# Genetically Engineered Microorganisms for Bioremediation Processes

**Manmeet Kaur and H. S. Sodhi**

## 1 Introduction

Although a diverse and particular microbial population can completely eradicate specific contaminants from the environment, the majority of toxins disintegrate slowly and so therefore tend to accumulate. Many of these contaminants have chemical characteristics that make it difficult for bacteria to degrade them (Dejonghe et al., 2000). Microorganisms have yet to develop relevant catabolic mechanisms to remove these chemicals due to their distinctiveness. Complex mixtures of pollutants are resistant to standard degradation mechanisms, or the communities of microbes that are responsible for this degradation are too small or inactive to properly convert these compounds (Bruins et al., 2000).

Using exogenous microorganisms to boost indigenous populations is one way to expand populations of microorganisms capable of precise pollutant breakdown. Bioaugmentation is a technique that involves introducing microorganisms that have been genetically modified or those that have been naturally endowed with the necessary genes (Gentry et al., 2004). This method can also be used to deliver plasmids containing sufficient genetic material to native microbes. New strains with beneficial bioremediation characteristics have been created as a result of recent advancements in molecular biology adapted to microorganisms. One of these is the development and control of novel pathways:

- Extending the substrate intervals of pathways without producing harmful metabolites
- Modifying the selectivity and affinity of catabolic enzymes
- Improving the genetic stability of catabolic activities (Paul et al., 2005)

M. Kaur (✉) · H. S. Sodhi
Department of Microbiology, Punjab Agricultural University, Ludhiana, India
e-mail: manmeet1-mb@pau.edu

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2023

R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_5

The instability of the infused genetic material may restrict their application in the environment, despite the efficiency of GEMs in terms of bioremediation (Velkov, 2001). GEMs' ability to transport genetic material in a regular manner is essential to their function. Second, despite the fact that genetic material transmission is a common occurrence among indigenous species, it is regarded as a negative phenomenon. Scientists are investigating GEM durability, tenacity, and competitiveness, as well as the risks associated with their release into the environment. Table 1 shows how genetic engineering techniques can be used to improve bioremediation. In limited environments, these genetically modified bacteria have been shown to break down a variety of contaminants. However, biological and environmental issues, as well as bureaucratic constraints, make field testing GEM complicated. Before GEM can deliver a suitable clean-up solution at a cheaper cost, these challenges must be addressed.

## 2 Advancement and Implication of Genetically Engineered Microorganisms in Bioremediation

Pathway design and change of substrate affinity, enzyme specificity, expression and cellular location have resulted in innovative strains with important properties. It's also led to the development of new technologies for detecting GEMs and pollutants in the environment.

### *2.1 Gene Transfer Strategies*

Xenobiotic substances can remain in the environment due to a variety of factors, and organisms are not involved in the degradation processes of such molecules. There is a lack of proper catabolic routes, inferior catabolic ability of pre-existing pathways, completely inadequate potential for substance uptake due to retention and hydrophobicity in soil due to progressing (Suidan et al., 2005). To circumvent these constraints, microorganisms can be genetically engineered, with the potential to develop strains capable of enormous in situ bioremediation (Furukawa, 2000a, 2000b).

**Table 1** Genetic engineering for biodegradation of pollutants (Paul et al., 2005)

| Microorganism | Modification | Contaminants |
|---|---|---|
| *Pseudomonas* sp. B13 | Pathway | Mono/dichlorobenzoate |
| *P. putida* | Pathway | 4-ethyl benzoate |
| *P. putida* KT2442 | Pathway | Toluene |
| *Pseudomonas* sp. FR1 | Pathway | Methylbenzoate |
| *E.coli* JM109 | Substrate specificity | Benzene, toluene |

Using traditional methods, bacterial strains with enhanced potency to bioremediate harmful chemical metals have indeed been established.

