

# A Review On Environmental Pollution And Enhancement Of Biodegradation By Genetic Engineering

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## ABSTRACT

*Environmental Biotechnology started with waste water treatment at the turn of 19/20<sup>th</sup> Century and provides services to remove contaminants from waste water and soil, to monitor pollutants and toxic materials in the environment and captures valuable products from wastes. This review we briefly summarize related recent progress on isolation of microorganisms for pollutant degradation in extreme conditions and the study of genes and enzymes related to biodegradation pathways. This paper also reviews various parameters like effect of metal ion, pH, carbon sources etc. and information on enhancement of biodegradation of genetic Engineering was also mentioned in detail.*

**Keywords:** *Environmental pollution, Industrial pollution, Microbial pollution, Genetic engineering, Metabolic pathways.*

## I. Introduction

### A. Environmental biotechnology

The Environmental biotechnology started with wastewater treatment within the turn of the 19/20th century [1] and provided services for the removal of contaminants from wastewater and soil, to monitor pollutants and harmful materials within the environment and to recovery valuable products from waste [2]. In recent decades, it's offered many methods for environmental pollution and has become a technology for sustainable development.

Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds [1]. Indeed, biodegradation is the process by which organic substances are broken down into smaller compounds by living microbial organisms. Biodegradation provides useful strategies to wash up many sorts of and xenobiotic pollutants like polycyclic hydrocarbon (PAH), organic metals, and halogenated or sulfur compounds[2–3]. The combination of genetics and process engineering, biodegradation and decomposition

pathways are useful and therefore the regulatory mechanisms for removing many organic pollutants are elucidated, and lots of engineering strategies are developed to harness this power.

Up to 13 million lives might be saved annually by reducing environmental risks, in step with the planet Health Organization's first country-by-country analysis of the impact of environmental factors on human health ([www.who.int/quantifyingehimpacts/countryprofiles/en/index.html](http://www.who.int/quantifyingehimpacts/countryprofiles/en/index.html)). Environmental pollution could be a great concern to all or any countries round the world [4].

## B. Environmental Pollution

Environmental pollution is not a new phenomenon, yet it remains the world's greatest problem facing humanity, and the leading environmental causes of morbidity and mortality. Man's activities through urbanization, industrialization, mining, and exploration are at the forefront of global environmental pollution. Environmental pollution by technological revolution may be a major problem facing the globe today and there's an increasing awareness of the very fact that a clean environment is important for better health of living organisms. Environmental pollution is an unfavorable alteration of our surroundings led to particularly by the commercial revolution. Industrial projects have a profound influence on society and therefore the environment not only in terms of advantages but also in risks and hazards. Man has been paying the value of destruction he has produced in his environment and in natural action throughout the centuries. it's exact to mention that the economic pollution added by different industries is causing a good threat to human, animals additionally on plants and formed Industrial pollution. Developments of recent technologies and anthropogenic activities have affected environmental quality in an exceedingly lot of how. The harmful effects on the environment and health of living organisms are resulting from man lack of awareness. As industrial wastes are disposed into the environment with no proper monitoring and management, which become hazardous for living organisms. Industrial wastes disposed into air, water and soil, mostly without proper treatment can cause severe health impacts of air, water and soil pollution, which cause hazards to living organisms found in such disposal sites (World Bank, 1992). Increases in developmental technology in developed industries, different chemicals became an important matter for human development. a vital use of chemicals is to regulate different agricultural pests. On one hand use of those chemicals shows the positive influence on agricultural products while, on the opposite hand, toxic chemicals show harmful effects to the environment and damage human health. Hence, water, air and soil pollution affecting natural environment, plant growth and other living organisms, including human health has become a very important environmental issue especially in industrial area. The environment consists of air, water, land, plants and animals. of these constituents are interdependent on each other and maintain revolution of the 19th century is especially chargeable for environmental pollution. This environment al a balance in nature. Sometime this balance is lost thanks to various reasons, one amongst them is that the environmental pollution, which happen mainly through differing kinds of industries. Figure-1 shows an image of Environmental pollution.



Fig.1: Environmental Pollution

Water is that the alternative name of life and without water life is impossible to continue. Because of increase of the quantity of population within the earth on a daily basis have caused of rapidly increased in demand for water, and increase in occurrences of pollution of various water sources, environmental risks to humans and other life beings are enhanced. In such issues, pollution is a vital and essential issue within the world which needs ongoing evaluation and revision. The statistical data counted that quite 14,000 people died daily and 700 million Indians don't have any access to a correct toilet, and 1,000 Indian children die of diarrhea sickness each day. On the opposite hand, of 90% of china's cities suffer from a point of pollution and nearly 500 million people lack access to safe drinking water.



