreactor under controlled aeration with significant yield of terpenoids [20]. In automated plant propagation systems, various multiple shoot cultures have been successfully grown at Plant Biotech Industry Ltd., Israel. Micropropagation of pineapple was scaled up in bioreactor using clusters in a periodic immersion system [21]. C. roseus shoot cultures were cultivated in shake flasks and achieved ajmalicine production 2.4-fold higher compared to leaves of 1-year-old naturally grown plants [22]. Shoot cultures of *A. annua* cultivated in three types of bioreactors demonstrated that hyper growth was obtained in mist

bioreactor than airlift bioreactor [23]. Two fold more production of flavonoids was produced by cell cultures of *Saussurea medusa* in a 20 L airlift bioreactor [4].

Bioreactors for Hairy Root Cultures and Production of Bioactive Compounds

Hairy roots are obtained after the successful transformation of a plant with *Agrobacterium rhizogenes*. Hairy root cultures have received considerable attention from plant biotechnologists for the production of bioactive compounds. Hairy root cultures can be indefinitely propagated on a synthetic medium without growth regulators [24]. A major characteristic property of hairy roots is that they are able to produce bioactive compounds concomitantly with growth. Hence, it is possible to get a continuous source of production from actively growing hairy roots. Specific bioreactors have been designed and developed for mass propagation in order to overcome the limiting factors existing for biomass and bioactive compounds production. Various physical and chemical parameters can be controlled in bioreactor with a view to enhance yield. Two-phase systems have also been used to facilitate the release and recovery of the bioactive compounds in the culture medium [7]. This technology helps to continuously remove the compounds from the medium and helps to prevent the feed back repression of the synthesis.

Environment in stirrer tank reactor is characterized by high shear, which damages the roots, poor control of critical gas concentrations, and insufficient nourishment of the biomass at high concentration due to poor liquid circulation. There are four primary environmental factors, which affect aeroponic hairy root cultures: moisture, temperature, mineral nutrient and gas phase composition mainly carbon dioxide, ethylene and oxygen [25]. One of the major advantages of aeroponics is the complete control of gases in the culture environment. This is important for the maintenance of healthy root cultures because gases play a major role in hairy root metabolism. Hairy roots may be oxygen limited unless cultures are aerated with oxygen enriched air. Oxygen depletion triggers anaerobiosis and the production of ethylene. Carbon dioxide has been shown to stimulate root growth of many species. Periodic exchange of culture medium in bioreactor and longer culture period increased the growth rate and produced ginsenoside by *Panax ginseng* hairy roots [8].

Conclusion

Many multinational pharmaceutical companies have started their production units in India with a view to produce bulk amounts of plant-based drugs. Currently field grown plants are being used for extraction and purification process to achieve the fine powder. The continuous production of anti-cancer, anti-AIDS and other important life saving drugs requires large number of plants thus leading to the problem of depletion of several elite varieties of medicinal plants. Automated, bioreactor-based systems may be the method of choice for the mass propagation of shoots and hairy roots for the production of bioactive compounds.

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Bioenergy and Organic Manure Generation from Biodegradable Waste in Nisargruna Biogas Plant

Dr. S. P. Kale *is engaged in research on pesticide degradation and environmental pollution for last 27 years. He is the Chief Scientific Investigator of IAEA project on "Distribution, Fate and Effects of Pesticides on Biota in the Tropical Environment". He was the Chief Coordinator of an IAEA-BARC workshop on Use of Radiotracers in Pesticide Residue Analysis during December 1999. He has worked with Prof. Dr. Fuehr, Director, Institute of Radioagronomy, Juleich, Germany on use of lysimeters to study the fate of ^{14}C or otherwise labelled compounds on field scale. He is working on another IAEA project on Marine Pollution with respect to polyaromatic hydrocarbons, polycyclic benzenes and chlorinated hydrocarbons. He has developed NISARGRUNA plant for solid waste management. This has generated lot of interest in the last few months. He is also engaged in training M.Sc. and post doctoral students on applications of radioisotopes in Biology and Agricultural research. He is the recognized Ph. D. Guide for University of Agricultural Sciences, Dharwad, University of Mumbai and University of Pune. He is the Editorial Board Member of Environmental Technology, University of London (Selper Press), London. He is a popular science writer in Marathi.*

Introduction

Waste disposal is one of the major problems being faced by all nations across the world. It is more than a menace in our country. This is especially painful because our cultural heritage teaches us that waste and wealth are two sides of same coin. We worship the broomstick along with Laxmi on Diwali day. Science has also preached similar things but we tend to forget certain natural laws either due to their commonness or because of poor perception. The law of conservation is probably the most important of these laws. We are aware that nothing can be created or destroyed in this world. There is only a change in form. If we try to live in accordance with this law then we will realise that waste is not a problem but a part of life. We need to pay as much attention to it as we pay to the other necessities of life. In short, waste should not be a wasted resource!

The daily per capita solid waste generated in our country ranges from about 100g to 500g. Thus a small town with a population of 100000 would generate about 10 to 50 tonnes of waste daily. Metropolitan cities like Mumbai, Chennai, Delhi and the likes generate staggering amounts of waste. If we carefully analyse this waste we will realise that more than 60% of it is biodegradable. Waste like glass, metals and paper can be recycled. The biodegradable waste if handled properly would maintain the natural balance of essential elements and thereby promote more harvests from nature. Careful thought has to be given to the aspect of waste management on the national level. The hour demands that we educate the masses about the importance of waste management and segregation so that we may draw maximum benefit out of this resource.

Nisargruna Plant

Disposal of biodegradable waste can be achieved by several means. Vermiculture has been used in recent past in urban areas. One of the economic ways would be to raise community NISARGRUNA biogas plants developed at Bhabha Atomic Research Centre, Mumbai. It is based on

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Nisargruna Plant at INS Kunjali

biodegradable waste. They would serve many purposes.

1. Environmental friendly disposal of waste, which is need of hour considering mass pollution everywhere.
2. Generation of fairly good amount of fuel gas which will definitely support the dwindling energy resources.
3. Generation of high quality manure which would be weedless and an excellent soil conditioner. This is very important for replenishing fast decreasing resources of productive soils. It may be noted that need for replenishing the soil with high quality organic manure has been identified in tenth five-year plan.
4. It would reduce the menace of street dogs and other nuisance animals.
5. It would help in removing the garbage hills from rural and urban areas and help in achieving the dream of "Garbage-free Vasundhara".

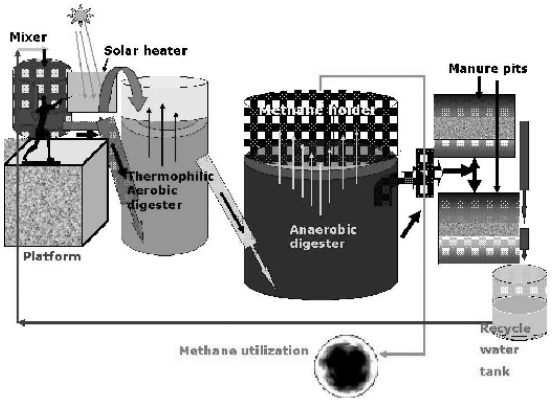
Conventional biogas plant developed mainly for processing gobar has been popularised by KVIC, India over last few decades. There are six models presently propagated by KVIC. They include KVIC floating drum type, Deenbandhu, Pragati KVIC plant with ferrocement digester, KVIC plant with fibre reinforced plastic gasholder and FLXI. Most of the modifications among these plants are structural rather than functional. These models are designed for small scale handling of gobar waste in villages. Against an estimated potential of 12 million family type biogas plants, only over 3 million plants have been installed in the country till recently and a large number of them are not functioning properly. So a vast potential still remains to be explored and this is only for one specific waste (gobar). Nisargruna concept can even help the existing biogas plants making it more efficient.

What makes Nisargruna Plant different from Conventional Gobar Gas Plant?

There is a definite need for developing means to handle enormous amounts of biodegradable waste that is generated daily. The waste organic matter that is generated in the kitchens can be processed using the modified biogas plant. One of the natural agencies, which will play an important role in this utilisation, is the tiny part of the huge world of tiny microbes. What is so special of these microorganisms? They can thrive in extreme environments where ordinarily no one can even imagine that there would be life. An organism that can happily grow in an extreme environment is an extremophile. The discovery of extremophiles has pumped a new life into the biotech industry and dreams of stock options in the minds of field biologists. The extreme environments include physical extremes like pressure, temperature and radiation and geochemical extremes like desiccation, salinity, pH and low redox potentials.

Thermophiles are the extremophiles that can thrive superbly at high temperatures. They have developed such enzyme systems that can help the organisms to not only survive at higher temperatures but also grow and reproduce. They have ability to use sulphurous waste and convert it into non-toxic products. Since the environment for such microorganisms sustains higher temperatures, many spoilage and pathogenic organisms cannot survive in such extreme conditions. Therefore it would be ideal if we could make use of these organisms to degrade the kitchen waste to remove more toxic elements and then subject it to the traditional biogas plant for methane generation. What we need to do is to maintain the high temperature in the predigestor tank.

In our country sunlight is available almost everywhere throughout the year except for some days in the months of July-August during monsoon.



