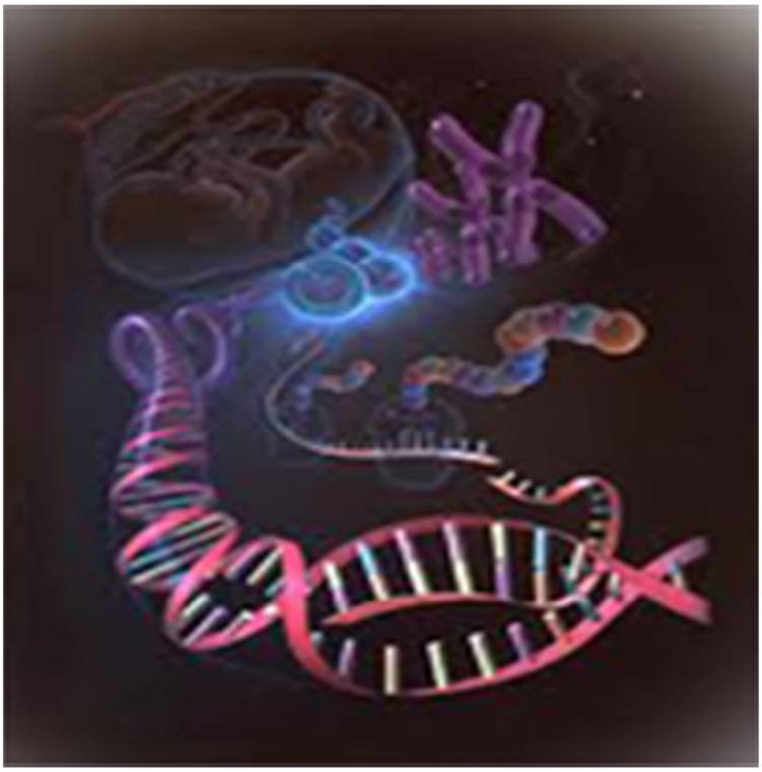
IJLBPR

ISSN 2250 - 3137
Vo1. I, No.1, January 2012

International Journal of Life Sciences Biotechnology and Pharma Research



www.ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

Int. J. LifeSc. Bt & Pharm. Res. 2012

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol.1, Issue. 1, January 2012

Review Article

ROLE OF ENVIRONMENTAL BIOTECHNOLOGY IN DECONTAMINATING POLLUTED WATER

Upma Singh[1], Bina Rani[2], A K Chauhan[3*], Raaz Maheshwari[3], M K Vyas[4]

Corresponding Author: A K Chauhan, ✉ *dr.ashok.k.chauhan@gmail.com*

Water pollution problems, due to industrial effluents in aquatic environments (lakes, rivers, estuaries & coastal waters), are increasing day by day as industrial effluent waste is degrading ecosystem, global water cycle and environment. Applications of biological agents (organisms or their components), along with physical, chemical & engineering processes to maintain, protect and restore the environment are involved in Environmental Biotechnology (EB). Analytical analysis and tests like biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS) and total solids (TS) etc. are required to assess the pollution load & strength of waste to be treated in most efficient manner. Biological treatment, bioremediation and natural attenuation have been a rapidly growing area of science over the past decade. Natural attenuation is viewed as the best solution for cleaning up many waste sites and will save billions of dollars in cleanup costs. The biotreatment, bioremediation and Natural Attenuation area have both basic research and field application foci for the EB. The field application foci are co-metabolic techniques, biogeochemical assessment techniques, and modeling of attenuation and environmental fate. There is further scope for research for modification and applications in physical and chemical engineering processes (like creation of more channels for treatment of effluent wastes, increase in retention time of effluent during various stages so that more biodegradation & bioremediation can be achieved) and applications of genetically improved better strains of microbes to control effluent pollution more efficiently.

Keywords: Water pollution, Effluents, Biodegradation, Bioremediation, Microbes, Natural attenuation

INTRODUCTION

Environmental Biotechnology (EB), plays extremely important role in water pollution management, is the multidisciplinary integration of sciences and engineering in order to utilize the huge biochemical potential of microorganisms,

---

[1] School of Applied Science, Gautam Buddha University, Greater Noida, UP.

[2] Department of Engineering Chemistry & Environmental Engineering, PCE, Sitapura, Jaipur.

[3] Department of Chemistry, SKGC, Sikar, Rajasthan.

[4] Department of Engineering Chemistry & Environmental Engineering, GEC, Bikaner, Rajasthan.

plants and parts thereof for the restoration and preservation of the environment and for the sustainable use of resources. EB is utilized to develop advanced technologies based on biological systems to improve efficiency and reduce or utilize waste to benefit a wide range of industries and the environment, involves research (Meadows *et al.* 1972) and commercial efforts in three core areas namely biofilm prevention and dispersal, rapid in-field microbial detection and control and bioprocesses such as bioremediation and industrial wastewater treatment. EB brings together the multidisciplinary skills of researchers, engineers and industry participants to bring out novel technologies in environmental, industrial, agricultural, veterinary, mining and medical applications. EB is the multidisciplinary integration of sciences and engineering in order to utilize the huge biochemical potential of micro-organisms, plants and parts thereof for the restoration and preservation of the environment and for the sustainable use of resources. Some impressive studies on industrial applications of biotechnology are published in two OECD reports (OECD 2001; OECD 1998), which summarized, that biotechnology has the potential of a reduction of operational and/or capital cost for the realization of more sustainable processes (OECD). However, until today the sustainability of technical processes is more the exception than the rule and therefore so-called "End-of-Pipe"-technologies are absolutely necessary for the treatment of production residues. A disturbance of the equilibrium of the natural cycles of carbon, nitrogen, phosphate, sulfur or halogen compounds causes an ecological imbalance and endangers nature. In the Brundtland- report "Our common future" (Hauff 1987) a discussion was started about "sustainable development". The practical realization of this concept was suggested at the "Conference on Environment and Development" of the United Nations in Rio de Janeiro in 1992 and enforced as an action programme in the so-called Agenda 21. A sustainable development to maintain the basis for future generations is contraindicated by exploitation of non-regenerative energy and material resources and a shortening of life cycles (e.g. in information technologies). By the obligate demand for recycling of waste components, which is fixed in European Council Directive 91/156/EEC and e.g. translated to the German waste law (KrW/AbfG 1996), production and the use of commodities should minimize the amount of wastes. The practicability of this approach must be demonstrated in industrialized countries and then should be adopted by less developed or developing countries. EB initially started with wastewater treatment in urban areas at the turn of the 19/20th century (Hartmann 1999) and has been extended among others to soil remediation, off gas purification, surface and groundwater cleaning, industrial wastewater purification, deposition techniques of wastes in sanitary landfills and composting of bioorganic residues, mainly in the second half of the 20 century. The processes of EB for liquid, solid and gaseous waste treatment will help students and professional experts to obtain fast fundamental information and an overview over the biological background and general process alternatives (Jordening and Winter 2005).