Fig.2: Water Pollution

Water pollution in developing countries, developed countries still struggle with pollution problems further (Figure-2). The foremost recent national report on water quality within the U.S, 45 percent of assessed stream miles, 47 percent of assessed lake acres, and 32 percent of assessed bays and estuarine square miles were classified as polluted. Generally, pollution is rooted in water bodies of toxic chemicals and biological agents which exceed what's naturally found in water and should pose a threat to human health and therefore the environment. The polluted water caused serious problems for human health further as hampered ecological and environmental agents. Moreover, the range of health risks from temperature change include direct, indirect (mediated), and diffuse and delayed effects. The health risks posed by temperature change are now getting down challenging the talents, creativity, and policy engagement of researchers, policy analysts, and stakeholders. On the identical way, studies identified that the massive number of chemicals released into the river which caused for environmental risk round the geographic region area. it's concluded that 49% of the basin presently have soil loss greater than the tolerable rate, thus indicating that there are zones where the erosion process is critical, meaning that both management and land-use haven't been used appropriately in these areas of the basin. The impact of bizarre events on the sediment dynamics in rivers. The rise within the number of utmost precipitation events and other unusual weather events in Norway strongly suggests that weather are changing. Moreover, the provision of sediment increased with the increasing soil moisture content within the area. because the ground became saturated, more active slope processes caused erosion rates to extend markedly. The combined effects of temperature change and human impact on sediment transport in rivers appear to boost downstream sediment delivery. This study conducted supported the survey of common empirical studies on the causes of environmental risk through polluted water over the geographical area round the world. during this study, we conducted the overall search by the name of "Environmental Risk and River pollution and causes of environmental risk and impact of polluted water. From this search we found huge numbers of the article abstract, which we've read to see which articles have to be included within the review of this paper. After reading through most of the articles were found are as case study approach and analysis of research.

### C. State of Groundwater

Groundwater is the largest liquid freshwater resource of the Earth. It plays a crucial role in human sustenance and global food security by supporting irrigated agriculture<sup>1</sup>. At present, India is undergoing a "groundwater drought". The country comprises <3% of the terrestrial area and hosts about 19% of the global population. It also covers more than 30% of the global irrigated land and consumes the largest volume of global groundwater resource (higher than the sum of the total groundwater abstraction of United States and China, the second and third countries, respectively, in the country-wise groundwater. The country is witnessing a rapid rise in population, urbanization and change in anthropogenic water use, cropping pattern and lifestyle leading to unsustainable abstraction of available groundwater (e.g. 245 billion cubic meters (BCM) irrigational groundwater abstracted from India alone during 2011 only), which is at least 25% of the total global groundwater withdrawal. These result to groundwater withdrawal to availability ratio being higher than 0.8 in most parts of the country. The country has been placed in the top of the list of groundwater depletion (GWD) with 33.9% of the global GWD linked with food production and trade.

### D. River Pollution in India

Water pollution in India has now reached a juncture. Almost every river system in India is now polluted to a substantial extent. As assessed by the scientists of the National Environmental Engineering Research Institute (NEERI) Nagpur, nearly 70% of water in India is polluted. Pollution in river Ganga has been studied by a substantial number of scientists. Physico-chemical characterization of the identical was studied in Mirzapur and in Varanasi. Both the works culminate into a standard conclusion that the physico-chemical properties of Ganga water has degraded continuously and still it's following the identical suit. The reports favour the presence of an oversized number of pathogenic and non-pathogenic microorganisms in much beyond their excess limit. Physico-chemical properties of water of Hoogly estuary at various points was conducted. Agrawal and conducted pollution studies in Ganga and Yamuna at Allahabad.

## 2. Major Industrial Pollutants

### E. Major Industrial Pollutants

Environmental pollution by different types of industries occurs in different forms but can usually be thought of as gaseous and particulate pollutants that are discharged from different industries (Figure-3).