This natural source of energy can be effectively used for providing the thermophilic microorganisms their natural environment. This energy is used to heat water and by controlling the proportion of hot water in the predigestor tank, one can achieve the desired temperature that can be easily sustained for about a day. This would provide favourable surroundings for the potential use of thermophiles to degrade the waste and sustain the culture. In places where sunlight may be a problem, part of methane generated in the system can be used for provision of hot water. Thus the system is self-sustainable and effective.

Another important aspect in the smooth running of a NISARGRUNA plant based on solid waste is how effectively one can avoid the choking of the plant. This choking may occur due to thick biomass that may be inaccessible to the microorganisms for digestion. The logical solution to such a problem is to convert the solid waste into slurry that would be far more accessible for the microbial action. A high power mixer to convert the solid waste into slurry can achieve this purpose. The afore mentioned two modifications will certainly improve design of the traditional biogas plant.

Design of NISARGRUNA Plant

Six NISARGRUNA plants have been installed at and around Anushaktinagar area in Mumbai for environmental friendly disposal of about 20 tonnes of waste generated in this area. A little introduction to the structure of plant would be appropriate at this juncture to understand the finer points of its operation.

The biogas plant has following components.

1. A mixer/pulper (5 HP motor) for crushing the solid waste
2. Premix tanks (3)
3. Predigester tank
4. Air Compressor
5. Solar heater for water heating
6. Main digestion tank (35 m^3)
7. Gas delivery system
8. Manure pits (4)
9. Tank for recycling of water and water pump
10. Gas utilisation system

Processing in NISARGRUNA Plant

The waste generated in kitchens in the form of vegetable refuge, stale cooked and uncooked food, extracted tea powder, waste milk and milk products can all be processed in this plant. Based on our understanding of thermophilic microorganisms in particular and microbial processes in general, there are two important modifications made in the conventional design of the biogas plant at BARC. We have introduced a 5 HP mixer to process the waste before putting it into the predigestor tank. The waste is converted into slurry by mixing it with water in a 1:1 ratio. Usually this is the failure point as solid waste is difficult to digest and can easily clog the system. The other modification is use of thermophilic microbes for faster degradation of the waste. The growth of thermophiles in the predigestor tank is assured by mixing the waste with hot water and maintaining the temperature in the range of 55-60^{o}C. The hot water supply is from a solar heater. Even few hours' sunlight is sufficient per day to meet the need for hot water.

After the predigestor tank the slurry enters the main tank where it undergoes mainly anaerobic degradation by a consortium of archaebacteria belonging to *Methanococcus* group. These bacteria are naturally present in the alimentary canal of ruminant animals (cattle). They produce mainly methane from the cellulosic materials in the slurry. The undigested lignocellulosic and hemicellulosic materials are then passed on to the settling tank. After about a month, high quality manure can be dug

out from the settling tanks. There is no odour to the manure at all. The organic contents are high and this can improve the quality of humus in soil, which in turn is responsible for the fertility.

As the gas is generated in the main tank, the dome is slowly lifted up. It reaches a maximum height holding 55 m^3 of gas. This gas is a mixture of methane (70-75%), carbondioxide (10-15%) and water vapours (5-10%). It is taken through GI pipeline to the lamp posts. Drains for condensed water vapour are provided on the pipeline. This gas burns with a blue flame and can be used for cooking as well.

The gas generated in this plant is used for gas lights fitted around the plant. The potential use of this gas would be for cooking purposes. It can also be used to produce electricity. The manure generated is high quality and has been used for our nursery and gamma field at BARC.

It must be stressed that the success of this biogas plant depends a great deal on proper segregation of the kitchen waste. The materials that can pose problems to the efficient running of the plant are coconut shells and coir, egg shells, bones and plastic pieces. Steel utensils like dishes, spoons etc. are likely to appear in the waste bags from hotels and household kitchens. While bones, shells and utensils can spoil the mixer physically, coir and plastic can have detrimental effects on microbial consortium in the predigester and main digestion tanks which could be disastrous for the plant. Hence it is necessary that following precautions may be taken while collecting the kitchen waste. There should be a separate container for coconut shells, coir, egg shells, and bones. These will not be processed in the biogas plant. There should be separate containers of small volumes (5 L capacity) to collect the wet waste (spoilt or stale cooked food, waste milk products etc.). The vegetable refuse like peels of various vegetables, rotten potatoes, and tomatoes, coriander leaves etc. may be collected in garbage bags of 5-kilo capacity. It must be noted that such segregation is of utmost importance for smooth running of the biogas plant.

Thus the efficient disposal of kitchen waste can be ecofriendly as well as cost effective. While calculating the cost effectiveness of such waste disposal one has to consider more than monetary aspects. The dumping of uncooked food in unmanned areas may not be very civilized. It can also lead to growth in the population of nuisance animals. It is undoubtedly unhygienic and can pose a threat to the habitat. These factors will add to the value of such plants. Using the natural friends in the form of thermophiles, methanogenic microorganisms and their consortia we can certainly handle the kitchen waste and a variety of other biodegradable wastes.

It must be noted that BARC NISARGRUNA plant is suitable as a community plant rather than for individual dwellings. City corporations, big hotels, government establishments, housing colonies, residential schools and colleges, hospitals, power plants, Agricultural Produce Market Committees and large factories can easily set up such plants and process their wastes in a most environment-friendly way. It would generate employment as well, and it would easily be self-sustainable looking at the fertiliser and gas output. Though its initial cost may be relatively higher than conventional gobar gas plant, the BARC model will be more reliable and enduring due to modifications made in it to avoid choking and the variety of biodegradable wastes it can handle. Instead of comparing it with gobar gas plant, we must consider this NISARGRUNA plant as an independent unit. That way we can avoid certain unrealistic comparisons.

Science of NISARGRUNA Plant

NISARGJYOTI (biogas) microbes consist of a large group of complex and differently acting microbe species, notably the methane-producing bacteria. The whole process of formation of NISARGJYOTI (biogas) can be divided into three steps: hydrolysis, acidification, and methane formation. Various types of bacteria are involved in these processes.

Hydrolysis

In the first step (hydrolysis), the organic matter is enzymolyzed externally by extracellular enzymes (cellulase, amylase, protease and lipase) of microorganisms in the predigestor. Bacteria decompose the long chains of the complex carbohydrates, proteins and lipids into shorter parts.

For example, polysaccharides are converted into monosaccharides. Proteins are split into peptides and amino acids.

Acidification

Acid-producing bacteria, involved in the second step, convert the intermediates of fermenting bacteria into acetic acid (CH_3COOH), hydrogen (H_2) and carbon dioxide (CO_2) in the predigestor. These bacteria are aerobic and facultatively anaerobic and can grow under acid conditions. We have isolated about 21 *Bacillus* species so far from the predigester slurry. An air compressor would be required to keep the conditions in the predigestor aerobic. To produce acetic acid, they need oxygen and carbon. For this, they use the oxygen solved in the solution or bounded-oxygen. Hereby, the acid-producing bacteria reduce the compounds with a low molecular weight into alcohols, organic acids, amino acids, carbon dioxide, hydrogen sulphide and traces of methane. The pH of the raw slurry falls from 7.5 to about 3-4 in the predigestor.

Methane Formation

Various types of methanogenic bacteria: The spherically shaped bacteria are of the methanosarcina genus; the long, tubular ones are methanothrix bacteria, and the short, curved rods are bacteria that catabolize furfural and sulfates. The total length of the broken bar at the top left, which serves as a size reference, corresponds to 1 micron. Methane-producing bacteria, involved in the third step, decompose compounds with a low molecular weight. For example, they utilize hydrogen, carbon dioxide and acetic acid to form methane and carbon dioxide. Under natural conditions, methane-producing microorganisms occur to the extent that anaerobic conditions are provided, e.g. under water (like in marine sediments), in ruminant stomachs and in marshes. They are obligatory anaerobic and very sensitive to environmental changes. In contrast to the acidogenic and acetogenic bacteria, the methanogenic bacteria belong to the archaebacter genus, i.e. to a group of bacteria with a very heterogeneous morphology and a number of common biochemical and molecular-biological properties that distinguish them from all other bacteria in general. The main difference lies in the make-up of the bacteria's cell wall.

The separation of two stages in methane production helps in improving the purity of methane gas, thereby increasing its fuel efficiency. We have registered the purity of methane as high as 90% in peak summer season. However the average composition round the year would depend upon how effectively we can maintain the predigestor temperatures.

Parameters and Process Optimization

The metabolic activity involved in microbiological methanation is dependent on the following factors:

- Substrate temperature
- Available nutrients
- Retention time (flow-through time)
- pH level
- Nitrogen inhibition and C/N ratio
- Substrate solid content and agitation
- Inhibitory factors

Each of the various types of bacteria responsible for the three stages of the methanogenesis is affected differently by the above parameters. Since interactive effects between the various determining factors exist, no precise quantitative data on gas production as a function of the above factors is available. Thus, the discussion of the various factors is limited to their qualitative effects on the process of fermentation.

Substrate Temperature

Aerobic fermentation by thermophilic bacteria is in principle possible between 40°C and approximately 70°C. The third stage anaerobic process can occur at lower range of temperature (3°C to 60°C).

Changes in Temperature

The processes of acidification and bio-methanation are very sensitive to *changes* in temperature. The degree of sensitivity, in turn, is dependent upon the temperature range. Brief fluctuations not exceeding the following limits may be regarded as still uninhibitory with respect to the process of fermentation: psychrophilic range: ±2°C/h; mesophilic range: ± 1°C/h; thermophilic

range: ± 0.5°C/h. The temperature fluctuations between day and night are no great problem for plants built underground, since the temperature of the earth below a depth of one meter is practically constant.

