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Use of Biotechnology for Cleaning Up Our Environment.

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ABSTRACT

Bio-Technology encompasses a wide range of specialized discipline right from age old fermentation process to the latest techniques of genetic engineering. It has received tremendous attention in recent years due to its potentialities and applications in aquaculture, agriculture, immunology, chemical production, industrial processes, pollution control etc. But modern environmental protection is unthinkable without biotechnology. Biotechnological methods are indispensable in fields of soil, waste water and exhaust air purification. Besides microbial and system biology contribute to increase in efficiency of purification and biowaste recycling plants. These days biotechnology is being put to varied uses. One of the potential uses of it is in the environmental protection and in conserving the natural resources and endangered plants of economic and medical uses. Biotechnology not only plays important role in remedying environmental damage but also in detection of environmental damage. But, any advanced technology is often accompanied by benefits to mankind as well as risks to the environment. Therefore, for efficacious and safe utilization of biotechnology, it is essential to select safe and useful genetically engineered microorganisms for applications to waste treatment processes of special environmental significance.

Keywords: biotechnology, environment, genetic engineering.

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INTRODUCTION

Since the evolution of man on earth, he has always been dependent on nature for all his basic needs. With rapid industrialization, urbanization and economic growth, man has started living in two different worlds. The first and foremost world is natural world of plants, animals, soil, air, and water. Gradually as his needs increased, he has created another world of comfort composed of variety of production systems and process with the use of science and technology and other social and political organizations. Now in 21st century, these two worlds have become the need of life. But this demand for new technological society has created a stress on man's life. His ambition for limitless comfort has led him towards the exploitation of nature's wealth and resources indiscriminately and rapidly. Man's greed for resources and his desires to conquer nature has placed him in front of the environment. Besides, man designed production processes also generate by-products, wastes and effluents which are difficult to biodegradable. But what nature gets in return to this-contamination by various human activities that generate pollutants of many kinds. Some of the areas where biotechnology has proved very effective in environmental clean-up include:

Landfill Technologies: Solid wastes account for an increasing proportion of the waste generated by urban societies. While a part of this volume consists of glass, plastics, and other non-biodegradable material, a considerable proportion of this is made of decomposable solid organic material, like food wastes from large poultry and pig farms. In large non-urbanized communities, a common method for disposing off such biodegradable waste is the low-cost Anaerobic Landfill Technology. In this process, the solid wastes are deposited in low-lying, low value sites. The waste deposit is compressed and covered by a layer of soil every day. These landfill areas house a wide variety of bacteria, some of which are capable of degrading different types of wastes. The only shortcoming in this process is that these bacteria take a considerably long time to degrade the waste. However, modern biotechnology has enabled scientists to study the available bacteria, which are involved in the degradation of the waste – including hazardous substances. The most efficient strains of these bacteria can be cloned and reproduced in large quantities, and eventually applied to the specific sites. This ensures rapid degradation of the waste material.

Composting: Composting is an anaerobic microbiologically driven process that converts organic wastes into stable sanitary humus like material. This material can then be safely returned to the natural environment. This method is actually low moisture, solid substrate fermentation process. In large-scale operations using largely domestic solid wastes, the final product is mostly used for soil improvement. In the more specialized operations using raw substrates (like straw, animal manure etc.), the compost (final product) becomes the substrate for the production of mushroom. The primary aim of a composting operation is to obtain final compost with a desired product quality in a limited time period, and within limited compost. The basic biological reaction of the composting process is the oxidation of the mixed organic substrates to produce carbon dioxide, water and other organic by-products. However, it is important to ensure that a composting plant functions under environmentally safe conditions. Composting has long been recognized not only as a means of safely treating solid organic wastes, but also as a technique of recycling organic matter. This technique will increasingly play a significant role in future waste management schemes, since it enables the reuse of organic material derived from domestic, agriculture and food industry wastes.

Bioremediation: Various products (chemicals) generated by the modern technologies are posing a great threat to the natural breakdown processes and the natural mechanisms of maintaining ecological balance. Many of these pollutants are complex in nature, and are hence difficult to break down. Such pollutants are accumulating in the natural environment to an alarming rate. The application of biotechnology has helped in the environmental management of such hazardous contaminants by bioremediation. This process is also referred to as bio-restoration or bio-treatment. Bioremediation involves the use of naturally existing microorganisms to speed up the breaking down of biological substances and degradation of various materials. This process adds substantial momentum to the process of cleaning up. The basic principle of bioremediation is the breaking down of organic contaminants into simple organic compounds like carbon dioxide, water, salts and other harmless products.