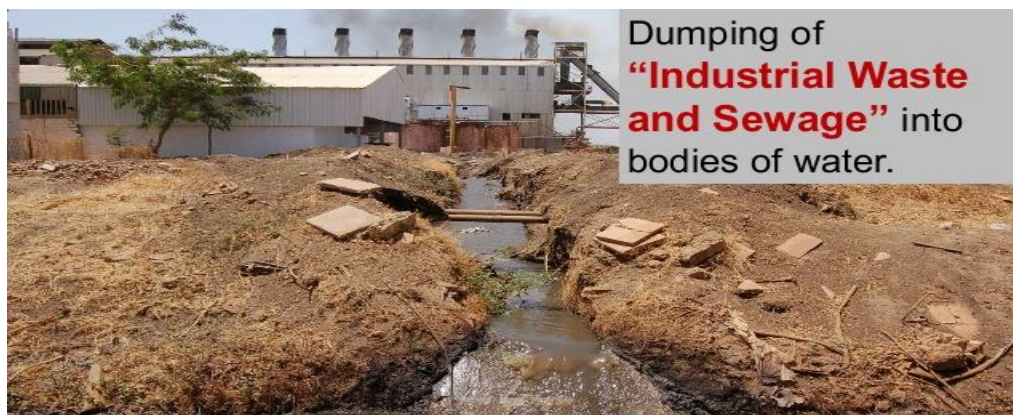


Fig.3: Industrial Pollution

and become a part of the earth's atmosphere. The gaseous pollutants of commercial polluted environment include dioxide ( $\text{SO}_2$ ), different oxides of nitrogen ( $\text{NO}_x$ ), ozone ( $\text{O}_3$ ), monoxide (CO), sulfide ( $\text{H}_2\text{S}$ ), fluoride (HF) and diverse gaseous sorts of metals. Such contaminants are released from large industrial estates like fuel fired power plants, smelters, industrial boilers, petroleum refineries and manufacturing facilities additionally as from different industrial units. Air pollutants can directly or indirectly affect plants via leaves or soil and water acidification respectively it's been reported that the majority plants show physiological and ecological changes before exhibiting visible damage to leaves. The most diffuse and harmful pollutants in industrial area are gas ( $\text{SO}_2$ ), nitrogenoxides ( $\text{NO}_x$ ), carbon mono oxide (CO), troposphere ozone ( $\text{O}_3$ ) and heavy metals, similarly as suspended particulate. Various air pollutants are identified as phytotoxic agents. Phytotoxicity of gas ( $\text{SO}_2$ ) has been recognized for a couple of century effects of ozone ( $\text{O}_3$ ) for over 30 years, acidic precipitation for pretty much 20 years, and effects of elevated levels of nitrogen compounds, nitrogen oxides ( $\text{NO}_x$ ) and ammonia ( $\text{NH}_3$ ) within the last decade. Importance of other pollutants like peroxyacetyl nitrate (PAN), fluorides 20 or heavy metals has also been recognized. Suspended particles can often reduce the quantity of sunlight, disrupting the expansion of photosynthetic plants and microorganisms. Industrial pollution could be a significant issue affecting the standard of other environmental resources still because the human-made structures and services within the area. Polluted air of business area can harm resources in several manners counting on the toxicity of the pollutant, environmental conditions and also the nature or depending sensitivity of resources. the consequences of pollution on vegetation became a matter of great concern in 1890s when pollutant was first recognized as a phytotoxicant. Since then many airborne compounds are characterized as phytotoxic, among them are fluoride (HF), ozone ( $\text{O}_3$ ), peroxyacetyl nitrate (PAN) and dioxide ( $\text{NO}_2$ ). Reviewed and reported that gas increased the sulphur content of vegetation within the upper Columbia valley at a distance of about 54 miles from the trial smelter. Most of the suspended industrial atmospheric pollutants finally subside and decide on the bottom surface, causing soil and pollution. The similarly industrial pollutants also return to soil when polluted water is employed for irrigation. The increased rate of industrialization also created the matter of pollution. Hence the bottom water, which is taken into account safe from pollution becomes contaminated with lead, cyanides, mercury, solvent and hydrocarbon compound, hospital and pharmaceutical industrial wastes.

### F. Sources of pollution

The pollutants come from three prominent sources- (i) sewage discharged into the river, (ii) industrial effluents discharged into the river with none pretreatment and (iii) surface get away from agricultural land, where chemical fertilizers, pesticides, insecticides and manures are used. This makes the river water unsafe for drinking and bathing. About 1500 substances are listed as pollutants in freshwater ecosystems and a generalized list of pollutants includes acids and alkalies, anions (e.g. sulphide, sulphite, cyanide), detergents, domestic sewage and farm manure, food processing water, gases chlorine, ammonia, heat, metals (cadmium, zinc, lead), nutrients (phosphates, nitrates), oil and oil dispersants, organic toxic wastes (formaldehydes, phenols) pathogens, pesticides, polychlorinated biphenyls and radionuclides, additionally to oxidizable materials, domestic sewage contains detergents, nutrients, metals, pathogens and a range of other compounds. Now on a daily basis an oversized number of things are being employed for the study of pollution. A modification in biology of polluted water. Silicon and nitrate in water was studied. Biological character with reference to physico-chemical properly in ponds was studied. Effluents of enormous and tiny scale industries, agricultural runoff and city sewage are marked as sources of pollution during various researches. Effect of sewage on the standard of river Ganga in Kanpur was studied. Heavy metals in sewage sludge are found. Chemistry of urban runoff water had been studied. Similar study within the sewage of Ahmedabad was conducted. Effect of disposal within the chemistry of water bodies. Biology of

sewage was studied. Pollution aspect of sewage overflow was studied. Change in chemistry of Chambal river because of sewage was studied. Chemistry of runoff water containing birds and animal waste was studied. Crude agricultural practice is taken into account as a crucial source of pollution. Pesticides in river water are detected. Herbicides employed in agriculture were also detected in river water. The above has shown positive test for the presence of huge number of pesticides and heavy metals in grains, fruits, vegetables and milk. These components would have reached to such target directly or indirectly and accumulated thanks to biomagnification. Remains of pile (burning of dead bodies) increases organic matter in river. Industries generate a major quantity of wastewater which ultimately finds its way to stream or rivers. Industrial discharges containing toxic and dangerous substances contribute to the severe pollution within the aquatic systems. Industrial development is basically due to the assembly of chemicals leading to the generation of toxic and unsafe substances which are continuously on the rise during the previous few decades Table-1 shows Toxic chemical production in India during 1960 to 1987.