Available Nutrients

In order to grow, bacteria need more than just a supply of organic substances as a source of carbon and energy. They also require certain mineral nutrients. In addition to *carbon, oxygen* and *hydroge*n, the generation of bio-mass requires an adequate supply of *nitrogen, sulfur, phosphorous, potassium, calcium, magnesium* and a number of trace elements such as *iron, manganese, molybdenum, zinc, cobalt, selenium, tungsten, nickel* etc. "Normal" substrates such as agricultural residues or municipal solid waste usually contain adequate amounts of the mentioned elements. Higher concentration of any individual substance usually has an inhibitory effect, so analyses are recommended on a case-to-case basis to determine what amount of whichever nutrients, if any, still needs to be added.

Cost Efficiency

The process parameters such as *retention time, process temperature, substrate quality,* and *volumetric load* determine, among others, the cost efficiency of the biological processes. But as each m^3 digester volume has its price, heating equipment can be costly and high quality substrates may have alternative uses, the cost-benefit optimum in NISARGJYOTI (biogas) production is almost always below the biological optimum.

Retention Time

If the retention time is too short, the bacteria in the digester are "washed out" faster than they can reproduce, so that the fermentation practically comes to a standstill. This problem rarely occurs in agricultural NISARGRUNA systems. We have observed that the gross retention time in predigestor is 3-4 days while that in methanogenic tank is 10-12 days. In the conventional gobar gas plants the retention time is about 30-35 days. For liquid manure undergoing fermentation in the mesophilic temperature range, the following approximate values apply: liquid cow manure: 20-30 days; liquid pig manure: 15-25 days; liquid chicken manure: 20-40 days; animal manure mixed with plant material: 50-80 days. Thus the NISARGRUNA system scores over the conventional systems as the efficiency for a given volume of waste increases by at least 3 times. It also increases the scope of the plant from specific waste material to that of a varied nature.

pH Value

The pH of incoming waste material generally is in the range of 7 to 8. In the predigestor tank mainly acid production takes place and pH drops to 4 to 5. This acidic slurry flows in the methanogenic tank where the acids are rapidly utilized. The methane-producing bacteria live best under neutral to slightly alkaline conditions. The incoming slurry has about 15% of the total volume in the methanogenic tank. This kind of dilution would not affect the pH range in this tank. Once the process of fermentation has stabilized under anaerobic conditions, the pH will normally take on a value of between 7 and 8.5.

C/N Ratio

Microorganisms need both nitrogen and carbon for assimilation into their cell structures. Various experiments have shown that the metabolic activity of methanogenic bacteria can be optimized at a C/N ratio of approximately 8-20, whereby the optimum point varies from case to case, depending on the nature of the substrate. If there is a lot of paper to be processed in the NISARGRUNA plant, the C/N ratio being less, there will have to be some supplementation needed to increase nitrogenous quantity.

Economics of NISARGRUNA PLants

Labour intensive decentralized NISARGJYOTI (biogas) units, on the regional level, improve income distribution amongst income brackets and reduce regional disparities, enhancing the attractiveness of rural life. The design of NISARGRUNA plant does not involve any imported materials and hence is truly indigenous. The indigenous nature of NISARGRUNA means less of external diseconomies, which may arise in consequence of varying exchange rates.

In a macro-economic level these effects are significant and only unfold themselves fully if NISARGRUNA plants are introduced over a wide area i.e. for closed settlement areas. This is the NEED OF HOUR and hence quite practical in our context. It also refers primarily to NISARGRUNA plants as an improvement for inferior sanitary and hygienic conditions for members of the poorer classes. These are problems, which cannot be solved on an individual basis but only by collective decisions and measures. How far NISARGRUNA plants in a definite case are the suitable and advantageous solution to a problem has to be discovered with reference to alternative sectoral measures. The macro-economic evaluation needs to account for effects of benefits within the fields:

- Energy and organic manure
- Zero garbage, zero effluent
- Elemental balance
- Environment
- Health Sector
- Employment

Energy

Many developing countries, especially like ours, base their energy consumption upon traditional energy sources (wood, plants and crop residues and animal waste, as well as animal traction and human muscle power). Biomass energy use varies widely in developing countries from as little as 5% in Argentina to over 90% of the total supply of energy sources in countries like Ethiopia, Tanzania, Rwanda, Sudan and Nepal. In the case of wood, plant and animal waste, according to local necessities, the energy source is collected and used. Surplus of energy sources is traded informally on the local and regional level. In so far estimations on the potential effects of NISARGJYOTI (biogas) use instead of the use of traditional energy sources do not have any impact on government's budget, presuming the non-existence of taxes on traditional energy sources. Negative consequences on the income of the local traders may result, presuming less demand on traditionally traded energy sources, causing a slump of its prices. On the other side NISARGJYOTI (biogas) users may continue with trading of traditional energy sources on more distant markets (or even will be encouraged to trade on regional levels), not willing to forego secure earnings. Consequently, the substitution effect of NISARGJYOTI (biogas) results primarily in environmental benefits due to less consumption of i.e. firewood, leading to less deforestation (under the presumption of a declining or constant price of firewood). Commercially or monetarily traded sources like petroleum, coal and natural gas on the other hand have impacts on the balance of payments and therefore influence governmental budgets.

The macro-economic effect of a NISARGJYOTI (biogas) use provides a substitution for kerosene. This may reduce government's earnings due to decreasing duty income. On the other side as petroleum import dependency decreases, the internal economy of the nation becomes more stable. Although only less than 10% of a country's commercial energy is consumed by the rural population, the effects of NISARGJYOTI (biogas) use, substituting systems for generation, transmission and distribution of electricity shall be mentioned. The macro-economic benefits of a NISARGRUNA plant result in its self-efficiency and reliability (benefits from avoidance of blackouts and supply interruptions) and in less costs for networks and distribution infrastructure.

Organic Manure

On the assumption that the slurry of the NISARGRUNA plant is used as organic manure and, when spread on the fields, it increases the crop production and the economies' benefit amounts to a higher supply of organic manure given the same output level of crops. Moreover, the substitution of commercial fertilizers with organic manure produced by NISARGRUNA technology reduces the impacts on balance of payments (assuming a dependence on imports of chemical fertilizers). The consequence of reliance on digested dung and residues (in a NISARGRUNA plant) is that valuable nutrients and organic matter are led back to the soil in an improved stage, rising agricultural productivity and soil stability (combating devegetation and desertification). The higher productivity of crop production results in higher yields, maybe keeping pace with the increase in population (maybe: because one has to estimate the balance of population fluctuations).

Soil Protection and Reforestation

A unique feature of NISARGRUNA technology is that it simultaneously reduces the need for firewood and improves soil fertility. It compensates for soil erosion. Traditionally, wood fuel claims the largest proportion of biomass fuels (in some regions up to 90%) used in developing countries, where about 40% of the total wood cut annually is used for domestic purposes (cooking and heating). Estimating an average per capita consumption of 3 kg of wood per day for energy (cooking, heating and boiling water) in rural areas in Asia and Africa, the daily per capita demand of energy equals about 13 kWh which could be covered by about $2m^3$ of NISARGJYOTI (biogas). A NISARGRUNA plant therefore directly saves forest, assuming that not only deadwood is collected for fuel. Firewood consumption in rural households is one of the major factors contributing to deforestation in developing countries. Most firewood is not acquired by actually cutting down trees, but rather by cutting off individual branches, so that the tree need not necessarily suffers permanent damage. Nonetheless, large amounts of firewood are also obtained by way of illegal felling. In years past, the consumption of firewood has steadily increased and will continue to do so as the population expands - unless adequate alternative sources of energy are developed. In many developing countries such as India, the gathering of firewood is, strictly speaking, a form of wasteful exploitation. Rapid deforestation due to increasing wood consumption contributes heavily to the acceleration of soil erosion. This goes hand in hand with overgrazing which can cause irreparable damage to soils. In the future, investments aimed at soil preservation must be afforded a much higher priority than in the past. It will be particularly necessary to enforce extensive reforestation. Without any effective political measures, the problem of deforestation and soil erosion will become more and more critical. As the population increases the consumption of firewood will increase more steeply. Without NISARGRUNA (biogas) the problem of deforestation and soil erosion will steadily become more critical as firewood consumption rises relative to higher density of population. The demand for nourishment also rises accordingly, which means that constant extension of agricultural land increases at the expense of forested areas. The soil erosion associated with deforestation in its advanced state reduces quantitatively and qualitatively the potential of agricultural land. Finally, this leads to future increases in the cost of food production. Moreover, the advancing soil erosion increases the frequency and extent of floods and their disastrous consequences.

The widespread production and utilization of NISARGJYOTI (biogas) is expected to make a substantial contribution to soil protection and amelioration. First, NISARGJYOTI (biogas) could increasingly replace firewood as a source of energy. Second, NISARGRUNA (biogas) systems yield better fertilizer. As a result, more fodder becomes available for domestic animals. This, in turn, can lessen the danger of soil erosion attributable to overgrazing. According to the ICAR paper (report issued by the Indian Council of Agricultural Research, New Delhi), a single NISARGRUNA system with a volume of 100 cft ($2.8\ m^3$) can save as much as 0.3 acres (0.12 ha) woodland each year. Taking India as an example, and assuming a NISARGJYOTI (biogas) production rate of 0.36 m^3/day per livestock unit, some 300 million head of cattle would be required to produce enough NISARGJYOTI (biogas) to cover the present consumption of firewood. This figure is somewhat in excess of the present cattle stock. If, however, only the amount of firewood normally obtained by way of deforestation (25.2 million trees per year) were to be replaced by NISARGJYOTI (biogas), the dung requirement could be satisfied by 55 million cattle.