Bioremediation can help clean up the environment in two ways: Promotion of microbial growth in situ (in the soil) can be achieved by addition of nutrients. The microbes acclimatize themselves to these toxic wastes (so called nutrients). Over a period of time, the microbes use up these compounds, thus degrading these pollutants. Another option is to genetically engineer microorganisms, which can degrade organic pollutant

molecules. For instance, bioremediation engineers from an American organization used the 'Flavobacterium' species to remove pentachlorophenol from contaminated soil. The use of microbes has also proved efficient in cleaning up toxic sites. An American microbiologist has discovered a GS-15 microbe, which can eat up uranium from the wastewater of a nuclear weapon manufacturing plant. The GS-15 microorganisms convert uranium in water into insoluble particles that precipitate and settle at the bottom. These particles can subsequently be collected and disposed off. GS-15 bacterium also metabolizes uranium directly, thus yielding twice as much energy as it would generate normally in the presence of iron. This organism has a very fast growth rate, and can be extremely useful in waste treatment of uranium mining. Bioremediation employs biological agents, which render hazardous wastes into non-hazardous or less hazardous compounds. Even the dead biomass houses some fungi that can trap metallic ions in aqueous solutions. This is due to their special cell wall composition. Many fermentation industries produce fungal biomass on unwanted by-products, which can be used for this purpose.

The biomass of the fungus *Rhizopus arrhizus* can absorb 30-130 mg of cadmium/gm of dry biomass. Fungus has ions in its cell-wall like amines, carboxyl and hydroxyl groups. 1.5kg of mycelium powder could be used to recover metals from 1 ton of water loaded with 5 grams of cadmium. 'Algasorb', a product patented by the Bio-recovery Systems Company, absorbs heavy metal ions from wastewater or ground water in a similar manner. Trapping dead algae in silica gel polymeric material produces Algasorb. It protects algal cells from being destroyed by other microorganisms. Algasorb functions in the same manner as commercial ion exchange resin, and heavy metals can be removed on saturation.

Controlling pollution at its source itself is an extremely effective approach towards a cleaner environment. Heavy metals like mercury, cadmium and lead are often present as pollutants in the wastewater of modern industry. The effects of mercury as pollutant have been known for quite some time now. These metals can be accumulated by some algae and bacteria, and thus removed from the environment. For instance, 'Pseudomonas aeruginosa' can accumulate uranium and 'Thiobacillus' can accumulate silver. Several companies in the US sell a mixture of microbes and enzymes to clean up chemical wastes including oil, detergents, paper mill wastes and pesticides. Of late, plants are also being used to clean up metal infested sites. These plants absorb the metals in their vacuoles. This process is referred to as Phytoremediation. The metals can be recovered by burning the plants. This practice of growing such trees near the industrial plants that release heavy metals in the environment has proved extremely effective.

Biosensors: Biosensors are biophysical devices that can detect and measure the quantities of specific substances in a variety of environments. Biosensors include enzymes, antibodies and even microorganisms, and these can be used for clinical, immunological, genetic and other research purposes. The biosensor probes are used to detect and monitor pollutants in the environment. These biosensors are non-destructive in nature, and can utilize whole cells or specific molecules like enzymes as biomimetic for detection. Their other advantages include rapid analysis, specificity and accurate reproducibility. Biosensors can be created by linking one gene to another. For instance, mercury resistance gene (*mer*) or toluene degradation (*tol*) gene can be linked to the genes coding for proteins showing bioluminescence within a living bacterial cell. The biosensor cell, when used in a particular polluted site, can signal by emitting light – which would suggest that low levels of inorganic mercury or toluene are present at the polluted site. This can be measured further by using fibre-optic fluorimeters. Biosensors can also be created by using enzymes, nucleic acids, antibodies or other reporter molecules attached to synthetic membranes as molecular detectors. Antibodies specific to a particular environmental contaminant can be coupled to changes in fluorescence so as to increase the sensitivity of detection. In India, the Central Electrochemical Research Institute at Karaikudi has developed a glucose biosensor based on enzyme glucose oxidase. This enzyme is immobilised on a electrode surface acting as an electro-catalyst for the oxidation of glucose. The biosensor in turn gives a reproducible electrical signal for glucose concentration as low as 0.15 mM (millimolar), and works for several weeks with no apparent degradation of the enzyme. Another similar application of the biosensors is 'Bio-monitoring', which may be defined as the measurement and assessment of toxic chemicals or their metabolites in a tissue, excreta or any other related combination. It involves the uptake, distribution, biotransformation, accumulation and removal of toxic chemicals. This helps minimise the risk to industrial workers who are directly exposed to toxic chemicals.