**Table 1: Toxic chemical production in India (During 1960 to 1987):**

INDUSTRIES	POLLUTION RELEASED			
	1960	1970	1980	1986-1987
<b>Pesticides</b>	1.46	3.00	40.68	56.20
<b>Dyes and Pigments</b>	1.15	13.55	30.85	-
<b>Organic chemicals and Petrochemicals</b>	580	17100	24100	42500
<b>Fertilizers</b>	153	1059	3005	7000
<b>Steel</b>	1500	3400	8000	9000
<b>Non-Ferrous</b>	8.5	34.6	82.9	123.4
<b>Caustic Soda</b>	101	304	457	764
<b>Pharmaceuticals</b>	1.23	1.79	5.07	-

### 3. Microbial pollution

Microorganisms are reported to be present in sediments of ocean. Some microorganisms are helpful in removal of nutrients from the water bodies. Underground water has also been reported to contain bacteria. The reaching of microbes even to the underground water could be an alarm because we have got more or less spoiled most of the surface water but water which is a heritage should be protected (Figure -4).

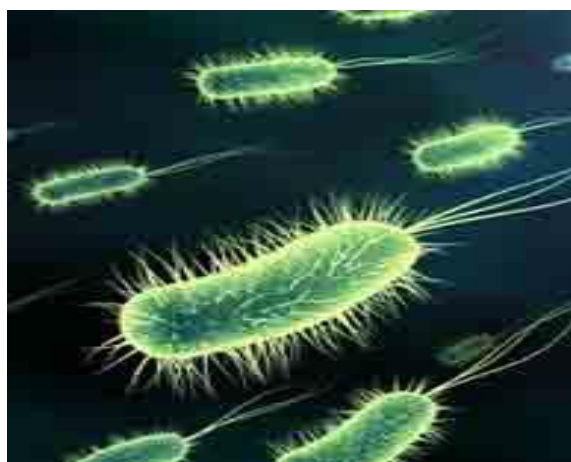


Fig.4: Microbial Pollution

### G. Microorganisms for pollutant biodegradation

Because microorganisms have diverse catabolic pathways for breaking down many persistent toxic compounds under gentle conditions with no emission and few byproducts, biodegradation was believed to own great potential for pollutant treatment. Efforts were first put into isolating various microorganisms for pollutant degradation or decomposition from environmental genetic pools. A spread of microorganisms are isolated for transforming and metabolizing vast numbers of recalcitrant toxic compounds including trichloroethylene, chloroform, solvent, toluene, phenols, chlorinated phenols, polychlorinated biphenyls, and polyaromatic

hydrocarbons [3]. Microorganisms or enzymes with unusual characteristics like organic solvent resistance or high salt resistance, which were believed to be useful for in place biodegradation of some pollutants in extreme conditions, attracted much attention worldwide [4]. by Chinese. In recent years, microorganisms with excellent degradation abilities, even under extreme conditions, are isolated scientists (Table 1). Moreover, genomics and metagenomics offer opportunities to get new functions of microorganisms, even in unculturable environmental microorganisms [5]. These technologies are applied in environmental biotechnology in China (i.e., a genomics study of an oil-degrading strain of *Geobacillus thermo denitrificans* NG80-2), and considerable new findings are likely to be obtained within the future [5].

#### 4. Biodegradation Engineering

While the metabolic pathways and their regulatory mechanisms were being unveiled, improvement of pollutant biodegradation was achieved by gene-splicing, process engineering, and signal transduction engineering. So as to effectively utilize these enhancement strategies, a far better understanding of the biodegradation processes through kinetic and modeling studies, including advanced monitoring technology, are required (Figure-5).