If NISARGRUNA plants were developed to take care of mammoth biodegradable waste generated in the country and supplement the existing biogas plants, probably we would end up in positive environmental balance. This would mean more forestation, lesser soil erosion and better soil fertility.

Firewood consumption could be reduced to such an extent that – at least under the prevailing conditions - a gradual regeneration of India's forests would be possible. According to empirical data gathered in India, the consumption of firewood in rural households equipped with a biogas system is much lower than before, but has not been fully eradicated. This is chiefly attributable to a number of

technical and operational shortcomings. Nisargruna can help in reducing these shortcomings. In order to predict the direct monetary savings to an economy, two procedures are to be carried out: If the forest has not previously been used economically, shadow pricing has to be based on the valuation of saved biodiversity, respectively on the capacity of reducing the effects of global warming. If the forest has been used economically, several procedures of shadow pricing can be carried out, like: Value of saved forest via price of firewood. Given the price of cut firewood on the local market, the savings of forest by substitution of NISARGJYOTI (biogas) can be determined by multiplication of the number of trees cut, its tree growth ratio per year and the average price of firewood.

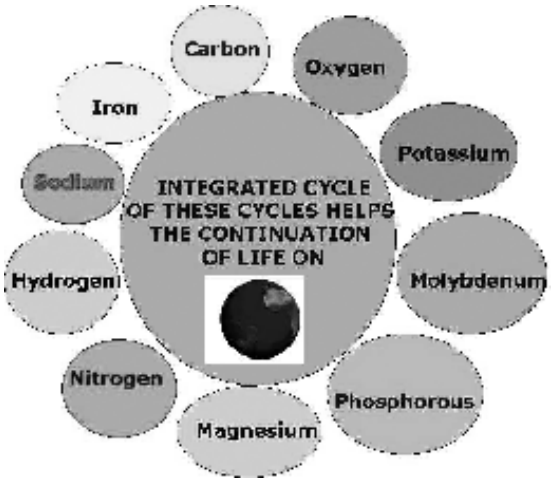
Value of saved forest as an area for nourishment (hunting, collecting fruits, etc.): The value of the forest equals the sum of income forgone from these activities. The correct shadow pricing would be based on the prices of the goods on the formal consumer markets (i.e. price of meat). Value of saved forest as a recreation area The value of the forest equals the sum of the incomes obtained by charges for admission to National Parks, Wildlife Areas, etc.

Zero Garbage Zero Effluent

NISARGRUNA plant processes biodegradable waste. There is no garbage coming out of the plant. The volume and weight reduction achieved through NISARGRUNA plant reduces the need of landfill sites and dumping yards to a very large extent. After the waste is processed in the mixer and the slurry passes in the aerobic predigestor tank, it is not seen at all. Thus the biodegradable waste is totally nullified. Recycling of water by provision of sand filters in slurry tanks contributes to economic use of water till the last drop. Good quality water is required only for solar heaters. Water requirements of NISARGRUNA plant may be satisfied to a larger extent by effluents from sewage treatment plants if available in the vicinity. Thus the project achieves the aim of Zero Garbage Zero Effluent.

Elemental Balance

Law of conservation energy and law of conservation of matter are strictly followed by NATURE. NISARGRUNA plant helps NATURE to maintain various biogeochemical cycles of elements. Solid waste management done in a traditional way (!) does not help the maintenance of these cycles. Incineration is totally against these laws. We must realize that microorganisms play an important role in these cycles. Their strategic position in all these cycles can only be acknowledged in processes similar to NISARGRUNA.



Health Sector

In order to estimate the impacts on the health sector, benefits arise on the individual level, as well as on the level of the society. NISARGRUNA plants serve as methods of disposal for waste and sewage and in this way directly contribute to a better hygienic situation for individual users. By collecting centrally dung and by connecting latrines, open storage is avoided. Apart from this, pathogens are extensively eliminated during the digestion process. All in all quite an improvement of sanitation and hygiene is achieved and therefore a NISARGRUNA plant can contribute to a higher life expectancy. In the People's Republic of China this effect became apparent in the bilharziosis, worm and gastro-disease endangered areas where the number of people suffering was greatly reduced. Theoretically, a reduction in the frequency of disease

comprises economically a saving in medicine and consultation costs. Regarding the leakage of health services in rural areas, another approach to savings is suggested: Labour productivity rises due to elimination of potential disease-causing agents due to the better hygiene situation in consequence of NISARGRUNA plants. Applied to individual NISARGRUNA projects, these economic effects cannot be credited directly to NISARGRUNA projects in monetary terms, as there are plenty of influences on the health sector. If the main goal of a NISARGRUNA plant is to achieve a higher standard of hygiene, one possible method of shadow pricing would be the answer to the question: Which alternative investment in providing the same result of hygiene equals the positive hygiene results of a NISARGRUNA plant?. The evaluation of sanitary and hygienic effects can be made i.e. by means of the alternative costs for a purifying plant.

Employment

During construction of NISARGRUNA plants unless the investors build these themselves, there are effects on regional/local income and employment which subsequently continue. Permanent jobs, unless users participate, are created for the operation personnel and indirect effect result in contracts with local and regional companies for the service and maintenance of a plant including the mixers, gas burners in the households, compressors and generators. This will have direct effects on agricultural, environmental, health and waste management sectors. The utilization of NISARGJYOTI (biogas) contributes to an enlarged range of energy fuels offered on the market. In this way the local basis of the energy supply can be extended and secured, and it also simplifies the setting of additional commercial activities where the factor energy has so far proved to be a problem.

In our country NISARGRUNA plants can provide good employment opportunity for the lower strata. The rag pickers in various cities could be uplifted using this technology. Unskilled labour is required for handling the waste in NISARGRUNA plants, removal and processing of manure and maintaining the plant. The need of very large number of such plants nationwide opens up a possibility of good employment opportunities. Some redeployment also will be involved in the process. Various self-help groups could be encouraged to run these plants.

Why Nisargruna Technology should be adopted Nationwide?

NISARGJYOTI (biogas) gained by a three-step digestion process (hydrolysis, aerobic and anaerobic) containing 60-80 per cent methane and 10-20 per cent carbon dioxide (remaining part would be moisture) makes it a potential source of renewable energy. Given a heating value of about 5.5 kcal/m^3, its uses for electricity generation, as a heat resource, for internal combustion engines, boilers, as a supplementary fuel for diesel engines or substitution of firewood for cooking purposes in rural areas are widely reported. Especially the economic benefits of NISARGJYOTI (biogas) utilization in selected agro-industries (palm oil mills, tapioca starch factories and alcohol distilleries) amount to savings due to electricity generation by NISARGJYOTI (biogas), fertilizer savings and rising productivity in agriculture. Moreover, the environmental benefits due to substitution of energy sources based on wood (firewood, charcoal) or on fossil energy sources can be outstanding.

Without any doubt - even if there would be constructed only one NISARGRUNA plant in a country - the following valuable assets of NISARGJYOTI (biogas) use from the environmental point of view can be determined. *As CO_2 generation by burned NISARGJYOTI (biogas) only amounts to 80 per cent of the CO_2 generation of fired fuel oil (per kWh electrical energy) and is even more advantageous in relation to coal (about 50 per cent), the environmental benefits of NISARGJYOTI (biogas) in relation to fossil fuels are indisputable.* Due to the high cohere efficiency of wood (0.7 kg CO_2 per kWh gross energy), the substitution of the wood based biomasses by NISARGJYOTI (biogas) rise the national and global storage capacity of CO_2.

Facing more and more the challenging phenomena of global warming and setting global standards of polluting potentials, environmental external economies are getting steadily very important issues and may stimulate a government to start investing in appropriate energy technologies rather than to follow the conventional way to solve the problem of generating energy in remote areas by

rural electrification based on fossil fuels. A financially viable and well-structured joint implementation concept may help to generate (financial) facilities to governments in order to invest in energy generation, based on sustainable energy sources. How far and to which partner (of the partnership) the positive effects of the project shall be ascribed to, may be determined politically. In the long run each saving of irreparable damage to the environment helps to save the world as a whole.

Environmental Biosensors

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Introduction

Stricter regulations and a greater public awareness of environmental issues have necessitated the need to monitor wider range of analytes in air, water and soil, and to do so with greater frequency and accuracy. Analysts currently have a range of portable analytical techniques at their disposal for monitoring across a variety of environmental analytes. More recently, biosensors have emerged as another promising technology in the analyst's armory, especially for applications requiring continuous monitoring. The term biosensor is

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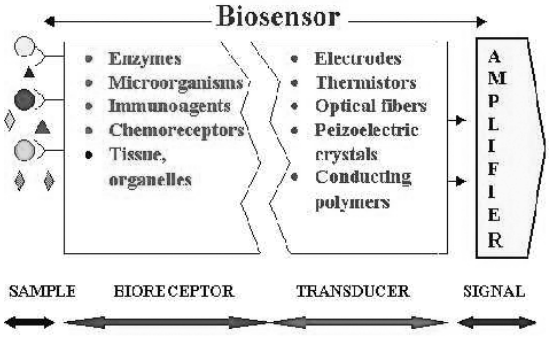

Fig. 1 Components of biosensor: Schematic diagram

defined as a sensor incorporating biological elements such as enzymes, antibodies, receptors proteins, nucleic acids, cells, or tissue sections - as the recognition element, coupled to a transducer. Specific interactions between the analyte and the biorecognition element produce a physico-chemical change, which is detected and measured by the transducer (Fig. 1). The amount of signal generated is proportional to the concentration of the analyte, allowing for both quantitative and qualitative measurements in time [1].