Biodegradation of Xenobiotic Compounds: Xenobiotics are man-made compounds of recent origin. These include dyestuffs, solvents, nitrotoluenes, benzopyrene, polystyrene, explosive oils, pesticides and surfactants.

As these are unnatural substances, the microbes present in the environment do not have a specific mechanism for their degradation. Hence, they tend to persist in the ecosystem for many years. The degradation of xenobiotic compounds depends upon the stability, size and volatility of the molecule, and the environment in which the molecule exists (like pH, susceptibility to light, weathering etc). Biotechnological tools can be used to understand their molecular properties, and help design suitable mechanisms to attack these compounds.

Oil Eating Bugs: Accidental oil spills pose a great threat to ocean environments. Such spills have a direct impact on marine organisms. To counter this problem, scientists have now developed living organisms to clean up the oil spills. The most common oil-eating microorganisms are bacteria and fungi. Dr Anand Chakrabarty, a leading US-based scientist of Indian origin, has successfully created bacterial forms which can degrade oil into individual hydrocarbons. These bacteria include *Pseudomonas aureginos*, where a gene for oil degradation has been introduced into the *Pseudomonas*.

Once the oil has been completely removed from the surface, these engineered oil-eating bugs eventually die, as they can no longer support their growth. Dr Chakrabarty was the first scientist to obtain a patent for such live organisms. *Penicillium* species has also been found to possess oil degrading features, but its effect needs much more time than the genetically engineered bacterium. Many other microorganisms like the *Alcanivorax* bacteria are also capable of degrading petroleum products.

Designer Bugs: More than hundred thousand (one lakh) different chemical compounds are produced in the world every year. While some of these chemicals are biodegradable, others like chlorinated compounds are resistant to microbial degradation. To tackle these Polychlorinated Biphenyls (PCBs), scientists have now isolated a number of PCB-degrading bacterial (*Pseudomonas pseudoalkali*) genes KF 707. A whole class of genes, referred to as bph-making enzymes, has also been isolated. These enzymes are responsible for the degradation of PCBs. Other genetically engineered bacteria are also degrading different ranges of chlorinated compounds. For instance, an anaerobic bacterial strain *Desulfitobacterium* sp. Y51 dechlorinates PCE (Poly chloroethylene) to cw-12-dichloroethylene (CDCE), at concentrations ranging from 01 – 160 ppm.

Japanese scientists have come up with a technology called 'DNA shuffling', which involves mixing the DNA of two different strains of PCB degrading bacteria. This results in the formation of chimeric bph genes, which produce enzymes capable of degrading a large range of PCBs. These genes are further introduced in the chromosome of original PCB-degrading bacteria, and the hybrid strain thus obtained is an extremely effective degrading agent. Genes have also been isolated from bacteria that are resistant to mercury called as mer genes. These mer genes are responsible for total degradation of organic mercurial compounds. The bph genes and tod-genes for toluene degrading bacteria (*pseudomonas putida* FI) have shown similar gene organisations. Both these genes code for enzymes which show a sixty per cent similarity. By exchanging the subunits of the enzymes it is possible to construct a hybrid enzyme. One such hybrid enzyme created is hybrid deoxygenase which is composed of TodCl – Bph A2 – Bph A3 – Bph A4. This was expressed in *E.coli*. It was observed that this hybrid deoxygenase was capable of faster degradation for Trichloroethylene (TCE) based compounds. The todCl gene from toluene degrading bacteria has been successfully introduced, in the chromosome of bacterial strain KF707. This strain then resulted in efficient de-gradation of TCE. This KF707 strain could also be grown on toluene or benzene etc.

Biomining: Among the oldest industries in the world, mining is the source of alarming levels of environmental pollution. Modern biotechnology is now being used to improve the environment surrounding mining areas through various microorganisms. For instance, a bacterium *Thiobacillus ferrooxidans* has been used to back out copper from mine tailings. This has also helped in improving recovery. This bacterium is naturally present in certain sulphur-containing materials, and can be used to oxidise inorganic compounds like copper sulfide minerals. This process releases acid and oxidising solutions of ferric ions that can wash out metals from the crude ore. These bacteria chew up the ore and release copper that can subsequently be collected. Such methods of bio-processing account for almost a quarter of the total copper production world-over. Bio-processing is also used to extract metals like gold from very low-grade sulfidic gold ores.