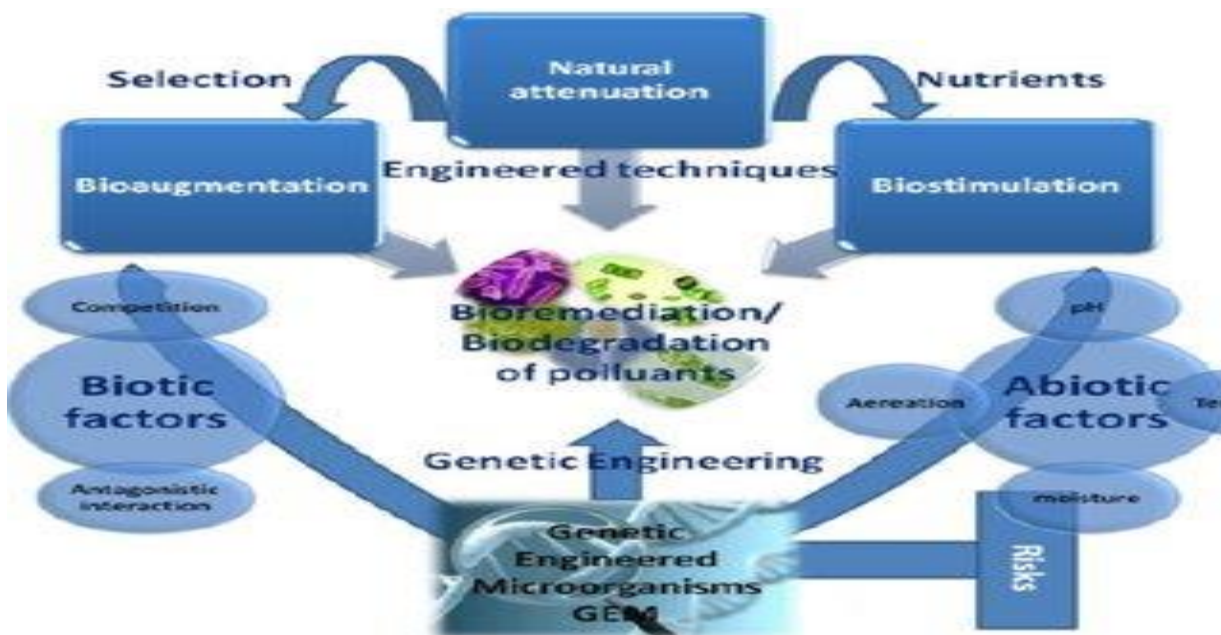


Fig.:5 Biodegradation Engineering

#### 5. Enhancement of biodegradation by Genetic Engineering

In light of the depiction of several pollutant biodegradation pathways at the genetic level, genetically engineered microorganisms with enhanced degradation abilities are constructed. Biotechnology has successfully improved biodegradation by reducing/eliminating the substrate transport limitation across the cell wall, transforming/reducing the buildup of toxic intermediates or expanding the substrate spectrum of the bacteria existing in China. Synthetic organophosphates (OPs), which account for 38% of all pesticides used globally, end in worldwide health problems and uncountable poisonings annually [6]. Bacterial degradation is economical and effective for the detoxification of OPs [6,7]. However, the transport limitation of OPs across the cytomembrane may be a barrier to whole-cell degradation efficiency, and it becomes a bottleneck during this biodegradation process [7]. Using various surface display systems, functional OPH (organophosphorus hydrolase) has been co-displayed with green fluorescent protein on the surface of *E. coli* [7,8]. As *P. putida* JS44 was an efficient PNP degrader, that can't degrade any OPs [6]. The co-expression of genes with different degradation abilities in an exceedingly single bacterium may be a simple and effective method to construct that sort of superbug. Chemical pesticide OPs, carbofuran, carbamates and pyrethroids usually coexist in agricultural ecosystems [9,10]. An engineered strain that may simultaneously degrade OPs.

#### 6. Effect of Parameters

##### I. Effect of metal ion

In recent years,  $Fe^{3+}$  has been found to possess the power to reinforce the biodegradation/decomposition of pollutants. During the anaerobic treatment of azo dyes by *Shewanella decolorationis* S12, which is linked to plasma membrane electron transport [11], the decolorization efficiency was significantly influenced by  $Fe^{3+}$ . The decolonization efficiency of dyestuff was increased by

about 30% after addition of 6mM  $\text{Fe}^{3+}$  [11-12]. Interestingly, the decomposition of triphenyltin (TPT) by purified PCH are often greatly enhanced by  $\text{Fe}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Cu}^{2+}$ . The decomposition rate of TPT by FePCH was 3.4-fold beyond that of PCH alone after a 12 h reaction. During the decomposition of TPT by FePCH, formation of  $\text{HO}\cdot$  was detected by electron paramagnetic resonance (EPR) and probed with carboxylic acid. The formation of  $\text{HO}\cdot$  was also confirmed because the mechanism of enhancement of TPT decomposition by  $\text{Fe}^{3+}$ . Using NMR and ESI-MS/MS, the existence of TPT-Fe PCH ternary complex within the reaction mixture was suggested. These results indicate that  $\text{Fe}^{3+}$  could enhance organotin decomposition by PCH [13].

#### J. Effect of pH and aeration

Both have significant effects on biological processes. Therefore, these factors are intensively studied in past decades; their effects on the performance of aerobic granules are reviewed by Liu et al. [14]. Here, recent progress in learning about the results of pH on bacterial community shift from an ecological perspective is going to be reviewed. In an enhanced biological phosphate removal (EBPR) process with sequential anaerobic-aerobic treatment, pH was an analytical factor affecting phosphate removal efficiency and stability of microbial community structure [15]. By sequencing the 16S rDNA of clones from a reactor at pH 7.0, the 108 clones comprised 24 operational taxonomy units (OTUs) with 16 associated with and eight unrelated to known sequences. When pH was slightly shifted to six.5, however, only 18 OTUs with 1 unrelated to known sequences were obtained among 103 clones, and it only removed 93% TOC and 17% P from the wastewater. Surprisingly, 14 OTUs at pH 7.0 (64.8% of the population), most of which are likely chargeable for the phosphate removal, were absent within the reactor sludge at pH 6.5.