The EB scientists and engineers provide expertise by involving microbial ecology and environmental engineering and integrated technology is useful for Environmental Remediation Technology Program, the Energy Resources Program, and the Climate Change and Carbon Management Program. As EB, houses all of the Ecology offices and laboratory facilities, therefore it is highly useful and focuses on research in real-time direct environmental assessment and biological treatment, bioremediation, and natural attenuation. Production of bioenergy and biochemicals from industrial and agricultural wastewater (Angenent 2004) is a better step. Science of metabolic engineering (Bailey 1991) plays an important role in EB. Engineering hydrogen sulfide production and cadmium removal by expression of the thiosulfate reductase gene (phsABC) from *Salmonella enterica* serovar typhimurium in *Escherichia coli* (Bang *et al.* 2000) like results are opens scope for future. Enhanced bioaccumulation of heavy metals by bacterial cells displaying synthetic phytochelatins (Bae *et al.* 2000) and genetic engineering of *Escherichia coli* for enhanced uptake and bioaccumulation of mercury (Bae 2001) was also successful. Enhanced mercury biosorption by bacterial cells with surface- displayed MerR (Bae *et al.* 2003) was also achieved in experimental conditions. Detection of heavy metal ions at femtomolar levels using protein-based biosensors (Bontidean 1998) and tuning biphenyl dioxygenase for extended substrate specificity (Bruhlmann and Chen, 1999) were also achieved successfully.

Directed evolution of toluene ortho monooxygenase for enhanced 1-naphthol synthesis and chlorinated ethene degradation (Canada 2002); bacterial cell surface display of organophosphorus hydrolase for selective screening of improved hydrolysis of organophosphate nerve agents (Cho *et al.* 2002); altering the substrate specificity of organophosphorus hydrolase for enhanced hydrolysis of chlorpyrifos (Cho *et al.* 2004); molecular evolution of an arsenate detoxification pathway by DNA shuffling (Crameri *et al.* 1997); genome shuffling improves degradation of the anthropogenic pesticide pentachlorophenol by *Sphingobium chlorophenolicum ATCC 39723* (Dai & Copley 2004); an *Escherichia coli* chromosomal *ars* operon homolog is functional in arsenic detoxification and is conserved in gram-negative bacteria (Diorio et al 1995) are some other successful experiments and evidences as far as application of EB is concerned Wonders of life. Stories from life sciences research (from the Fourth and Fifth Framework Programmes) documents provide economically viable information (European Commission 2002). Experiments like retrofitting existing chemical scrubbers to biotrickling filters for H_2S emission control (Gabriel & Deshusses 2003); alteration of the substrate range of haloalkane dehalogenase by site-directed mutagenesis (Holloway *et al.* 1998); microbial pathway prediction via a functional group approach (Hou *et al.* 2003); genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* (Jimenez *et al.* 2002); novel metal-binding proteins by design (Klemba 1994); functional analysis of a variety of chimeric dioxygenases constructed from two biphenyl dioxygenases that are similar in structurally but different in functionally (Kimura *et al.,* 1997); enhanced arsenic accumulation in engineered bacterial cells expressing ArsR (Kostal *et al.* 2004); enhanced degradation of polychlorinated biphenyls by directed evolution of biphenyl dioxygenase (Kumamaru *et al.* 1998); engineering a recombinant *Deinococcus radiodurans* for organopollutant degradation in radioactive mixed waste environments (Lange *et al.,* 1998); metabolic engineering of *Pseudomonas putida* for the simultaneous biodegradation of benzene, toluene, and *p*-xylene mixture (Lee *et al.,* 1994); selection of cadmium specific hexapeptides and their expression as OmpA fusion proteins in *Escherichia coli* (Mejare *et al.* 1998); the MerR heavy metal receptor mediates positive activation in a topologically novel transcription complex (O'Halloran *et al.* 1989); two-dimensional electrophoretic analysis of protein production during growth of *Pseudomonas putida* F1 on toluene, phenol, and their mixture (Reardon & Kim 2002); rapid evolution of a protein *in vitro* by DNA shuffling (Stemmer 1994); metabolism of polyhalogenated compounds by a genetically engineered bacterium (Wackett *et al.* 1994); metabolic engineering of an aerobic sulfate reduction pathway and its application to precipitation of cadmium on the cell surface (Wang *et al.* 2000) and rhizoremediation of trichloroethylene by a recombinant root-colonizing *Pseudomonas fluorescens* strain expressing toluene *ortho*monooxygenase constitutively (Yee *et al.* 1998) are some well establish experiments that opened new ways in the field of EB.

APPLICATIONS OF ENVIRONMENTAL BIOTECHNOLOGY (EB)

In Aerobic granulation i.e. wastewater treatment technology of the future, optimization of wastewater treatment systems by manipulating the microbial composition and properties of biosolids involves. The benefits of granulation include a dramatic increase in the throughput of a biosolid processing facility thus reducing the immediate need for plant managers to upgrade their assets. In addition, with the combined use of nutrient removal processes, it is envisaged that the entire footprint of a sludge processing plant will be greatly reduced.