Biosensor Technology

The two main elements in a biosensor are a biological recognition element or bioreceptor and a signal transducer. The bioreceptor is a biomolecule that recognizes the target analyte and can be divided into three distinct groups: biocatalytic, bioaffinity, and microbe-based systems. Biocatalysis-based biosensors depend on the use of pure or crude enzymes to moderate a biochemical reaction. For environmental applications, enzyme-based reactions involve enzymatic transformation of a pollutant or inhibition of enzyme activity by the pollutant. Enzyme inhibition approaches tend to cater for a larger number of environmental pollutants, usually of a particular chemical class such as pesticides and heavy metals. However, such methods require the use of substrates and in some cases the biosensor may need to be reactivated due to the inhibition.

Bioaffinity-based biosensors rely on the use of proteins or DNA to recognize and bind a particular target. For environmental applications such systems depend primarily on the use of antibodies. This is due to the ready availability of monoclonal and polyclonal antibodies directed toward a wide range of environmental pollutants, as well as the relative affinity and selectivity of these recognition proteins for a specific compound or closely related groups of compounds. Nucleic acid-based affinity and electrochemical biosensors for potential environmental applications have recently been reported. Application areas for these include the detection of chemically induced DNA damage and the detection of microorganisms through the hybridization of species-specific sequences of DNA [2].

Microbial biosensors use microorganisms as the biological recognition element. These generally involve the measurement of microbial respiration, or its inhibition, by the analyte of interest. Compared to enzyme-based approaches, microorganism-based biosensors are relatively inexpensive to construct and can operate over a wide range of pH and temperature. The broad specificity of microbial biosensors to environmental toxins make them particularly applicable for general toxicity screening like biological oxygen demand (BOD) or in situations where the toxic compounds are well defined, or where there is a desire to measure total toxicity through a common mode of action. Biosensors have also been developed using genetically modified microorganisms (GMOs) that recognize and report the presence of specific environmental pollutants [1].

A signal transducer is the second essential component of a biosensor. It converts the recognition event into a measurable signal. The transducer can take many forms depending upon the parameters being measured. The most well developed classes of transducers are potentiometric, amperometric, conductometric, optical, acoustic or piezoelectric etc. These utilize various electrochemical responses to measure changes in the electrical properties of the biological recognition element. Most of the reported potentiometric biosensors for detection of environmental pollutants have used enzymes that catalyze the consumption or production of protons. Phosphoric and carbamate pesticides can be evaluated through the use of a pH

electrode that measures the activity of acetylcholinesterase [1]. The activity of the enzyme is affected by the presence of pesticides. In another application, heavy metals can be measured using the inhibition of enzyme urease, coupled to an ammonia selective electrode [3].

Amperometric biosensors are based on monitoring the current associated with oxidation or reduction of an electroactive species involved in the recognition process. The current produced is linearly proportional to the concentration of the electroactive product, which in turn is proportional to the non-electroactive enzyme substrate. An example of this configuration would be acetylcholinesterase coupled to an amperometric sensor used to detect hydrogen peroxide as described in the following reaction:

$$\text{Acetylcholine} + O_2 + H_2O \xrightarrow{\text{Acetylcholinesterase}} \text{Choline} + \text{Acetate}$$

$$\text{Choline} + 2O_2 + H_2O \xrightarrow{\text{Choline Oxidase}} \text{Betaine} + H_2O_2$$

Compounds of environmental concern, measured using amperometric and electrochemical electrodes include 2,4-Toluene diamine (2,4-T) [4] polychlorinated biphenyls (PCBs), triazines and various toxins such as serin and soman [5].

Enzyme reactions, which produce or consume ionic species, can be monitored conductometrically depending on the total ionic strength of the media. Thin film interdigitated planar conductometric electrodes have been used to measure heavy metals [6]. Glucose oxidase, alcohol oxidase, butyril oxidase and urease have been immobilized on transducer surfaces and used as bioactive elements for detection of Ag^{+}, Hg^{+2} and Pb^{2+} [7].

Transducers based on optical detection techniques have also been used in the field of biosensors. These may employ linear optical phenomenon, including fluorescence, phosphorescence, polarization, rotation, interference, surface plasmon resonance (SPR), total internal reflection fluorescence (TIRF), etc. or non-linear phenomena, such as second harmonic generation [8]. Advantages of optical techniques involve the speed and reproducibility of the measurement. Optical transducers have been used for affinity-based biosensors, and for a few enzyme- as well as microbial biosensors for environmental applications. A microbial-based optical biosensor for the detection of organophosphate pesticide is being developed in our laboratory. Fiber optic immunosensor for 2,4,6-trinitrotoluene (TNT) and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) have also been developed [9].

Piezoelectric crystals have also been used in biosensor applications. The vibration of piezoelectric crystals produces an oscillating electric field in which the resonant frequency of the crystal depends on its chemical nature, size, shape and mass. By placing the crystal in an oscillating circuit, the frequency can be measured as a function of the mass. The detection limit of mass bound to the electrode surface is about 10^{-10} to 10^{-11} g. These transducers have been coupled with various biomolecules especially antibodies to detect analytes, including microbial load, formaldehyde, cocaine and parathion. Direct as well as indirect antibody-based piezoelectric sensors have been reported for the atrazine and 2,4-D [2].

The basic requirement of a biosensor is that the biological material should bring the physico-chemical changes in close proximity of a transducer. In this direction immobilization technology has played a major role [1]. Immobilization not only helps in forming the required close proximity between the biomaterial and the transducer, but also helps in stabilizing it for reuse. The biological material is immobilized directly on the transducer or in most cases, in membranes, which can subsequently be mounted on the transducer. Selection of a technique and/or support would depend on the nature of the biomaterial and the substrate and configuration of the transducer used [10,11].

Some of the widely used immobilization techniques include adsorption, entrapment, covalent binding and cross-linking [12,13]. Immobilization of enzymes and whole cells through adsorption perhaps is the simplest of all the techniques. Enzymes have been immobilized through adsorption on a variety of ion exchange, hydrophobic and affinity surfaces [12]. Most of these techniques have the drawbacks of weak adhesion as well as complexity of the process. Novel techniques have

Table 1 - Biosensors applied for the determination of pollutants in real samples

Analyte	Sample source	Transducer, recognition element
Pesticides	River water	Optical, immunochemical
Phenols	Wastewater	Electrochemical, enzymatic
Linear alkyl benzene sulphonate (LAS)	River water	Electrochemical, bacteria
Toxicity	Wastewater	Electrochemical, bacteria
Toxicity	Wastewater	Optical, bacteria
Alkanes	Groundwater	Optical, bacteria
Estrogens and xenoestrogens	Real water samples (lake and a sewage plant)	Optical, human estrogen receptor (EC)
BOD	River water	Optical, Pseudomonas sp.
Zinc dichromate chromate	Soil (extract)	Optical, bacteria
Mercury arsenite	Soil (extract)	Optical, Pseudomonas sp.
Daunomycin, PCBs, aflatoxin	River water (preconcentrated)	Electrochemical, DNA
Chlamydia trachomatis (DNA)	River water (preconcentrated)	Electrochemical, DNA

been developed in our laboratory for immobilizing viable or non-viable cells through adhesion on a variety of polymeric surfaces including glass, cotton fabric and synthetic polymeric membranes using polyethylenimine (PEI) [14]. This technique is gaining importance in the introduction of enzymes and microbes on transducer surfaces [15].

Synthetic polymers are also used for the entrapment of the biological materials in a membranous form for biosensor applications. Some of these include polyacrylamide, polyurethane-based hydrogels, photo cross-linkable resins and polyvinyl alcohol (PVA). Natural polymers used for the entrapment of the biomolecules include alginate, carrageenan, low-melting agarose, chitosan, etc. [1, 10,11]. These polymers are known to be very useful in obtaining viable cell-immobilized systems. Apart from these, a number of polymeric membranes can be prepared using radiation polymerization under frozen conditions. This helps in not only controlling the shape, size and porosity of the membranes but also minimizes the damage to the enzymes by thermal denaturation encountered in the chemical polymerization approach [16].

Commercial Biosensors

Although most biosensors systems have been tested only on non-real samples (such as in distilled water or buffer solutions), a few biosensors applied to real samples have appeared in recent years. Some representative examples of their application to the determination of different classes of key pollutants and environmental quality parameters, such as biological oxygen demand (BOD), toxicity or endocrine effects, in a variety of matrices are listed in Table 1 [17]. The application of biosensors to real samples must be a necessary step before their commercialization, which is, in general, the aim of the device development. Results must also be validated by comparison with those obtained with standard protocols in order to get the acceptance of end users.

Most commercial biosensors developed are focused in clinical applications, such as for glucose and lactate. Prospective biosensor market for food, pharmaceutical, agriculture, military, veterinary and environment are still to be explored. A brief list of commercially available biosensors for environmental applications is listed in Table 2.