(a) *Catabolic Pathways*

Aromatic compounds are a diversified collection of pollutants in soil and water, making them a strong contender for bioremediation with modified bacteria. Reineke (1998) investigated how patchwork assembly may be employed to create chloro-aromatic breakdown-complete recombinant strains. This technique gathers a comprehensive system capable of mineralizing a given chemical by combining pathways from several bacteria into a single recombinant host. Hrywna et al. cloned and produced the ohb operon from *P. aeruginosa* and the fcb operon from *Arthrobacter globiformis* (both encode enzymes that may metabolize chloro-benzoic acids) in *Comamonas* testosterone strain VP44 (1999).

The genes that metabolize chlorinated biphenyls into ortho- and para-CBAs are identified in the host strain. Using plasmids with the ohb and fcb operons to evolve the host resulted in a mono-chlorobiphenyl mineralizing strain. A *Burkholderia* sp. strain transferred DNT genes for the 2,4-dintirotoluene breakdown pathway into *Pseudomonas fluorescens* ATCC 1740 (Monti et al., 2005). When 2,4-DNT was employed as the primary nutritional supply for the recombinant strain, it was entirely digested, and the carbon produced was co-metabolized by the cell. The recombinant strain outperformed *Burkholderia* in terms of breaking down DNT at relatively low temperatures and non-toxicity to a particular species under specific surroundings. Genes from the *Comamonas* sp. strain CNB1 have also been transcribed and generated in *E. coli* to establish a purported preferred oxidation–reduction pathway for 4-chloronitrobenzene and nitrobenzene (Wu et al., 2006).

(b) *Engineered Bacteria: Enhanced Bioremediation of Mixed Waste and Metals*

Radionuclides, heavy metals and organic compounds are a few of the pollutants found in trash heaps. Bioremediation of organics in such environments is arduous due to the radiation from these radionuclides, which is hazardous to most microorganisms. *Deinococcus radiodurans* is an excellent host for genetic engineering procedures using mixed waste because of its increased tolerance to rapid ionizing radiation exposure. The modified strain was shown to successfully oxidize 3,4-dichloro-1-butene, chlorobenzene and toluene in a highly incinerating environment (Lange et al., 1998).

Renninger et al. (2004) utilized an integral strategy for uranium bioremediation by upregulating polyphosphate kinase in modified *Pseudomonas aeruginosa*. Underneath the impact of the tac-lac promoter, endogenous genes for polyphosphate synthesis and degradation were reproduced in a plasmid with a broad host range. When compared to the control strain, the transformed strain accumulates 100 times the quantity of polyphosphate. A substantial amount of phosphorus is liberated when the polyphosphate is degraded, which couples with the uranyl group and condenses at the cell membrane. Heavy metal bioremediation genetic change has also been carried out on *E. coli*. Crameri et al. (1997) used the arsenate resistance operon from *Staphylococcus aureus* and DNA shuffling techniques to build an

**Table 2** Engineered bacteria involved in remediation of heavy metals

| Microorganisms | Gene | Heavy metals | Reference |
|---|---|---|---|
| HgR *E. coli* | *mer A* | Hg | Gomes et al. (2013) |
| *Salmonella choleraesuis* strain 4 A | *SmtAB* | Pb | Naik et al. (2012) |
| *Deinococcus radiodurans* strains | *mer A* | Hg | |
| *Achromobacter* sp. AO22 | Hg reductase expressing mer gene | Hg | Nagata et al. (2009) |
| *Enterobacter* sp. CBSB1 | *Gcsgs* | Pb, Cd | Qiu et al. (2014) |

arsenate detoxification route. The genetic modified bacteria have a significant metabolizing capability and have been shown in controlled settings to assimilate a range of pollutants. The genetically modified bacteria used in bioremediation are listed in Table 2.

## *2.2 Variations of Genes That Encode Biodegradative Enzymes*

Engineered strains with superior bioremediation capabilities can be created since genes can be transferred from one species to another. These genes can be managed to support degradative proteins by increasing their specialized activity, quantity and site-directed mutation as well as directed evolution (Ang et al., 2005). In vitro mutagenesis and recombinant DNA can also be applied to generate hybrid genes that code for beneficial fusion proteins, or to contribute different transcriptional promoters and translational start sites to enhance enzyme expression.