#### K. Effect of carbon source

The biodegradation of pollutants may well be improved by augmenting with additional carbon sources or using intermediates as inducers. Phosphorus and nitrogen in effluents which bring eutrophication of water bodies can be efficiently removed by microorganisms supplemented with available biodegradable carbon sources [16]. Augmentation of biological phosphorus removal can be achieved by adding alkaline fermentative short-chain fatty acids (SCFAs) as carbon sources. In two sequencing batch reactors, phosphorus removal efficiency of around 98% was reached with fermentative SCFAs, and 71% was reached with ethanoic acid [15-17]. Investigation of the effect of pH on bacterial community dynamics and merchandise distribution during vegetable waste fermentation suggested that pH remarkably influenced the bacterial community and resulted in shifting of the merchandise distribution [18]. These results imply that pH may be a significant think about pollutant treatment processes which these changes are implemented by affecting bacterial community structure. Obviously, process parameters significantly influence the biodegradation,

### 7. Opportunities for genetically engineered microbes

The application of GEMs requires strains that actually solve problems with the implementation of bioremediation processes for removal of recalcitrant compounds that are of environmental concern. The TCP-degrading *Pseudomonas* strains described above are probably the most effective samples of genetically engineered (or metabolically engineered) bacteria that grow on a recalcitrant chlorinated chemical. 20–67 These strains were obtained by a mix of classical microbiology, protein engineering including directed evolution, and metabolic engineering. Furthermore, laboratory-scale studies with these GEMs provided proof of principle for continuous treatment of TCP contaminated water in packed bed bioreactors. 20,67 Other groups have also investigated the event of bioremediation organisms by gene-splicing. 8–13 However, to our knowledge, full-scale applications of genetically engineered strains for the bioremediation of xenobiotic compounds haven't been reported up to now. What hinders full scale implementation of those and other GEMs for practical cleanup operations? First, compared to pure or mixed natural cultures with a reputation of outstanding catabolic activity, engineered strains with better biodegradation potential towards important environmental chemicals are rare. a serious problem is that the technical difficulty of constructing microorganisms that degrade compounds which are really recalcitrant and that no catabolic activity is found in pure or mixed cultures that are conveniently obtained by classical adaptation or enrichment methods. TCP could be a relatively simple compound, with only 1 hydrolytic reaction separating it from 2,3-dichloro-1-propanol that microorganisms may be easily isolated. The structure differs only by one chloromethyl substituent from DCA, that microorganisms are also found everywhere the world. Yet, a good body of protein engineering and metabolic engineering was required to get GEMs that degrade TCP and use it for growth. 20,67 just in case of important compounds just like the trichloroethanes, trichloroethene or various chlorinated pesticides, the task becomes increasingly complicated, and most past achievements with less recalcitrant compounds (like chlorinated aromatics) are scientifically impressive but of low practical value. a heavy issue is that GEMs for bioremediation don't always utilize the target substrate likewise obviously, whether or not the pathway seems simple biochemically. Unwanted side reactions of chlorinated compounds can easily yield reactive products, and toxic effects of such reactive intermediates formed during metabolism of halogenated aliphatic are reported, also for the compounds discussed during this perspective. 40,65,74 the event of rapid tools for synthetic biology and genome engineering (e.g. DNA synthesis, combinatorial and multiplex methods for gene recombination and integration) will definitely accelerate the pace at which known bottlenecks may be solved, but the identification of those bottlenecks remains a tedious and time consuming task. Like with directed evolution, the employment of high-throughput approaches in metabolic engineering may circumvent a number of these design problems since the speed at which functional genetic diversity will be created will still increase. Sometimes, biosafety is

mentioned as a reason for modest progress with the appliance of genetically engineered organisms (GMOs) for environmental cleanup. 16,43,67 There are different aspects to this: ecological, health-related and regulatory. Regarding ecological effects, one should consider the functional properties related to the genetic changes that are introduced in engineered bioremediation organisms, including the way such changes could impact ecological behavior. It seems impossible that introduction of some catabolic genes encoding enzymes that act specially on xenobiotic compounds that ought to not occur in nature anyway (like TCP) would have ecological effects beyond persistence of such organisms in polluted environments and improved degradation of the target compounds. The most concern is also the formation of reactive side- or end products, prefer it occurs during stepwise anaerobic reductive dechlorinating of tetra- and trichloroethene. Introduction of the catabolic genes of interest within the chromosome of a GEM and avoiding transposons, plasmids, and antibiotic resistance markers, is suggested and possible. This could prevent additional mobilization of genetic material between replicons and organisms, a process that widely happens in nature. Avoiding pathogenic hosts and antibiotic resistance markers also will prevent health-related side effects. The regulatory context is extremely much passionate about regional, national and international provisions. In most European countries, release of GMOs is prohibited unless permission is obtained. However, we don't seem to be tuned in to any granted or non-granted request for permission to use genetically engineered microorganisms in bioremediations schemes. The sole well documented case seems to be the discharge of a bioluminescent GEMs for monitoring purposes. 75 Acceptance of the utilization of GMOs within the environment – a minimum of in Europe – is low. Consequently, procedures require detailed risk assessment studies and are time-consuming, probably not considered definitely worth the effort if there's no large gain. However, a true good thing about using recombinant organisms can only be expected if these GEMs outperform natural organisms – of which there are probably still no proven examples, but the laboratory scale TCP removal by engineered strains described above. Regulatory and responsibility issues can delay innovative clean-up actions for up to tens of years. US-EPA legislation demands positive results of pilot tests for a given technology to be implemented. To the simplest of our knowledge and not surprisingly, bioremediation of TCP-contaminated groundwater by engineered microbes has not been considered to this point. At Tyson's dump site, groundwater that's apparently freed from suspended solids is currently pumped and treated using two 20 m<sup>3</sup> granular atomic number 6 columns. Over the 5 year treatment period a median of 700 g of volatile organic carbon was removed daily, with TCP being the first chlorinated organic chemical.