Biological Treatment, Bioremediation, and Natural Attenuation

Biological treatment, bioremediation and natural attenuation have been a rapidly growing area of science over the past decade. The acceptance of natural attenuation as a solution for cleaning up contaminated sites and DOE's recognition that they will have long-term stewardship issues that they must address at the most contaminated sites has greatly increased the urgency for basic and applied research related to microbial ecology and biogeochemistry. This type of research is truly enabling for natural attenuation since characterization, predictions, and verification monitoring requires a strong scientific basis. Natural attenuation is viewed as the best solution for cleaning up many waste sites and will save billions of dollars in cleanup costs. Bioremediation, both *in situ* and *ex situ* have also enjoyed strong scientific growth, in part due to the increased use of natural attenuation, since most natural attenuation is due to biodegradation. Bioremediation and Natural Attenuation are also seen as a solution for emerging contaminant problems, e.g. endocrine disrupters, landfill stabilization, mixed waste biotreatment, and biological carbon sequestration. The types of contaminants that EB investigators have expertise with include chlorinated solvents, petroleum hydrocarbons, polynuclear aromatic hydrocarbons, ketones, TNT, inorganic nitrogen (NO_3, NH_4), tritium, Pu, Np, Cr, and U. The Biotreatment, Bioremediation and Natural Attenuation area has both basic research and field application foci for the EB. The basic research foci are co-metabolism, biotreatability, biotransformation kinetics, and modeling of biogeochemical processes. The field application foci are co-metabolic techniques, biogeochemical assessment techniques, and modeling of attenuation and environmental fate.

Basic Research Areas of EB

Co-metabolism: EB scientists are recognized leaders in the field of co-metabolic pollutant transformation. Research conducted by scientists at EB has demonstrated that co-metabolism is a dominant process for the degradation of PAHs, chlorinated solvents, and fuel oxygenates. Co-metabolic processes are difficult to study and require novel experimental approaches. One approach under development is the use of genetic probes to identify the presence of metabolic pathways implicated in co-metabolic processes. In this research, physiological responses are being linked to gene probe signatures to develop methods for identifying co-metabolic potential in environmental samples. The second approach uses a kinetic evaluation of partial transformation reactions to compare and characterize bacterial enzyme systems implicated in co-metabolism. Co-metabolic processes are competitive enzyme reactions and can be tested and modeled as such. Both these approaches are yielding unique and valuable advances in our understanding and application of co-metabolic and partial transformations.

Biotreatability: EB does a number of types of treatability tests for contaminants in soil and water, using soil columns, respirometers, bioreactors, and field respiration tests. Real-time direct biogeochemical techniques are being developed to provide the most direct possible methods to measure the rates of biodegradation and the effect stimulants and environmental conditions have on both the functional microbial components and the biogeochemistry of the environment being studied. For example, mixed waste biotreatment includes both the engineering of bioreactors for the treatment of high strength waste streams and understanding how natural attenuation or active bioremediation can be applied to mitigate the impact of the weapons legacy. Past projects have examined how bacterial blooms on solvents may affect the environmental fate of actinides in the subsurface. Current studies are examining the biotreatment of tritiated solvent wastes and the transformation of uranium on bacteria surfaces.

Biotransformation Kinetics: EB is linking engineering and microbiology in an integrated program to examine biotransformation kinetic for pollutant clean-up and microbial product formation. The kinetic program examines microbial response and activity as a function of substrate concentrations and time. The results of kinetic studies are being used in reactor design and operations analysis. Future research will focus on the application of kinetic approaches to predicting microbial population response to pollutant inputs.

Modeling: EB currently does not have a modeling program, but is developing collaboration with modeling groups within ESD. Modeling is targeted as an area for increased collaboration in the next three years. In particular, there is a need to link biodegradation kinetic information with subsurface fate and transport of pollutants. Modeling is also needed to further understand the control and operation of co-metabolizing biological reactors.

Engineering Biosorbents for Heavy Metal Removal

Immobilization of heavy metals into biomass or precipitation through reduction to lesser bioactive metal species, such as metal sulfide are the major mechanisms employed by nature (microorganism, animals and plants) to counteract heavy metal toxicity. These natural mechanisms can be easily exploited to optimize biosorbents that are more efficient for heavy metal removal. In one example, a sulfide-dependent metal removal strategy was developed by engineering the sulfate reduction pathway into a robust bacterium *E. coli.* The resulting strains produced significantly more sulfide and removed more than 98% of the available cadmium under anaerobiosis. Further improvement in metal precipitation was achieved by engineering effective sulfate reduction under aerobic conditions. *E. coli* expressing both serine acetyltransferase and cysteine desulfhydrase overproduced cysteine and converted it to sulfide. The resulting strain was effective in aerobically precipitating cadmium. This aerobic approach of metal precipitation is particularly attractive as large-scale processes could be implemented under aerobic conditions. The challenges are to incorporate these genetic modifications into a robust environmental microbe that could survive and thrive under the required operation conditions. Similar success in engineering enhanced biosorbents has been achieved by displaying metal-binding peptides onto the cell surface. These peptides emulate the structure of phytochelatins, metalchelating molecules that play a major role in metal detoxification in plants and fungi.