(A) *Alterations of Enzyme Affinity and Specificity*

Oxygenases are enzymes that aid in the reduction of oxygen by adding one or two oxygen atoms to the oxidized substrate. They can use this method to ionize a range of organic pollutants (Parales et al., 2002). Anaerobic breakdown pathways, such as the anaerobic conversion of TCE to vinyl chloride, can generate toxic metabolites (Ensley, 1991). Oxidase protein engineering can be used to improve the effectiveness of oxidative pollution elimination. Furukawa et al. (2004) highlighted research that demonstrated how oxygenases involved in the dissolution of a wide range of aromatic compounds might be adjusted in terms of substrate specificity relaxation and degradation rate acceleration. Exchanging genes encoding homologous components from adjacent animals, swapping parts of such genes, DNA shuffling and site-directed mutagenesis of critical amino acids were among the biochemical processes used to achieve these changes. The laccase gene (mtL) from the fungus *Myceliophthora thermophia* is expressed by *S. cerevisiae*. Laccase can assist in the biodegradation of polyaromatic hydrocarbons and the breakdown of

lignin (PAHs). Through error-prone PCR and in vivo shuffling, direct evolution produced a 170-fold more active mtL encoded laccase than the wild type (Bulter et al., 2003).

**Methods for Upregulating Non-modified Coding Regions**
The Palk promoter from *Pseudomonas* sp. P51 was used to make *E. coli* with the chlorobenzene dioxygenase (CDO) gene. In this strain, the lac promoter regulated CDO gene transcription, resulting in three times the amount of CDO produced by an identical recombinant *E. coli* strain. Furthermore, when compared to the lac promoter, the Palk promoter has better transcriptional control. The resultant strain was capable of catalysing benzonitrile and other aromatics cis-dihydroxylation as well as contributing to biodegradation (Yildirim et al., 2005). Utilizing PCR primers arbitrarily inserted 4–17 bp upstream of the dszB start codon, Reichmuth et al. (2004) generated a list of dszB hybrids with distinct ribosome binding sites. When integrated with the rest of the dsz operon, a proportion of these alterations resulted in nine-fold greater dibenzothiophene to hydroxybiphenyl transformation than the wild-type dszB ribosome-binding site strain.

(B) *Fusion proteins having a distinct significance*

The enzyme organophosphorus hydrolase (OPH) degrades organophosphate insecticides. Using genetically modified *E.coli*, OPH fusion proteins with domains that allow them to be produced on the cell surface were developed (Wang et al., 2005). In a model bioreactor, the cloned cells digested organophosphorus insecticides well, providing them a significant advantage over intracellular expression because the cell membrane hindrance to substrate transit would not have been an issue (Mulchandani et al., 1999). In recombinant *E. coli*, a heterologous bacterial OPH gene was coupled to a signal sequence to stimulate its extrusion to the periplasm. When contrasted to a modification in cytosolic OPH expression, this resulted in a 1.8-fold rise in OPH activity (Kang et al., 2006). It means that during organophosphate breakdown, OPH's periplasmic expression defies substrate dispersion restrictions.

## 3 Stability and Survivability of Genetically Engineered Microorganism and Genetic Transmission to Bacteria

The propensity of a strain to reproduce and disseminate its modified genotype in naturalistic situations and the extent to which it can transmit undesired genes to native species are the key considerations when using bacterial GEMs as biosensors or bioremediators (Pieper & Reineke, 2000). As a result of these considerations, studies into the survival, tenacity and conflict of GEMs released into the wild have been conducted.

# 4 Survivability of Genetically Engineered Microorganisms

In order to be effective under the environment, a bacterial GEM must be able to live and reproduce in such settings. In this regard, inoculum size, growth rate and environmental circumstances such as spatial dispersion and the presence of competing microorganisms and predators are all key determinants. The spatial distribution of injected GEMs in the environment is important because it affects how they interact with the indigenous microbial population and other ecology variables (Dechesne et al., 2005). A bacterium that has been taken from its natural habitat is more likely to survive when placed back into that ecosystem than one that has spent days in a laboratory. A plasmid-bearing GEM's rate of multiplication is a crucial predictor in its survival and implantation in the milieu. Plasmid-free cells are thought to have a significant advantage over plasmid-bearing cells due to plasmids' ability to boost metabolic strain (Diaz-Ricci & Hernandez, 2000). While they compete for nutrients with natural flora, this could be a tripping topic in the field when it comes to creating substantial and lengthy GEM colonies for bioaugmentation (Top et al., 2002).