Table 2 - Commercially available biosensors for environmental applications

Company	Instrument	Analyte	Transducer & recognition elements	Web page
Texas Instruments Inc. USA	Spreeta	Toxicity assay, liquid quality & concentration.	Optical SPR, biofilm as recognition element	www.ti.com
XanTec Bioanalytics, Germany	Ibis	Toxicity assay from wastestream	SPR Based immunosensors	www.xantec.com
Remedios, Scotland	Remedios	Volatile hydrocarbon, non-volatile hydrocarbon, heavy metals	Optical Bioluminescence inhibition, whole cell	Www.remedios.uk.com
Aclara Bioscience, USA	eTag Assay System	Whole cells, pathogens detection,	Optical eTag fluorescent reporters linked to antibodies and peptides	www.aclara.com
Lincoln Ventures Ltd, New Zealand	Micredox	Environmental monitoring based on mediated cellular Respiration, For BOD and Toxicity (Cu, Cr, and As)	Amperometric, Whole cell based using microelectrodes	www.lincoltechnology.co.nz

Future Prospects

Some of the obstacles common to biosensor technology include: the diversity of compounds and the complexity of environmental samples. These hurdles also include: relatively high development costs for single analyte systems, limited shelf and operational life times for pre-manufactured biorecognition components and complexity in devising potentially portable biosensor systems. Nevertheless, there are a number of areas where the unique capabilities of biosensors might be exploited to meet the requirements of environmental monitoring. Advances in areas such as multi-pollutant-screening could allow these techniques to be more competitive. The present scenario demands for increased range of detectable analytes with portable device structure. Solving the resulting integration issues will require further convergence with associated technologies such as biochemistry, polymer chemistry, electronics, micro-fluidics and separation technology.

Micro-Electro-Mechanical Systems or MEMS technology is one of the promising areas that may be going to fulfill these demands in future. The technology is an integration of mechanical elements, sensors, actuators, and electronics on a common silicon substrate through micro fabrication technology. Biochips and sensor arrays for detection of a wide range of hazardous chemical and biological agents can be made out of these MEMS based devices, making it feasible for simultaneous detection of multiple analytes. This also brings the lab-on-chip concept. However, Immobilization and stabilization of biomolecules on these nanodevices may be a greater challenge. Some of the works in these areas have already been initiated. Utilization of molecular recognition ability of biomolecules like avidin-biotin or streptavidin-biotin in conjunction with a lithographic technique is being investigated for the micro immobilization of enzymes on silicon wafers for biosensor applications [18]. Immobilization of enzymes on silicon supports has attracted attention in biosensor chip technology and a variety of classical techniques have been proposed [2].

There are interesting possibilities within the field of biosensors. Given the existing advances in biological sciences, coupled with advances in various other scientific and engineering disciplines, it is imminent that many analytical applications will be replaced by biosensors. A fruitful fusion between biological sciences and other disciplines will help to realize the full potential of this technology in the future.

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Eco-friendly Methods for the Management of Insect Pests of Agricultural Importance

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Introduction

When synthetic chemical pesticides were introduced in 1940s, they were hailed as miracles in the field of pest control. However, it did not take long to recognize the deadly consequences associated with these miraculous chemicals. There are now many evidences that some of these chemicals pose potential risk to humans and other life forms in the environment [1-3]. Though no segment of the population is completely protected against exposure to pesticides, a disproportionate burden is shouldered by the people of developing countries [4]. The worldwide deaths and chronic illnesses as a result of pesticide poisoning number about 1 million per year [5]. India accounts for one third of pesticide poisoning cases in third world. The first pesticide poisoning report was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion. Since then there are many such incidents and this rampant use of chemicals, under the adage, "if little is good, a lot more will be better" has played havoc with human and other life forms.

In fact we are forced to use these hazardous chemicals due to the unabated growth of human population. We cannot afford to incur pest-associated losses, which are globally estimated at 14% of total agriculture production [6]. World population may reach 8.3 billion by the year 2025 and hopefully it may stabilize at about 11 billion towards the end of the 21st century. Today there are around 800 million people (200 millions of them are children) who do not have access to sufficient food to meet their requirements [7]. In such a situation the major question that confronts scientists is how to protect crop losses due to pest attack without polluting the environment. Eco-friendly methods of pest management seem to be the only solution. Even though these alternate eco-friendly methods have great potential in management of insect pest, they are not yet fully exploited. Now with the advancement of science, these alternate eco-friendly insect control methods will play a vital role in insect pest management strategy. Presently available methods include biological control using parasites, predators and pathogens; cultivation of resistant crop varieties; semiochemicals which include pheromones and allelochemicals like allomones or kairomones, insect hormones, botanical insecticides and autocidal control methods like Sterile Insect Technique.

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Biological Control

Biocontrol agents include a wide variety of life forms like vertebrates, invertebrates and microorganisms. These beneficial species are common in most natural communities and, although their presence is often unnoticed, they help to maintain the "balance of nature" by regulating the density of their host or prey population. Insect species often become "pests" when this ecological balance is disrupted by natural events or human interventions like indiscriminate use of pesticides. Biological pest control strives to reestablish this balance. In conventional usage, biological control usually refers to the practice of rearing and releasing parasites and predators, or using biopesticides based on various insect pathogens.

Biopesticides

Like human beings, insects are subjected to infections by entire array of microorganisms like bacteria, virus, protozoa, fungi and nematodes. Perhaps the greatest potential for future progress in biological control lies in improving the success of microbial pathogens. Many of these organisms are highly desirable as biocontrol agents and attack narrow range of insect hosts. They are non-hazardous to humans or domestic animals and pose no threat to the environment. In the past few years, there has been significant progress in the development of microbial insecticides. These are commercial suspensions of spores, toxins, or virus particles that can be mixed with water and sprayed onto crops, just like conventional insecticides. In many cases, microbial insecticides are better than conventional insecticides because they suppress pest populations without eliminating natural populations of predators and parasites. A great deal of research is still needed before we begin to benefit from the full potential of biopesticides. Presently sale of biopesticides accounts for only 2% of the worldwide market of agrochemicals and 90% products are based on single organism, *Bacillus thuringiensis*. This grim situation is mainly due to some of the shortcomings associated with biopesticides and unavailability of potent and efficient microorganisms. Fortunately, recent developments in biotechnology may soon enable us to create new strains of microbial pathogens that are more virulent, easier to mass-produce, and less sensitive to environmental conditions. The work like transformation of common soil microbe, *Pseudomonas fluorescens*, into a pathogen of soil-dwelling insects by implanting the delta-endotoxin gene from *Bacillus thuringiensis*, clearly indicates that there are major changes in biocontrol that lie just over the horizon.

Efforts at BARC have yielded promising results in isolating, identifying and proving the toxicity of pathogens like *Bacillus thuringiensis, Bacillus. sphaericus*, granulosis viruses and nuclear polyhedrosis viruses. During the past few years quite a good number of entomopathogens were isolated and tested for their pathogenecity. The spore bearing crystalliferous *Bacillus* isolates, ISPC 1 and ISPC8 were found to be the most promising ones. ISPC1 is effective against agricultural pests whereas ISPC8 is a mosquitocidal organism. Detail toxicity and bioefficassy of these isolates were studied in laboratory as well as in the field conditions [8]. For the last few years efforts are focused on developing low cost media for mass multiplication of Bt based biopesticides, screening UV protectant compounds, cloning and expression of Bt Cry gene and initiation of new insect cell lines from Indian insect pests. Among the various media tested, the one based on corn steep liquor was found to be the most suitable and economical for the production of Bt based biopesticides. For the production of virus-based biopesticides, a new project on development of insect cell lines of different insect pests has been started.

Parasites and Predators

Insects are their own worst enemies. The predators and parasites of other insects include members of over 300 families in 10 insect orders. They help to maintain the "balance of nature" by regulating the density of their hosts. There are three ways one can reestablish the balance by using natural enemies. They are importation, conservation and augmentation. Once predators and parasites are released into a new environment, there is a good chance that they will become established and provide a self-perpetuating form of control. This is the only control tactic that increases, rather than decreases, the species diversity within an agro ecosystem. Natural enemies usually take longer time to suppress a pest population than other forms of pest

control and farmers often regard this as a disadvantage. It also may be difficult to use natural enemies where pesticides are still in use. Predator species include spiders (Order Aranea) dragonflies (Odonata), lacewings (Neuroptera), beetles (Coleoptera) flies (Diptera), true bugs (Hemiptera) ants, wasps (Hymenoptera) thrips (Thysanoptera) and mantids (Mantodea), whereas parasites mainly include wasps (Hymenoptera), flies (Diptera) and nematodes.

In BARC we have intercepted with larval ectoparasite of bruchid (*Callosobruchus* species) and it was identified as *Dinarmus vagabundus* (Timberlake). Detailed investigations were carried out to find out the potential of using this parasite for arresting the multiplication of bruchids in storage [9]. This parasite was found to have very good searching ability. The tiny parasite females are able to locate the bruchid infested seeds from the healthy grains and able to parasitize the bruchid larvae feeding inside the grains [10]. This indicated the presence of kairomones associated with host plant or host larvae. When response of bruchid parasite towards possible kairomone sources were tested, it was found that full grown host larvae and their solvent extract elicited significant attraction.