Designer Strains for Enhanced Biodegradation

Using well-established tools from metabolic engineering and biochemistry, efforts have been made on engineering microbes to function as “designer biocatalysts,” in which certain desirable traits are brought together with the aim of optimizing the rate and specificity of biodegradation pathways. One common bottleneck is the transport of pollutants across the cell membrane, which limits the overall rate in many microbial biodegradation. An example is for a class of neurotoxic organophospates, which are used extensively as pesticides and chemical warfare agents. Although an enzyme, organophosphorus hydrolase (OPH), has been shown to degrade these pesticides (Richins *et al.* 1997) effectively, the use of whole cell detoxification is limited by the transport barrier of substrates across the cell membrane. Display of OPH onto the cell surface has been employed to bypass this transport barrier, resulting in 7-fold faster degradation compared to whole cells expressing OPH intracellularly. This simple approach typified the unique combination of chemical engineering principle with modern genetics, and has been similarly employed for other useful environmental applications such as the display of metal-binding proteins described earlier. Although only fairly simple enzymes or peptides are successfully displayed so far, continued development in this area should pave the way for the successful display of more complex enzymes, such as dioxygenases or monooxygenases, enabling a broader class of pollutants to be targeted. Recruiting different pathways into a designer microbe is another powerful approach to enhance biodegradation. Very often, these pathways are combined with other existing pathways to enable complete biodegradation. For example, construction of a hybrid strain which is capable of mineralizing components of a benzene, toluene, and *p*-xylene mixture simultaneously was attempted by redesigning the metabolic pathway of *Pseudomonas putida.* A hybrid strain carrying both the *tod* and the *tol* pathways was constructed and was found to mineralize a benzene, toluene, and *p*-xylene mixture without accumulation of any metabolic intermediate. Since the number of known biodegradation pathways is increasing everyday, this in combination with the increasing number of genome sequence (Whitfield 2004) elucidated for environmental microbes, should allow us to rationally combine useful pathways across species into any desirable combinations using tools available from metabolic engineering. In this respect, it will also be interesting to see whether multiple enzymes can also be displayed onto the surface, allowing sequential degradation to occur without any uptake limitation. The challenges here are to devise strategies that will allow not only multiple enzyme display, but also the display of complex enzymes without compromising integrity and viability.

Another promising approach for success remediation is the introduction of biodegradation pathways into microbes that thrive in the contaminated environment. *Deinococcus radiodurans* is a soil bacterium that can survive acute exposures to ionizing radiation of 15,000 Gy without lethality. A recombinant *D. radiodurans* strain (Daly *et al.* 1994) expressing toluene dioxygenase was shown to effectively oxidize toluene, chlorobenzene, in a highly irradiating environment. The recombinant strains were also tolerant to the solvent effects of toluene at levels exceeding those of many radioactive waste sites. The prospect of using this strategy to alleviate the toxicity of radionuclides and heavy metals and to provide efficient treatment for a variety of organic wastes is very promising. Similarly selective advantages can be achieved by exploiting the synergistic plant-microbe relationship in a rhizosphere. This strategy was recently reported using a wheat rhizosphere system for the detoxification of soil-borne trichloroethylene (TCE). The toluene *o*-monooxygenase (Tom) gene was introduced into *Pseudomonas fluorescents* 2-79, a bacterium that colonizes the wheat root, enabling the establishment of a bacterium-plant-soil microcosm. Treatment of TCE-contaminated surface and near-surface soil was demonstrated, with more than 63% of the initial TCE removed within 4 days. The most attractive aspect of this technology is the low cost associated since only expenses required for planting is necessary. This will also represent an excellent opportunity for chemical engineers working primarily with microorganisms to collaborate with others focusing on plant, combining the unique features of rhizoremediation with phytoremediation. A notable opportunity that has been so far overlooked by most chemical engineers is the production of valuable products and energy directly from wastewater and of particular interest is the possibility of biohydrogen and bioelectricity production. In most cases, only natural micro-organisms are exploited, resulting in fairly modest yields. However, this poor conversion also represents an excellent opportunity to employ the tools from metabolic engineering, protein engineering, molecular evolution, and system biology for the discovery of novel microorganisms with significantly improved efficiencies.

Enzyme Engineering for Improved Biodegradation

The ever-increasing information regarding the structure and function of enzymes and pathways involved in biodegradation of recalcitrant pollutants offers opportunities for improving enzymes or entire pathways by genetic engineering. Control mechanism and enzyme properties can be tailored by site directed mutagenesis, which is often guided by computer assisted modeling of the three-dimensional (3-D) protein structures. For example, site directed approaches have been applied to enlarge the binding pocket of haloalkane dehalogenase, resulting in several-fold faster dechlorination of dichlorohexane. However, no mutant tested could utilize the more bulky substrates, such as TCE, suggesting limitations using this structural based approach. Perhaps the use of computational methods to predict subtle and distal changes in the protein backbone without perturbing the overall protein structure could be used to further improve enzyme function and stability. Site-directed or rational approaches can often fail because it is known that mutations far from the active site can modulate catalytic activity or substrate recognition but are difficult to predict *a priori.* These methods are also restrictive because they allow the exploration of only a very limited sequence space at a time. This is clearly indicated by the creation of several chimeric enzymes guided by sequence comparison between two similar biphenyl dioxygenases. Although the resulting variants were capable of hydroxylating both double *ortho*- and *para*-substituted PCBs, combining the substrate range of the two parental enzymes, no new activity was observed. In this case, irrational approaches such as DNA shuf- fling, which allow the cross-breeding of genes between diverse classes of species, can be a preferable alternative to direct the evolution of enzymes or pathways with highly specialized traits. In two independent studies, the substrate range of biphenyl dioxygenases toward PCBs has been successfully extended using directed evolution. Variants were obtained by random shuffling of DNA segments between the large subunit of two wild type biphenyl dixoxygenases. Several variants had extended substrate ranges for PCBs exceeding those of the two parental enzymes. Similar attempts to extend the substrate specificity of toluene *ortho*-monooxygenase (TOM) have been successful. In both cases, DNA shuffling was combined with simple plate screening assays, resulting in rapidly degradation of virtually nondegradable substrates. These examples are perhaps the best reminder, suggesting that other important biodegradation enzymes could be similarly improved with this strategy since the number of related dioxygenases, monoxygenases, and hydrolases for different pollutants are virtually unlimited. Molecular evolution is probably the most useful way for evolving biodegradation enzymes for extended substrate specificities since microbial degradation (Pazos etb al. 2003) of xenobiotics is usually by co-metabolism and does not exert a natural selective pressure on bacteria. Computational methods that are useful to guide experimental design for directed evolution may be used to predict the optimal number of mutants that must be screened. Moreover, an optimal design of the parental DNA sequence set will allow a more focused probing of sequence space in only those regions that are likely to yield functional hybrids and should lead to a more efficient utilization of experimental resources, saving time and effort by reducing the number of evolutionary cycles.