## *4.1 Acquisition and Structural Fragility of Recombinant DNA*

Plasmid stability is strongly related to phenotypic stability in the field when a bacterium is genetically transformed using a plasmid. Medium composition, pH, oxygen availability, copy number, temperature and variation are all factors that affect the stability of plasmid vectors. Segregational plasmid instability occurs when one or both daughter cells refuse to endorse at least one plasmid during cell division. Due to alterations, deletions, and insertions to regions inside the plasmid vector, structural instability can interrupt the information on the plasmid vector without enabling the overall plasmid to be abandoned (Sharp et al., 1998).

## *4.2 Effect of Genetically Engineered Microorganisms on Microbiota*

The impact on ecosystem structure and function while transporting synthetic bacteria into agricultural regions is a critical challenge. Although molecular methods have been applied in research work in some instances, miniature investigations provide the preponderance of known information in this sector, indicating that the latter technique has a lot of potential.

### 4.3 Horizontal Transfer of DNA in Bacteria

Horizontal recombinant DNA transfer is sometimes confused with horizontal gene transfer, which is a widespread occurrence. Numerous studies have demonstrated the importance of horizontal gene transfer in bacterial evolution (Dennis, 2005). The exposure of microbial communities to organic contaminants is thought to be a crucial step in the development of unique biodegradative potential. Transposable elements, conjugative plasmids and integrative and conjugative transposons all appear to aid in the transmission of genes that code for biodegradative activity. Both the plasmid-containing cells, specific growth rate and different concentrations of substrate had a massive effect on the rate coefficients, showing that the cells' energy to facilitate transference was restricted (Rittmann et al., 2006). Horizontal gene transfer of recombinant genes can be done swiftly using the same methods as before. However, any horizontal DNA transfer from GEMs is predicted to occur mostly in non-recombinant individuals with the same genes at such reduced levels that any influence from GEMs is likely to be minimal.

### 4.4 Effects of Horizontal Recombinant DNA Transfer as Well as Other Heterologous Species on Native Flora

Horizontal recombinant DNA transfer occurs when GEMs are placed in polluted regions to induce bioremediation, and there is fear that this could have harmful environmental repercussions. Even if the imported strain does not sustain, plasmid transfer from an acquired GEM to an indigenous microbe can occur in extreme circumstances (Peters et al., 1997). In general, the impact of GEM importation on native microbial populations appears to be inconsistent, and each case must be evaluated separately. Dejonghe et al. (2000) investigated the exchange of two recombinant plasmid vectors that incorporate the 2,4-D breakdown pathway from host *P. putida* UWC3 to bacteria isolated in a 2,4-D-contaminated sandy-loam soil microcosm. Following the conjugative transmission of these genes to a variety of native bacteria and trans-conjugant growth, different communities emerged that were more effective in removing 2,4-D from the soil. DeFlaun et al. (1987) used recombinant copies of two naturally produced plasmids that generate 2,4-D breakdown enzymes to explore hgt in soil microcosms.

## 5 Suicidal Genetically Engineered Microorganisms

Because environmental safety is necessary, transposition vectors without antibiotic resistance genes must be developed, as antibiotic vectors are unsuitable for this purpose. Combining lethal genetically modified microorganisms with bacterial

contaminant systems is the most efficient strategy for mitigating risk coupled with transgenic microorganism ecological emission. GEMs have the power to destroy; nevertheless, fast advances in the manufacture of suicidal genetically altered bacteria will make it possible in the near future to use GEMs expressing suitable P450 for bioremediation of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) contaminated soil.