Semiochemicals

Semiochemicals are chemicals that mediate interactions between organisms. They are subdivided into allelochemicals and pheromones depending on whether the interactions are interspecific or intraspecific, respectively. Allelochemicals are allomones, kairomones or synomones. When the response of the receiver is adaptively favorable to the emitter it is called as 'allomone', when it is favorable to the receiver it is called 'kairomone' and when it is favorable to both it is called 'synomone'. Pheromones may be further classified based on the interaction mediated, such as alarm, aggregation or sex pheromone. It is the sex pheromones of insects that are of particular interest to agricultural integrated pest management practitioners.

Sex pheromones

Ever since sex pheromones were first discovered by A. A. Budenandt in 1959 (from silkworm moths, *Bombyx mori*), these chemicals have aroused great interest because of their potential as pest control agents. Sex pheromones can be used to detect the initial infestation leading to timely application of pest control measures or they can be used for insect control through disruption of insect matings. During the past 40 years, chemists have identified the sex pheromones for several insect species. Many of these compounds are now sold commercially. In some cases, pheromones are packaged (or encapsulated) in slow-release dispensers (rubber septa, hollow fibers, or rope wicks) that are used as lures in traps of various designs. At low densities, these pheromone traps are a valuable monitoring tool, providing information on the density and distribution of pest populations. At high densities, they can be used for mass trapping sexually active adults in efforts to reduce population density and lower pest's reproductive potential. Slow-release formulations of sex pheromones can also be used for mating disruption. By increasing the concentration of pheromone in an insect's environment, it may be possible to make everything smell like a prospective mate. Males wear themselves out courting inanimate objects or become habituated to the odor and stop responding to it.

Indigenous development of various aspects of pheromone technology and the utilization of pheromones in the control of insect species of economic importance are underway. Pheromones of insect pests attacking cotton crop are synthasized indigenously and tested under field conditions for mass trapping and mating diruption. The aggregating pheromone of red palm weevil, which plays an important role in SIT programme, works only in presence of food materials like sugarcane and dates. To replace the food sources, few attempts were made to test the various synthetic flavors of different fruits. Fruit flavors tried are apple, pineapple, mixed fruit, orange, strawberry, mango, banana, raspberry, lemon, and vanilla. All the flavors but for the last three, exhibited significant attraction in "T" olfactometer.

Insect Hormones

Chemicals that regulate developmental processes within an insect's body can sometimes be exploited as insect control weapons. These

compounds, often known as insect growth regulators (IGRs), can be used to stimulate development at inappropriate times or inhibit it at other times. The major groups of IGR compounds include: Chitin inhibitors, molting hormones and juvenile hormones. Chitin inhibitors like diflubenzuron and teflubenzuron inhibit the synthesis of chitin. They act rather slowly (2-5 days), but eventually disrupt any process that involves construction of new cuticle (e.g., molting, hatching, pupation). They are most effective when used against the immature stages of a pest. Diflubenzuron, currently registered under the trade name Dimilin, is used for controlling gypsy moths, boll weevils, and various other pests.

Molting hormone analogues, ecdysteroids stimulate the molting process by mimicking the action of molting hormone. Applied to the surface of an insect's body or incorporated into its food, these compounds work by initiating premature ecdysis during the immature stages of development Juvenile hormone (JH) and related compounds act as insect growth regulators by inhibiting the developmental changes associated with embryogenesis, morphogenesis, and reproduction. Several compounds (e.g., hydroprene, kinoprene, and methoprene) have been successfully incorporated into household products for controlling ants, flees and other household pests. Anti-juvenile hormone compounds (the precocenes) were first isolated in 1976 from a common houseplant (*Aegeratum houstonianum*). Precocenes are cytotoxic agents. They become activated by enzymes in the insect's corpora allata, selectively destroying these glands, and preventing all subsequent production of juvenile hormone. In immature insects, exposure to anti-JH compounds may result in premature (precocious) development of adult structures or behaviour. In adults, precocenes can cause sterility because the presence of juvenile hormone is necessary for normal production of eggs and sperm. Anti-JH compounds seem to be most effective against Hemipteran insects.

Insect resistant crop varieties: Breeding plants for resistance to insects is just another form of biological pest control. Breeders look for genetic traits (or combinations of traits) that reduce crop susceptibility to attack or injury by its insect pests. In general, there are three approaches that plant breeders use to develop resistant cultivars. They are antibiosis, antixenosis and tolerance. Developing resistant cultivars by traditional breeding methods can be a slow and uncertain process. However, with the advent of genetic transformation techniques based on recombinant DNA technology, it is now possible to remove some of the drawbacks from conventional techniques of evolving insect resistant crop varieties. Genetically engineered insect resistant crop varieties are viewed as one of the important alternatives to avoid crop losses and reduce the adverse impacts of pesticides on natural life supporting environment. These transgenic crop varieties will compliment positively in area wide integrated pest management program by replacing conventional synthetic broad spectrum insecticides, avoiding secondary pest outbreak and enhancing natural biological control. Recent advances in plant biotechnology have made it possible to transfer and express the novel foreign insect resistance genes in to crop plants. To develop insect resistant crop plants the first Bt toxin gene was cloned and transgenic plants were produced in mid 1980s. Since then several crop species have been genetically engineered to produce insect resistant crop varieties. Currently, bulk of insect resistant transgenic crops was planted in industrialized nations mainly USA, Canada, Australia and small percentages in China and Argentina. Recently Bt cotton has been introduced in India and it is grown in different part of the country.

Some attempts were made in BARC to develop the pest and disease resistant transgenic crop verities. Through the studies on interaction of bruchids, (*Callosobruchus maculatus* F.) with their host plants, four bruchid resistant sources from pigeonpea (*Cajanus cajan*) and *Vigna* species were identified. [11,12]. These resistant sources were included in our plant-breeding programme to develop bruchid resistant high yielding varieties of different pulses

Botanical Pesticides

The most of the botanical pesticides are chemicals produced by plants to discourage herbivorous organisms. Botanical pesticides are eco-friendly, economical, target-specific and biodegradable. For example, neem-based botanical pesticides have been used traditionally for many

years. Centuries before organic commercial insecticides were available, neem seeds, bark and leaf – were used in India to protect agricultural crops, household and stored grains. It is a well known practice in rural India to treat stored grains with neem oil or leaves. Intensive chemical investigation on neem seeds reveal that Azadirachtin, a complex and highly oxygenated compound belonging to tetranertriterpinoid class is the most potent antifeedant and growth disruptant to many insect species. Among the other natural insecticides rotenone from *Derris elliptica* (cube root) nicotine from tobacco leaf and pyrethrins from pyrethrum flowers (*Chrysanthemum cinerarifolium*) attained commercial importance. There are many other trees (besides herbs and shrubs), which are also useful as sources of botanical pesticides. Monoterpenoids of essential oils provide effective lead molecules in management of stored product insects, veterinary insects and insect pests of public-health importance. In BARC we have an ongoing programme to find out new effective botanical pesticides.We have shown that plants like *Xanthium strumarium, Blumea eriatha* and *Cida acuta* have significant insecticidal properties. [13-15].

Autocidal control or Genetic control

Some pest control tactics are designed to suppress a pest population by altering its genetic makeup and/or reducing its reproductive potential. These are also known as genetic controls because they affect the accuracy or efficiency with which a pest species passes its genetic material (DNA) from one generation to the next. Genetic control usually works in one of two ways: either by causing (inducing) reproductive sterility, or by incorporating new and potentially deleterious genes into the genetic makeup of a pest population. In effect, some members of a pest species are transformed into biological time bombs that eventually destroy other members of their own species. Because of the self-destructive nature of these tactics, they are sometimes called autocidal control.

Sterile insect technique (SIT) is one of the autocidal insect control techniques. Radiation makes insects sterile and thus affects reproduction. By taking advantage of this fact, E. F. Knipling suggested that it might be possible to suppress pest populations by flooding the environment with large numbers of males that had been rendered sterile by irradiation [16]. By maintaining a constant population of sterile males that was large in comparison to the normal males, Knipling calculated that the number of "normal" matings would decrease each generation until the population was forced into extinction. This method has been demonstrated to work successfully with several major insect populations in the world for control, elimination and quarantine. The first success story of SIT was eradication of screwworm fly (*Cochliomyia hominivorax*) from North America. This project started way back in 1954 and continued till 1984. Tsetse fly is another important veterinary insect pest, which is being controlled through SIT. The SIT programme to eradicate the tsetse fly from Zanzibar and Tanzania had made a considerable progress [17]. One of the most successful and long-term sterile insect release programs involves the pink bollworm in the San Joaquin Valley of California [18]. The highly successful programs in Australia, Mexico, Japan, and elsewhere have already proven that the SIT can be used effectively to control various species of fruit fly. IAEA/FAO helped Chile and Mexico to eradicate the Mediterranean fruit fly using SIT. The SIT technology is well developed for some other fruit flies like Caribbean fruit fly, Oriental fruit fly, Onion fruit fly, Mexican fruit fly, Cherry fruit fly and melon fruit fly. These programs showed a good success not only for controlling flies, but also for quarantine purpose in USA, Mexico and Japan [19].