Evolutionary and Genomic Approaches to Biodegradation

Evolutionary approaches are extremely useful for optimization of an entire biodegradation pathway comparing to step by-step modifications offered by rational design. This was recently demonstrated by the modification of an arsenic resistance operon using DNA shuffling. Cells expressing the optimized operon grew up to 0.5 M arsenate, a 40-fold increase in resistance. Moreover, a 12-fold increase in the activity of one of the gene products (arsC) was observed in the absence of any physical modification to the gene itself. The authors speculate that modifications to other genes in the operon affect the function of the *arsC* gene product. Such unexpected but exciting results are more likely to be realized using irrational approaches. This strategy is particularly attractive since the ultimate goal of many remediation approaches is for complete mineralization of the pollutants, and the concurrent optimization of an entire pathway will allow the efficient search for the correct coordination between a complex set of biodegradation reactions. Along the same line, recent advances in genome shuffling between species, which allow the exchange and recombination of diverse pathways into a single species, will further accelerate the discovery of novel microbes that are useful for the remediation of even a complex mixture of pollutants. The availability of bacterial genomes relevant to biodegradation in recent years has allowed the feasibility to study the complex interactions between cellular reactions from a genomic30 and proteomic31 level. A quantitative understanding of how cells function requires every gene and protein to be placed in their dynamic context, which entails the integrated consideration of many interacting components. From this perspective, a system biology approach is necessary to predict the functioning of an organism in a complex environment and to describe the outcome of the thousands of individual reactions that are simultaneously taking place in a microbial cell. So far, such prokaryotic models have been limited primarily to *E. coli* and a few pathogens. However, similar modeling approaches should be able to predict contaminant bioremediation by microorganisms that are known to predominate in polluted environments. Recently, de Lorenzo and coworkers presented a pioneering study on the characteristics of the “global biodegradation network”, in which they considered the global pool of known chemical reactions implicated in biodegradation regardless of their microbial hosts. The characteristics of this network support an evolutionary scenario in which the reactions evolved from the central metabolism toward more diversified reactions, allowing us to understand the evolution of new pathways for the degradation of xenobiotics and provide the basis for predicting the abilities of chemicals to undergo biological degradation, and for quantifying the evolutionary rate for their elimination in the future. This type of analysis, when coupled with the predictive approach for microbial catabolism using the University of Minnesota Biocatalysis/ Biodegradation Database (UM-BBD) as a knowledge base and various sets of heuristic rules, will lead to untapped and improved strategies for bioremediation. This represents an excellent opportunity for chemical engineers who are already involved with system biology, and will undoubtedly evolve into an important research direction within the next 5 years.

CONCLUSION AND DISCUSSION

Environmental Biotechnology (EB) plays very important role in waste water treatment and hence in pollution control. Already, the technology has been proven in a number of areas and future developments promise to widen its scope. Some of the new techniques now under consideration make use of genetically modified organisms designed to deal efficiently with specific tasks. As with all situations where there is to be a release of new technology into the environment, concerns exist. There is a potential for biotechnology to make a further major contribution to protection and remediation of the environment.

'White' biotechnology or industrial and EB is a broad and expanding field that includes making enzymes with a variety of industrial uses that include the manufacture of bioplastics and biofuels and using micro-organisms and plants for the treatment of wastes and abatement of pollution, a process known as bioremediation. Bacterial bioleaching, i.e. using specific bacteria to extract metals from ores or mine wastes sprayed with water, is a growing sector of the mining industry and several developing countries are already playing a key role in this area. Other research efforts are scanning the microbial diversity of various environments (e.g. the oceans and seas) and deciphering their genetic information aimed at isolating micro-organisms that could be used in the manufacture of drugs, enzymes and a wide range of bioactive compounds (Dupont 2004), as well as in bioremediation processes. As for materials and fuels that are not derived from petrochemical processes, researches are also underway for processes to improve and enhance the bioremediation of water, soils and ecosystems at large, and minimize the use of fossil-fuel energy. All these forms of 'white' biotechnology are poised to increase the field's positive social acceptance.

White biotechnology is part of the contribution of applied science to a healthier environment and to sustainable development (The Economist 2003). 'White' biotechnology or industrial and EB may now only seem to occupy a small niche but because of its enormous promise and potential, it may become as ubiquitous as those of the chemical industry today. Now, industrial chemistry is everywhere, from agrifood and pharmaceutical industries to fuel production to textile, fertilizer, paper industries, etc. (The Economist 2003). In June 2000, the Biomass Research and Development Act and the Sustainable Fuels and Chemicals Act, voted by the US Congress, allocated hundreds of million dollars to research projects jointly carried out by universities and industry; they also created the Biomass R&D Board, in charge of coordinating the action of federal administrations (commerce, energy, agriculture, etc.) (Reverchon 2002). Trypsin is an enzyme normally produced in the pancreas of animals that breaks down proteins as part of the digestion process. It is also a critical enzyme in the bioprocessing of proteins, such as insulin, where it is used to help cleave the protein into its active form. The proprietary technology offers an animal-free source of proteins as well as the capacity to produce large volumes that can be easily scaled-up or down (The Economist 2003). Enzyme production involves biotechnology (Sasson, 2000).