Biotechnology also offers the means of improving the efficiency of bio mining, by developing bacterial strains that can withstand high temperatures. This helps these bacteria survive the bio-processing which generates a lot of heat. Another option is to genetically engineer bacterial strains that are resistant to heavy

metals like mercury, cadmium and arsenic. If the genes that protect these microbes from heavy metals are cloned and transferred to the susceptible strains, the efficiency of bio mining can be increased manifold.

Pollution Control: With the help of modern biotechnology, naturally occurring biocatalysts can be used to detoxify harmful chemicals being released into the environment. Such biocatalysts have helped get rid of carcinogenic compounds like methylene chloride from industrial wastes. These special bacteria are exposed to the waste in a bioreactor, wherein the bacteria consume the noxious chemical and convert it to water, carbon dioxide and salts, thus completely destroying the chemical compound. A species of bacteria *Geobacter metallireducens* is also used to remove uranium from drainage waters in mining operations, and from contaminated ground waters. The isolation and subsequent characterisation of various important genes will help in developing strains that can degrade a wide range of pollutants. Using molecular manipulations can also help tailor bacteria to use them for removing specific toxicants.

Treating Industrial Wastes:

Wastes from the Pulp Industry: Wastes from the paper and pulp industries contain high levels of cellulose and lignocellulose, which pose massive treatment problems. Cellulose is extremely resistant to enzyme breakdown, and becomes resistant to both chemical and enzymatic attack when bound to lignin. Since lignin's and carbohydrates are interlinked in wood, it becomes difficult to delignify the pulp. Researchers have now developed enzymatic pulp bleaching, which prevents bleach waste formation by eliminating or reducing chlorine consumption. It also reduces the water in pulping and bleaching. This process involves the use of a xylanase producing organism *Bacillus stearthermophilus*, which is isolated from soil.

Microorganisms usually produce xylanases along with other polymers like cellulase and hemicellulose. Recombinant DNA technology is now being used to express only the xylanase genes in non-cellulolytic hosts. The first cellulase-free xylanase was reported from actinomycete *Chainia* from the deserts of Rajasthan. Various other xylanases were subsequently reported. Xylanases are being widely used because of their high temperature stability and high alkaline optimum. This property helps in its tight binding to the substrate. Alkaline xylanase has been reported from *Bacillus stearthermophilus*, which is active at pH 9, and 65° C. This has been tested for bleaching of wood pulp with promising results.

Another waste from wood pulping process is sulphite waste liquor, which contains ligno-sulphate (60%), sugar (36%) and a mixture of other organic compounds. This can be treated with yeast (*Candida albicans*), which ferments the sugar, producing nearly one ton of yeast for every two tons of sugar in the liquor.

Wastes from Dairy Industry:

The whey fluid is a substantial by-product in the manufacturing of cheese. Whey is left after the curd has been separated, and for every one kg of cheese produced, as much as nine litres of this fluid (whey) is generated. Though the whey contains potentially valuable nutrients, its use is limited to animal feed and some processed food like ice cream. With the world whey output approaching five million tons per annum, enormous waste disposal problems are beginning to haunt the dairy industry. When discharge into municipal sewage system would result in massive biological oxygen demand (BOD). This fluid has a lactose content of upto 4-5%, which is poorly metabolised by most of the organisms used in commercial fermentation. To make matters worse, whey is diluted (92% water), and involves high costs of collection.

Whey disposal is now being handled by various biotechnological approaches. These include:

1. Treating whey with proper strains of microbes and nutrients,
2. Direct fermentation of lactose to ethanol,
3. Using yeast like '*Kluyveomyces fragilis*' and '*Candida intermedi*',
4. Hydrolysis of lactose to glucose and galactose. (Fermentation results in sweet syrup, which is used in the food industry).

Wastes from Dye Industry:

The textile and dyestuff industries produce a number of dyes and pigments, which are released into the environment in effluent streams. Though most of the dyes are not toxic or carcinogenic to fish or mammals, some of them pose serious hazards.

Chemical methods for treatment of coloured effluents have proved successful, while the microbial removal of dyes and pigments is still very limited. Microorganisms have been found to degrade dyes only after adaptation to concentrations much higher than normally found in different streams.

Bio-scrubbing:

The discharge of noxious toxic and odorous gases is a serious environmental problem. Reduced sulphur compounds (thiosulphate, hydrogen sulphide) are generated from a variety of industrial processes in photographic and pulp industries, oil refining and purification of natural gases. These compounds are the by-products of anaerobic digestion of animal wastes with a high organic content. Most inorganic reduced sulphur compounds can be utilised either aerobically or anaerobically.