The SIT has been developed in BARC for red palm weevil and potato tuber moth. For red palm weevil, (*Rhynchophorus ferrugineus* Oliv) efficient and economical mass rearing methods based on sugarcane and artificial diet have been developed [20]. Detail radiological studies were carried out by using a ^{60}Co source and the optimum sterilizing dose for red palm weevil was found to be 15 Gy. Recently attempts have been made to demonstrate the feasibility of this technique to control red palm weevil under field conditions. The sterilizing dose for potato tuber moth was found to be 450Gy. Modifying factors like dose fractionation, temperature, diurnal rhythm and gaseous environment were tried for obtaining competitive sterile adults [21]. The ratio of ten or more sterilized males to one natural male was found to be effective ratio for suppressing progeny productions. Attempt

has also been made to test the F1 sterility approach to reduce radiation damage and increase competitiveness of sterile males. The feasibility of SIT in controlling multiplication of potato tuber moth in storage was assessed in specially made field cages.

In conclusion, the scientific literature available till today and our studies indicated that these ecofriendly alternate insect control methods will have a great future. Presently there are some shortcomings associated with these alternate techniques, however, with the advancement of science, these hurdles are slowly getting removed. In days to come these alternate eco-friendly insect control methods will play a vital role in saving pest related crop losses to feed the ever increasing human population.

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IANCAS Roundup

***Dr. M.S.M. Rawat**, is Head of Chemistry Department, HNB Garhwal University, Srinagar, Uttaranchal. He did his Post graduation from DSB College, Nainital (Agra University) in 1970 and Ph.D from IIT, Delhi in 1984 on 'Photochromism in Organic Compounds'. He became Reader in Chemistry in 1986 and Professor in 1990. He served as a Lecturer in Government Degree College, UP for 7 years before joining Garhwal University. He held many positions in the university as Dean, Students Welfare, Dean, Coordinator and Dean Science etc.His areas of research are Chemistry of Natural Products and Photochroism. He is currently working on medicinal plants, Lichens of Garhwal region and Biopesticidal plants. He has guided 20 students for D.Phill degree and published 70 papers in different journals. He was the invited Professor at Nagoya City University, Nagoya, 2002 on chemistry of Natural Products. He has attended several International Conferences and is a member and fellow of many scientific bodies.*

A 46th BRNS-IANCAS National Workshop was organized by IANCAS from April 29 to May 7, 2003 at the Department of Chemistry, HNB Garhwal Univeristy, Srinagar, Garhwal. The workshop was attended by Post Graduate teachers and other staff members of the science departments.

IANCAS donated a -ray spectrometer and a G.M. Counter to the Nuclear Chemistry laboratory of the college. These equipments have been used by the students doing M.Sc by research under the guidance of Dr.Rawat.

The following experiments were being conducted to the students on regular basis.

(1) Determination of solubility product of PbI_2
(2) Determination of iodine by isotope dilution analysis
(3) Characteristics of GM counter
(4) Energy calibration using gamma spectrometer.

It is intended to incorporate other experiments using radioisotopes in conformity with the revised syllabi of the University Grants commission.

The department also proposes to carry out heavy metal analysis of the hot water springs and biological accumulators of air pollutants by neutron activation analysis. Analysis of the ash content of the medicinal plants, though important, is not fully explored and the department is planning to take up the study of seasonal and altitudinal variations as a part of quality control.

The equipment received from IANCAS is being made available for other students in the university.

IANCAS LIFE MEMBERS AWARDED

Prof. S.P.Mishra, Banaras Hindu University & Prof.H.M.Agarwal, G.B.Pant University of Agriculture & Technology, Pant Nagar, the IANCAS life members, both representing the North-zone team, have won the DAE Golden Jubilee Science Quiz for College/University teachers on August 1, 2004. They have received certificates and cash awards from Chairman, AEC, DAE. The Quiz programme was conducted by the popular Quiz Master Sri.Siddhartha Basu.

Prof.Mishra has been awarded, the Prof.Frederic Joliot Curie Endowment Lecture Award, for his valuable contribution in the field of Nuclear and Radiation chemistry, by the Department of Chemistry, University of Pune on November 28, 2003.

IANCAS congratulates both the members on their achievement and wishes them to continue receiving such laurels in the future.

NUCLEUS

Elephants in Sri Lanka and Sumatra moved to high ground before the giant waves struck; they did the same in Thailand, trumpeting before they did so. According to a villager in Bang Koey, Thailand, a herd of buffalo was grazing by the beach when the animals 'suddenly lifted their heads and looked out to sea, ears standing upright'; they turned and stampeded up the hill, followed by bewildered villagers, whose lives were thereby saved. At Ao Sane beach, near Phuket, dogs ran up to the hill tops, and at Galle in Sri Lanka dog owners were puzzled by the fact that their animals refused to go for their usual morning walk on the beach. In Cuddalore District in Tamil Nadu, southern India, buffaloes, goats and dogs escaped, as did a nesting colony of flamingos that flew to higher ground. In the Andaman Islands 'stone age' tribal groups moved away from the coast before the disaster, having been alerted by the behaviour of animals.

How did they know? The usual speculation is that the animals picked up tremors caused by the under-sea earthquake. This explanation seems unconvincing to me. There would have been tremors all over Southeast Asia, not just in the afflicted coastal areas. And if animals can predict earthquake-related disasters by sensing slight tremors, why can't seismologists? Animals also seem to know when other kinds of calamities are about to strike. In my recent book The Sense of Being Stared At I summarise a large body of evidence of unusual animal behaviour before earthquakes, including those in Kobe in 1995 and Assissi in 1997 and recent quakes in California. In all cases there were many reports of wild and domesticated animals behaving in fearful, anxious or unusual ways several hours or even days before the earthquakes struck. The same is true of the 1999 earthquake in Turkey, with its epicentre near Izmit: dogs were howling for hours in advance, and many cats and birds were behaving unusually.

On 28 February 2001, a 6.8-magnitude quake struck the Seattle area, and once again animals behaved unusually beforehand. Some cats were said to be hiding for no apparent reason up to 12 hours in advance of the earthquake; others were behaving in an anxious way or 'freaking out' an hour or two before; some dogs were barking 'frantically'; and goats and other animals were showing obvious signs of fear.

No one knows how some animals sense earthquakes coming. Perhaps they pick up subtle sounds or vibrations in the earth; maybe they respond to subterranean gases released prior to earthquakes, or react to changes in the earth's electrical field. They may also sense in advance what is about to happen in a way that lies beyond current scientific understanding, through some kind of presentiment. Animals can also anticipate man-made catastrophes such as air raids. During WWII, many families in Britain and Germany relied on their pets' behaviour to warn them of impending air raids, well in advance of official notification. These warnings occurred when enemy planes were still hundreds of miles away, long before the animals could have heard them coming. Some dogs in London even anticipated the explosion of German V-2 rockets. These missiles were supersonic and hence could not have been heard in advance.

Unusual animal behaviour also occurs before avalanches. On 23 February 1999 an avalanche devastated the Austrian village of Galtür in the Tyrol, killing dozens of people. The previous day, the chamois (small goat-like antelopes) came down from the mountains into the valleys: something they never usually do. Through surveys in alpine villages in Austria and Switzerland, I found that the animals most likely to anticipate avalanches are chamois and ibexes, and also dogs. Although it is still unexplained, this ability would obviously be of survival value in mountain animals, and would be favoured by natural selection.

With very few exceptions, the ability of animals to anticipate disasters has been ignored by Western scientists, who dismiss stories of animal anticipations as anecdotal or superstitious. The Chinese, in contrast, have encouraged people in earthquake-prone areas to report unusual animal behaviour since the 1970s; and Chinese scientists have an impressive track record in predicting earthquakes. In several cases they issued warnings that enabled cities to be evacuated hours before devastating earthquakes struck, saving tens of thousands of lives.

By following the lead of the Chinese and paying attention to unusual animal behaviour, earthquake warning systems might be feasible in California, Greece, Turkey, Japan and elsewhere. Millions of pet owners and farmers in earthquake-prone areas could be asked to take part in this project through the media. They could be told what kinds of behaviour their pets and other animals might show if an earthquake were imminent: in general, signs of anxiety or fear. If people noticed these signs, or any other unusual behaviour, they could immediately call a telephone hotline or send a message via the internet.

A computer system could analyse the places of origin of the incoming calls. If there were an unusual number of calls it would sound an alarm, and display on a map the places from which the calls were coming. There would probably be a background of false alarms from people whose pets were sick, for example, and there might also be scattered hoax calls. But if there were a sudden surge of calls from a particular region, it could indicate that an earthquake was imminent. The same principles would apply to tsunamis.

To explore the potential for animal-based warning systems would cost a small fraction of current earthquake and tsunami research. By doing this research we would be sure to learn something, and could probably save many lives.

At present, many millions of pounds are being allocated for setting up tsunami warning systems. I hope that those responsible for spending this money will not ignore what animals can tell us.

(Dr Rupert Sheldrake is a biologist and author of The Sense of Being Stared At: and other aspects of the extended mind (www.sheldrake.org)

ERRATA

Members may please note the following corrections

1. The article on 'Chemistry in Fast Reactor Technology' that appeared in IANCAS Bulletin Vol.III, No.1 was coauthored by Dr.G.Periaswamy along with Dr.P.R.Vasudeva Rao.
2. The article on 'Neutron Detectors', that appeared in the bulletin Vol.III, No.3, was coauthored by D.B.Paranjape along with Dr.G.K.Gubbi.

The inadvertent lapse is regretted

Editor

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I, Dr.G.A.Rama Rao, hereby declare that the particulars given above are true to the best of my knowledge and belief.

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Sd/-
Dr.G.A.Rama Rao
Editor