India too is a player in biotechnology with the bulk of India's industrial biotechnology sector focuses on producing enzymes (Grace 2004) for the textile and paper industries. The sector was the second-largest exporter ($37 million in 2002-2003) to other Asian and European countries. In 2002-2003, this sector produced revenues of $72 million and was rapidly growing (Grace 2004). The example of vitamin-B2 synthesis illustrates how a new biotechnology process can benefit both environment and economics. This vitamin is generally produced using a complex eight-step chemical process. BASF AG's new process reduces it to a one-step process involving fermentation whereby the raw material is fed to a mould, which transforms it into the finished product, recovered as yellow crystals directly from the fermentation broth. The biotechnological process reduces overall costs by up to 40% and the environmental impact by 40% (CO2 emissions are reduced by 30%, resource consumption by 60% and waste by 95%). The synthetic pathway to the antibiotic cephalexin has also evolved from a multistep chemical process to a mild bio-transformation, based on a fermented intermediate linked enzymatically with a side chain to the final end product. The biotechnological process uses less energy and input chemicals, is water-based and generates less waste.

Metabolism of bacteria is amazingly versatile and nutritional versatility of microorganisms can also be exploited for biodegradation of environmental pollutants. This process is called bioremediation and is based on the capability of certain microorganisms to metabolize toxic pollutants, obtaining energy and biomass in the process. Ideally, the chemicals are transformed into harmless compounds such as carbon dioxide and water. Harnessing microorganisms to degrade harmful compounds is an attractive option for clean up of polluted environments. However, despite the apparent simplicity of microorganisms, the different strategies for dealing with pollutants are as diverse as the organisms themselves. The process of biodegradation must therefore be investigated on several levels; biochemical, genetic and physiological.

Small firms are also interested in the manufacture of biopolymers. Metabolix, a company based in Cambridge, Massachusetts, is working on the production of monomers by living organisms and on their polymerization by the same organisms. Some bacteria could store polyhydroxyalkanoate up to 80% of their weight. Polyhydroxyalkanoates (PHAs) are produced by either non-recombinant bacteria (e.g. Ralstonia eutropha) or recombinant ones (e.g. Escherichia coli to which genes of R. eutropha have been transferred). There is therefore a need to decrease the production cost and also increase productivity (Dufour 1999). Plants could produce the monomeric units of the plastic polymers, as some monomers are difficult to elaborate from oil. C. Chapple's work consisted of trying to stabilize some monomers and to produce them in high concentrations in plants. The viability of the whole process depended on the storage of sufficient amounts of the plant-made plastics in the cell vacuoles from which they could be extracted (The Economist 2003). Both transgenic Arabidopsis and oilseed rape produced only 2.5% of their biomass as plastic, which implied that more genetic transformations would be necessary to obtain commercially exploitable results. The extraction process was also an important element of a profitable production of plastics by transgenic crops (Dufour 1999). By the end of 2003, trials were expected to be carried out to test the production capacity of transgenic plants (Sasson, 2000).

The biofuels target for 2005 is 2 percent of vehicle fuel, rising to 5.75% by 2010 (The Economist 2004). Biofuel production is part of 'white' biotechnology. Ethanol, a biofuel, is produced from the fermentation of cane sugar. Another constraint relates to the balance between the market of petrol (gasoline) and gasoil: the European Union was importing 22 million tons of gasoil and exporting 20 million tons of gasoline, mainly to the USA. While the demand for gasoil was increasing by 2.5% annually, gasoline consumption was decreasing by 1.5% (Lauer 2004). Chile is the world's first-biggest producer of copper. While in 1990 the production target had been fixed at 2.5 million tons for the year 2000, this figure was superseded in 1995, and the production exceeded 5 million tons in the late 1990s (Bioplanet no. 6, July-August 2000, p.22). Since 1990 when Chile returned to democracy, there has been no privatization in the mining sector with 80% of the mines still presently owned by the state (Lazare 2004). Because of the importance of copper mining and production in Chile's economy, the country has often been nicknamed the 'Saudi Arabia of copper'. Copper's physico-chemical properties and multiple uses, as well as a strong demand for the product from Asia (mainly China) have resulted in a high rise of international prices (London's stock exchange) during the first half of 2004 (Lazare 2004). Codelco, the National Copper Corporation, is planning to raise its annual production up to 3 million tons in 2012 (Lazare 2004).

The efficiency of the procedure in mercury removal from polluted water down to a few ng/lt is high. Once the bacteria died, they were incinerated to recuperate the accumulated pure mercury (European Commission 2002). In the US, seven or eight similar companies in phytoremediation were already in existence in 2002, where the value of the potential market for phytoremediation was estimated at $100 million (Tastemain 2004). The same research team is well known for their "transgenic" work particularly in the transfer to plants of bacterial genes, coding for the conversion of toxic mercury into its less dangerous volatile form (Tastemain 2002). EB is useful to sequence the DNA of every organism in each sample (Whitfield 2004). Through better understanding of how myriads of bacteria combine and interact to influence the ocean, we shall be better able to monitor the conditions of these environments and possibly manipulate them to our advantage. It may even be possible to find micro-organisms that can produce drugs or act as energy sources (Whitfield 2004). In practice, however, there is too much diversity in most environments to sequence every gene of every species.

REFERENCES

1. Angenent L T, Karim1 K, Al-Dahhan1 M H, Wrenn B H, Domy´_guez-Espinosa R (2004), "PRoduction of Bioenergy and Biochemicals from Industrial and Agricultural Wastewater", *Trends. Biotechnol.* , Vol. 22, pp. 477-482.
2. Bailey J E (1991), "Toward a Science of Metabolic Engineering", *Science,* Vol. 252, pp. 1668-1691.
3. Bang S W, Clark D S, Keasling J D (2000), "Engineering Hydrogen Sulfide Production and Cadmium Removal by Expression of the Thiosulfate Reductase Gene (phsABC) from Salmonella Enterica Serovar Typhimurium in *Escherichia coli*", *Appl. Environ Microbiol.*, Vol. 66, pp. 3939-3944.
4. Bae W, Chen W, Mulchandani A, Mehra R (2000), "Enhanced Bioaccumulation of Heavy Metals by Bacterial Cells Displaying Synthetic Phytochelatins", *Biotechnol. Bioeng.*, Vol. 70, pp. 518-524.
5. Bae W, Mehra R, Mulchandani A, Chen W (2001), "Genetic Engineering of *Escherichia coli* for Enhanced Uptake and Bioaccumulation of Mercury", *Appl. Environ. Microbiol.*. Vol. 67, pp. 5335-5338.
6. Bae W, Wu C, Kostal J, Mulchandai A, Chen W (2003), Enhanced Mercury Biosorption by Bacterial Cells with Surface-Displayed Mer R.", *Appl. Environ. Microbiol.*, Vol. 69, pp. 3176-3180.