Pesticides:

Most commercially used chemical pesticides and fertilisers have proved hazardous beyond a certain threshold level. These chemicals, when degraded by microorganisms or ultraviolet light, release pollutants in the environment. Biotechnological tools can help in such situations.

Weed Control:

New herbicides have been developed, which will be selective to the target and harmless for the non-target organisms. Genetically engineered herbicide resistant plants have also been developed in a number of crops, which would help in the use of environment friendly herbicides. Genetically engineered insect resistant plants have also been successfully developed in certain crop species, thus suggesting the restricted use of pesticides in future.

Pest Control and Bio-pesticide:

Bacterial pesticides are now being synthesised by transferring bacterial gene (*Bacillus thuringiensis*) Bt into plants. This gene encodes a protein, which when ingested by feeding insects, results in the solubilisation of the insect's digestive tract (mid gut) and releases protoxins. This leads to disturbances in the equilibrium and ultimately kills the insect.

These 'biological pesticides' are being developed to target insect pests (ball worm and bud worm) by transferring the Bt gene into a soil bacterium (*Pseudomonas* species). Several American companies are involved in the development and marketing of biological pesticides and have come up with genetically engineered live bacteria for coating seeds before planting. Mycogen kills recombinant bacteria and applies them to the leaves of crop plants. Both these approaches protect the toxin from degradation by microorganisms and ultra violet light when applied to the crop plants.

Viral Pesticides:

Viral pesticides are environmentally safe and carry lower risk of toxicity. These pesticides can also be used against the pest strains, which have otherwise become resistant to chemical pesticides. A number of entomopathogenic viruses (virus infecting insects) have been used as safe and effective pesticides. These viruses kill specific pest species and have no adverse effects on useful insect pollinators, insect yielding useful products, parasites or predators. They are safe even in long scale spray operations.

Restoration of Denuded Areas:

Increasing human activity has created havoc in Earth's otherwise well- balanced ecosystem. More than half of the world's total land area is now being threatened by problems of salinity, acidity and metal toxicity. Biotechnological tools are being used for restoring the degraded ecosystem. Some of the methods based on plant biotechnology include reforestation, involving micro propagation and use of mycorrhiza.

Micro propagation has resulted in increasing plant cover, which in turn helps in preventing erosion and also adds climatic stability. Specific plant species have been planted in areas, which are more prone to denudation.

For instance, different species of plant Casuarina have been planted in nitrogen deficient soils, which will increase the soil fertility and enhance firewood production. Some plant species that can grow in high saline soils can also be planted in such areas. These species include Prosopis spiagera, Butea monosperma and Terminalia bellerica.

Biodiversity and Conservation:

Human activity has also proved devastating for the diversity of species, and the human induced extinction of species has been increasing at exponential rates. The need for expanding population with an unequal distribution of wealth has invariably resulted in unsustainable and exploitative use of existing resources. One of the major concerns today is the preservation of our existing flora and fauna (plants, animals and microbes).

Biotechnological applications have opened up new and improved methods of preserving plant and animal genetic resources, and have accelerated the evaluation of germ plasma collection for specific traits. Maintenance of a wide genetic base, which is an important element of biodiversity, is essential to the future of biotechnology and the sustainable use of biological resources. New technologies may increase the value of the world biodiversity if they allow increased use of the genetic diversity of both wild and domesticated species.

Plant tissue culture has been regarded as a key technology for increasing the production capability of many plants of selected varieties, so as to improve and increase their production and to prevent them from extinction.

However, the inherent nature of plant species is such that most crop genetic resources are conserved ex situ (outside the natural habitat). There are very few ex situ methods of preservation, which can distinguish the part of the plant to be conserved (whole organ, seeds, tissues or genetic material). But the newer biotechnological devices can help preserve the seeds as the preferred method of ex situ conservation. Here one has to overcome the problem of dormancy.

Another successful method of conserving biodiversity is the conservation of germ plasma by cryopreservation (freezing the tissue in liquid nitrogen at -196°C). The basic principle here is to bring the metabolic activity to a complete halt while keeping the tissue live (in a passive form).

Biotechnological tools have thus paved a way for restoring and preserving our biodiversity in multidimensional ways. These tools will definitely be the ultimate answer to the growing challenge of a depleting environment.

Bio-fertilizers:

These have also been used to reduce the cost of fertilizer applications and to reduce the environmental hazards caused by chemical fertilizers. Recently marine plants (seaweeds) have been used as bio fertilizers. They have proved to be very encouraging and thus reducing the burden of using chemical fertilizers.



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