Dense non-aqueous phase liquids (DNAPLs) are present at very deep levels, including in rock assures, so spreading of contaminants may continue for a protracted time. Apparently, the prices for running a groundwater treatment plant at the relatively low extraction rate of 5 m<sup>3</sup> h<sup>-1</sup> are still economically bearable. Bioaugmentation within the sense of introducing cultures with favorable degradation abilities is a longtime technology for in place groundwater treatment by reductive dehalogenation, eg. Just in case of pollution with chlorinated solvents like trichloroethene and tetrachloroethene. 76– 78 it had been also used for onsite groundwater treatment as explained above for the removal of DCA from extracted groundwater. In general, introducing organisms are helpful just in case of xenobiotic compounds which aren't rapidly degraded by individual strains or consortia of naturally evolved organisms that are already present. In situ and bioreactor bioaugmentation are often effective just in case cultures are available that use the target compound as a growth stimulating substrate, just in case of aerobic processes either as carbon source or electron donor. Introduction of host organisms carrying transmissible genetic material which will spread within the natural microbial communities may additionally stimulate biodegradation but is also less acceptable from a regulatory point of view. Stable and long-term establishment of a population of GEMs would force an condition for these organisms. To avoid the necessity of repeated inoculation with large amounts of cultures, introduced organisms mustn't only persist but also proliferate as long as pollutants are present. Such a distinct segment that enables growth will exist if the target compounds are present at concentrations that support growth and maintenance of the GEMs. If a species recalcitrant compound may be a minor component in a very stream of polluted groundwater, an ardent GEM will have difficulty establishing itself in an exceedingly bioreactor, especially if the host isn't an organism adapted to survival in a very complex ecosystem. Compounds often occurring as predominant pollutants are solvents and intermediates in chemical synthesis at places where leakages of storage tanks have occurred. Examples are DCA, TCP, chloroform, and trichloroethanes. Improper waste disposal of side products from chemical synthesis tends to steer to more complex mixtures of pollutants, where only electric battery of organisms would help. Obviously, the genetic construction of such strain collections would be an infinite task. The presence of recalcitrant chemicals, including solvents and pharmaceuticals in groundwater and surface water used for potable preparation could be a problem of skyrocketing concern. Biological removal of such compounds, which frequently occur at trace concentrations, will likely remain problematic even in to ascertain themselves in complex microbial communities. Low concentrations like dened in Alaska (groundwater cleanup standard for TCP is 0.0075 mg l<sup>-1</sup>) are unlikely to be met by biological methods. Furthermore, feed concentrations of below 1 mg l<sup>-1</sup> will delay the event of fine aerobic biolms unless the system is fed with huge quantities of water with considerable mass low of the chemicals into consideration. This fact is even more important for chlorinated chemicals, as an oversized fraction of the compound's mass (TCP is 71% chlorine by mass) doesn't contribute to growth. it's conceivable that top initial concentrations of a contaminant and huge amounts of inoculum would allow the event of an energetic biofilm within which desired activity stays long enough for prolonged treatment of low concentrations, but there's little experimental evidence for such a scenario. The prospects of application of specialized organisms, like the recently evolved DCA degraders and therefore the TCP degrading GEMs described above, are best just in case of concentrated waste streams and spills, with pollutants that have a high water solubility and may be extracted via a pump-and-treat approach, and within the absence of more cost and energy efficient technologies. the mixture of atomic number 6 Alters inoculated with special microorganisms which will degrade micro pollutants is additionally a stimulating prospect, 79 especially if concentrating effects of C and positive effects of immobilization on population



dynamics would act synergistically case suitable microorganisms would be found or constructed. Such decorated organisms will have little competitive advantage

### 8. Biodegradation gene cluster and metabolic pathway

Owing to developments in molecular biology and analytical chemistry, biodegradation pathways for many pollutants have been clearly elucidated. In recent years, the microbial degradation pathways for recalcitrant and xenobiotic pollutions have been intensively studied in China. Here, the biodegradation pathways of chloronitrobenzene, nitrophenol and hexachlorocyclohexane were taken as examples and reviewed in detail.