7. Bontidean I, Berggren C, Johansson G, Csorgi E, Mattiasson B, Lloyd JR, Jakeman K J and Brown N L (1998), "Detection of Heavy Metal Ions at Femtomolar Levels Using Protein-Based Biosensors", *Anal. Chem.*, Vol. 70, pp. 4162-4169.

8. Brühlmann F and Chen W (1999), "Tuning Biphenyl Dioxygenase For Extended Substrate Specificity", *Biotechnol Bioeng.*, Vol. 63, pp. 544-551.

9. Canada K A, Iwashita S, Shim H and Wood T K (2002), "Directed Evolution of Toluene Ortho Monooxygenase for Enhanced 1-naphthol Synthesis and Chlorinated Ethene Degradation", *J. Bacteriol., Vol.* 184, pp. 344-349.

10. Cho CM-H, Mulchandani A and Chen W (2002), "Bacterial Cell Surface Display of Organophosphorus Hydrolase for Selective Screening of Improved Hydrolysis of Organophosphate Nerve Agents", *Appl. Environ. Microbiol.*, Vol. 68, pp. 2026-2030.

11. Cho C M-H, Mulchandani A and Chen W (2004), Altering the Substrate Specificity of Organophosphorus Hydrolase for Enhanced Hydrolysis of Chlorpyrifos", *Appl. Environ. Microbiol.,* Vol. 70, pp. 4681-4685.

12. Crameri A, Dawes G, Rodriguez E Jr, Silver S and Stemmer W P C (1997), "Molecular Evolution of an Arsenate Detoxification Pathway by DNA Shuffling", *Nat. Biotechnol.*, Vol. 15, pp. 436-438.

13. Dai M H and Copley S D (2004), "Genome Shuffling Improves Degradation of the Anthropogenic Pesticide Pentachlorophenol by *Sphingobium chlorophenolicum* ATCC 39723", *Appl. Environ. Microbiol.,* Vol. 70, pp. 2391-2397.

14. Diorio C, Cai J, Marmor J Shinder R, DuBow M S (1995), "An *Escherichia coli* Chromosomal *ars* Operon Homolog is Functional in Arsenic Detoxification and is Conserved in Gram-Negative Bacteria", *J. Bacteriol.*, Vol. 177, pp. 2050-2056.

15. Daly M J, Ouyang L and Minton K W (1994), "*In vitro* Damage and *recA*-Dependent Repair of Plasmid and Chromosomal DNA in the Radioresistant Bacterium *Dinococcus radiodurans*", *J. Bacteriol.*, Vol. 176, pp. 3508-3517.

16. Dufour, J.-P. 1999. Des matières plastiques biodégradables en culture. Le Monde (Paris), 1 October 1999, p.28.

17. Dupont G (2004), Biocarburants: la France Peut-elle Rattraper Son Retard? Le Monde 2, 28 May 2004, p. 11, Paris.

18. European Commission (2002), "Wonders of Life Stories from Life Sciences Research (from the Fourth and Fifth Framework Programmes), Luxembourg, Office of Official Publications of the European Communities, p. 27.

19. Gabriel D and Deshusses M A (2003), "Retrofitting Existing Chemical Scrubbers to Biotrickling Filters for H_2S Emission Control", *Proc. Natl. Acad. Sci.,* Vol. 100, pp. 6308-6312, USA.

20. Grace J (2004), "Unveiling the Indian Biotechnology Industry, Australasian Biotechnology", *Journal of Australia's Biotechnology Organisation-AusBiotech*, Vol. 14, No.3, pp. 22-23.

21. Hartmann L (1999) "Historical Development of Wastewater Treatment Processes", in: *Biotechnology-Environmental processes I* (Volume Editor J Winter), pp. 5-16, Wiley-VCH, Weinheim.

22. Hauff V (Ed.) (1987), "Unsere Gemeinsame Zukunft, Der Brundtland-Bencht der

Weltkommission für Umwelt und Entwicklung. Eggenkamp Verlag, Greyen KrW/AbfG 1996. Kreislaufwirtschafts- und Abfallgesetz – Gesetz zur Förderung der Kreislaufwirtschaft und Sicherung der umweltverträglichen Beseitigung von Abfällen. Vom 27.9.1994. Bundesgesetzblatt BGBL I., p. 2705.

23. Holloway P, Knoke K L, Trevors J T and Lee H (1998), "Alteration of the Substrate Range of Haloalkane Dehalogenase by Site-Directed Mutagenesis", *Biotechnol. Bioeng.*, Vol. 59, pp. 520-523.

24. Hou B K, Wackett L P and Ellis L B (2003), "Microbial Pathway Prediction: A Functional Group Approach", *J. Chem. Inf. Comput. Sci.*, Vol. 43, pp. 1051-1057.

25. Jimenez J I, Minambres B, Garcia J L, Diaz E (2002), "Genomic Analysis of the Aromatic Catabolic Pathways from *Pseudomonas putida* KT2440", *Environ. Microbiol.*, Vol. 4, pp. 824-841.

26. Jördening H J and Winter J (2005), *Environmental Biotechnology: Concepts and Applications,* Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, ISBN: 3-527-30585-8

27. Klemba M, Gardner K H, Marino S, Clarke K D, Regan L (1994), "Novel Metal-Binding Proteins by Design", *Nat. Struct. Biol.,* Vol. 2, pp. 368-373.

28. Kimura N, Nishi A, Goto M and Furukawa K (1997), "Functional Analysis of a Variety of Chimeric Dioxygenases Constructed from Two Biphenyl Dioxygenases that are Similar Structurally but Different Functionally", *J. Bacteriol.,* Vol. 179, pp. 3996-3943.

29. Kostal J, Yang R, Wu CH, Mulchandani A and Chen W (2004), "Enhanced Arsenic Accumulation in Engineered Bacterial Cells Expressing ArsR", *Appl. Environ. Microbiol.*, Vol. 70, pp. 4582-4587.

30. Kumamaru T, Suenaga H, Mitsuoka M, Watanabe M, Furukawa K (1998), "Enhanced Degradation of Polychlorinated Biphenyls by Directed Evolution of Biphenyl Dioxygenase", *Nat. Biotechnol.*, Vol. 16, pp. 663-666.

31. Lange C C, Wackett L P, Minton K W and Daly M J (1998), "Engineering a Recombinant *Deinococcus radiodurans* for Organopollutant Degradation in Radioactive Mixed Waste Environments", *Nat. Biotechnol.*, Vol. 16, pp. 929-933.

32. Lauer S (2004), "Constructeurs Automobiles et Pétroliers Sont Réticents", *Le Monde,* 28 May, p. 11, Paris.

33. Lazare F (2004), "Le Cuivre du bout du Monde", *Le Monde,* 24-25 October, p. 12, Paris.

34. Lee J Y, Roh J R and Kim H S (1994), "Metabolic Engineering of *Pseudomonas putida* for the Simultaneous Biodegradation of Benzene, Toluene, and *p*-xylene Mixture", *Biotechnol. Bioeng.*, Vol. 4, pp. 1146-1152.

35. Meadows D. H., Meadows D. L., Zahn E., Milling P. (1972) Die Grenzen des Wachstums. Bericht des Club of Rome zur Lage der Menschheit. Stuttgart

36. OECD (1998), Biotechnology for Clean Industrial Products and Processes: Towards Industrial Sustainability, OECD Publications, Paris.

37. OECD (2001), *The Application of Biotechnology to Industrial Sustainability*, OECD Publications, Paris.

38. Mejare M, Ljung S and Bulow L (1998), "Selection of Cadmium Specific Hexapeptides and Their Expression as OmpA Fusion Proteins in *Escherichia coli", Protein Eng.*, Vol. 11, pp. 489-494.
39. O'Halloran T V, Frantz B, Shin M K, Ralston D M and Wright J G (1989), "The MerR Heavy Metal Receptor Mediates Positive Activation in a Topologically Novel Transcription Complex", *Cell*, Vol. 56, pp. 119-129.
40. Pazos F, Valencia A, de Lorenzo V (2003), The Organization of the Microbial Biodegradation Network from a Systemsbiology Perspective", *EMBO Rep.,* Vol. 4, pp. 994-999.
41. Reardon K F and Kim K H (2002), Two-Dimensional Electrphoresis Analysis of Protein Production During Growth of *Pseudomonas putida* F1 on Toluene, Phenol, and Their Mixture", *Electrophoresis*, Vol. 23, pp. 2233-2241.
42. Reverchon A (2002), Le vivant, nouveau carburant de l'industrie. Les bio-industries sont-elles viables économiquement? Chez Genencor, les chercheurs forgent les outils des usines du vivant. Le Monde, 10 September, p. II, Paris.
43. Richins R, Kaneva I, Mulchandani A and Chen W (1997), "Biodegradation of Organophosphorus Pesticides by Surface-Expressed Organophosphorus Hydrolase", *Nature Biotechnol.*, Vol. 15, pp. 984-987.
44. Sasson A (2000), Biotechnologies in Developing Countries: Present and Future, Vol. 3, Regional and Subregional Co-Operation, and Joint Ventures, UNESCO, Future-Oriented Studies, p. 1,103, Paris.
45. Stemmer WPC. Rapid evolution of a protein *in vitro* by DNA shuffling. *Nature*. 1994;370:389-391.
46. Tastemain C (2002), "Des Plantes Transgéniques Pour Dépolluer les sols Contaminés", *Le Monde,* 20 December, p. 27, Paris.
47. *The Economist* (2003), "Climbing the Helical Staircase, A Survey of Biotechnology", *The Economist*, 29 March, pp. 3-18, London.
48. *The Economist* (2004), "Industrial Biotech. Field of Dreams", *The Economist*, 10 April, p. 59, London.
49. Wackett L P, Sadowsky M J, Newman L M, Hur H G, Li S (1994), "Metabolism of Polyhalogenated Compounds by a Genetically Engineered Bacterium", *Nature*, Vol. 368, pp. 627-629.
50. Wang C L, Maratukulam P D L, Clark D S and Keasling J D (2000), "Metabolic Engineering of an Aerobic Sulfate Reduction Pathway and Its Application to Precipitation of Cadmium on the Cell Surface", *Appl. Environ. Microbiol.*, Vol. 66, pp. 4497-4502.
51. Whitfield J (2004), "Genomics, Microbial Soup of Life is Sieved for Treasure", *Financial Times*, 16 April, p. 11, London.
52. Yadav S K (2009), "Water Pollution Management Through Applied Environmental Biotechnology", *Proceedings of International Conference on Recent Advances in Environmental Protection,* St John's College, Agra, December 17-19.
53. Yee D C, Maynard J A and Wood T K (1998), "Rhizoremediation of Trichloroethylene by a Recombinant Root-Colonizing *Pseudomonas fluorescens* Strain Expressing Toluene *ortho*monooxygenase Constitutively", *Appl. Environ. Microbial.*, Vol. 64, pp. 112-118.

International Journal Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
E-mail: editorijlbpr@gmail.com or editor@ijlbpr.com
Website: www.ijlbpr.com

ISSN 2250-3137
9 772250 313001 01