#### L. Chloronitrobenzene

The chloronitrobenzene biodegradation pathway has been studied within the *Comamonas* sp. strain CNB-1 using 4-chloronitrobenzene (4-CNB) as a model compound [19]. Further analysis revealed that *cnb* gene clusters (*cnbB*-*orf2*-*cnbA*, *cnbR*-*orf1*-*cnbCaCbDEFGHI*, and *cnbZ*), which are located on the new IncP-1 plasmid pCNB1 (ca. 89kb), are to blame for 4-CNB biodegradation [20]. A partial reductive pathway for 4-CNB biodegradation by *Comamonas* sp. strain CNB-1 was proposed [20,21]. 4-CNB was first converted to 2-amino-5-chlorophenol [21]. 2-Amino-5-chloromuconic semialdehyde was then sequentially converted to 5-chloro-4-hydroxy-2-oxoacetate [20]. The genes accountable for these conversions are identified, and these reactions are confirmed [4-CNB to 2-amino-5-chloromuconic acid (catalyzed by *CnbA*, *CnbB*, *CnbCa*, *CnbCb*, *CnbD*, expressed in *Escherichia coli*); 2-amino-5-chlorophenol to 2-amino-5-chloromuconic semialdehyde (catalyzed by *CnbCa*, *CnbCb* purified from CNB-1 cells); [23]. Although this partial reductive 4-CNB biodegradation pathway are often proposed supported the above information, more solid evidence, like intermediate identification when biodegradation carried out, should be pursued to reveal actuality metabolic pathway in CNB-1, and a focus should be paid to the movement of 5-chloro-4-hydroxy-2-oxoacetate into the Tri Carboxylic Acid cycle or other metabolic processes.

#### M. Nitrophenol

*Alcaligenes* sp. strain NyZ215, isolated from activated sludge, can utilize ortho-nitrophenol (ONP) as its sole carbon, nitrogen and energy sources [24]. A completely unique ONP catabolic gene cluster consisting of *onpA*, *onpB*, and *onpC* was identified during this strain. *OnpA* and *OnpC* were characterized as ONP 2-monooxygenase and catechol 1,2-dioxygenase, respectively. Catechol was identified because the product of ONP biodegradation catalyzed by *OnpA* and *OnpB*. supported the bioinformatics analysis and experimental evidence presented above, ONP was proposed to be degraded to o-benzoquinone by *OnpA*; then, *OnpB* was suggested to catalyze the conversion of o-benzoquinone to catechol, and at last ring cleavage of catechol was catalyzed by *OnpC* [24]. Biodegradation of para-nitrophenol (PNP) was also studied in *Pseudomonas* sp. strain WBC-3 [25]. The *pnp* gene cluster which contains *pnpABCDEF* was identified. *PnpA* and *PnpB* were characterized as PNP 4-monooxygenase (conversion of PNP to para-benzoquinone) and p-benzoquinone reductase (catalyzes the reduction of p-benzoquinone to hydroquinone), respectively. Also, *pnpCDEF* was proposed to be involved within the mineralization of hydroquinone [27].

#### N. Hexachlorocyclohexane (HCH)

It is an organochlorine insecticide that persists in the surroundings with toxicity to birds, mammals, and other non-target organisms [26]. Commercial HCH has four predominant isomers (and), but only -HCH has insecticidal activity. However, and -HCH comprise quite 80% of economic HCH, and that they are extremely toxic to humans [26]. *Sphingomonas* sp. BHC-A, which may degrade all isomers of HCH, isolated, and eight *lin* genes (*linABCDEFRX*) accountable for HCH degradation are identified [26]. Using Gas Chromatography–Mass Spectroscopy analysis, the -HCH degradation pathway catalyzed by *LinAB* was elucidated and a number of other novel conversion reactions and intermediates were characterized [26]. -HCH is converted to -PCCH ( -pentachlorocyclohexene) by *LinA* or converted to TDOL (2,3,5,6-tetrachloro-1,4-cyclohexanediol) via PCHL (2,3,4,5,6-pentachlorocyclohexanol) by *LinB*, which are like previous reports [26]. A completely unique transformation of -PCCH to 1,4-TCDN (1,3,4,6-tetrachloro-1,4-cyclohexadiene, catalyzed by *LinA*) was also characterized. Furthermore, -PCCH and 1,4-TCDN will be converted to 2,3,5-TCDL (2,3,5-trichloro-5-cyclohexene-1,4-diol) and 2,5-DDOL (2,5-dichloro-2,5-cyclohexadiene-1,4-diol) by *LinB*, respectively. These novel conversions extend the catabolic ability of *LinA* and *LinB*, and also indicate the metabolic diversity of HCH biodegradation. Interestingly, TDOL and 2,3,5-TCDL, which may be degraded by strain BHC-A, couldn't be converted by *LinC*, *LinD*, *LinE*, *LinF*, and *LinX* [26]. These results suggest that other enzyme(s) are answerable for the conversions of TDOL and a couple of 3,5-TCDL; alternatively, it should be that other important factors in BHC-A metabolism are essential for these conversions in strain BHC-A. Hence this HCH (including and isomers) mineralization becomes more attractive and may be unveiled further

### CONCLUSION

The government have paid great attention to environmental biotechnology. Over the years, our understanding of biodegradation has significantly improved. All of the studies outlined above are significant for exploring efficient and sustainable

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biological processes for the cleanup of pollutants. In summary, biodegradation is still attracting technology for environmental pollutant treatment; the successes of biodegradation will encourage us to further unveil these processes and to explore more efficient and cost-effective biodegradation processes. More effort should be made to explore new microorganisms, enzymes, genes and to elucidate new biodegradation pathways.

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