Paul and his colleagues created a genetic model to anticipate the unpredictable behaviour of genetically modified microbes. Killer genes are activated when the chemical is no longer present, killing the GEM. Killer genes on plasmids have been proven to stop horizontal gene transmission by killing the microbial recipient during the transfer. The potential issues connected with introducing genetically engineered bacteria into the ecosystem are eliminated with this strategy. This is an approach for lowering the risks of genetically modified bacteria while also avoiding uncontrolled microorganism expansion for successful bioremediation. Authorities and scientists have failed to take into account most of these pollution technologies while constructing bioremediation microorganisms, which is unfortunate. GEMs can have one of two outcomes: the organism can do the required action and then totally remove the GEM from the surroundings, which is the desired conclusion. The organism can survive and grow instead of being killed, which is a less well-known possibility. Because recombinant bacteria that survive in the environment may have negative consequences on ecosystems, the first option is recommended (Paul et al., 2005).

## 6 Bacterial Plasmid Addiction System

Plasmids are recognized for containing genes that code for a number of helpful properties for the host in specific situations, such as resistance to hazardous substances, chemical breakdown ability, pathogenicity and toxin production. Plasmid copies are exchanged between generations during cell division, and plasmid-free isolates are unable to survive due to plasmid-encoded processes or plasmid addiction. Koyama et al. (1975) emphasized the importance of an addiction mechanism for proper plasmid maintenance in cells.

Poison–antidote, post-segregational killing, toxin–antitoxin and plasmid–addiction system are two terms used interchangeably. The terms killing–anti-killing and planned cell death are used to describe scenarios in which the host cell is purposely destroyed such that no plasmid survives cell division. The killing–anti-killing system requires the expression of two genes: a toxin/poison gene and a antitoxin/antidote gene. The half-life of the killer toxin is lengthy, while the half-life of the anti-killing toxin is brief. Antidotes work by neutralizing or suppressing the production of the toxins they're meant to counteract. In plasmid-free cells, toxin inactivation is based on the fact that toxin and antidote degradation rates differ. Toxin–antidote combos act as plasmid addiction mechanisms by eliminating plasmid-free cells from the population of plasmid-bearing cells (Pandey et al. 2005).

# 7 Techniques for Tracking GEMs

It's vital to locate and measure GEMs in a variety of microbial specimens to assess the potential liability of gene segments and their potential horizontal gene transfer to other existing microbial communities. There are several options in this field, but one that is real-time, convenient, reputable and cost-effective should be considered.

## *7.1 PCR-Based Techniques*

Counting the number of colonies that have formed on plates is a common way to identify GEMs. This method is straightforward, although it has limitations in terms of sensitivity and accuracy. These limitations can be solved with the use of molecular technology. A southern hybridization-based approach has limited sensitivity for detecting soil organism DNA, but PCR-based nucleic acid amplification of a sample measures both dead and live cells. The MPN-PCR method requires diluting soil samples in triplicate and comparing the occurrence of microbes in each concentration to a database. The viability of an organism is determined by the proficiency of target gene sequence amplification rather than the quantity of living cells. Similar to MPN-PCR, cPCR compares the value of final DNA in a sample to standard templates, providing details (Widada et al., 2002).

## *7.2 Fluorescent-Based DNA Hybridization Technique*

To detect the presence of a specific bacterium, unique fluorescent-labelled DNA probes of a particular strain are used. Researchers employed fluorescent labelling of a precise ribosomal RNA probe to distinguish and count *P. fluorescens* cells after they were introduced into a microcosm. Because ribosomal RNA rises with cell growth rate, the metabolic condition of the cells at any given time can be easily determined. Due to the hybridization phase, this method has a disadvantage in that it takes a lengthy time to finish the procedure (Boye et al., 1995).

## *7.3 Bioluminescence-Mediated Technique*

GEM's phenotypic features are detected through the selective abilities of recombinant organisms, such as bioluminescence or the formation of coloured compounds. The enzymes xyl E, lac A and gus A, respectively, are encoded by the genes xyl E, lac A and gus A. By encoding uroporphyrinogen III methyl transferase, a

genetically modified bacterium cloned with the lux, luc and cob A genes produced a bioluminescent product (Feliciano et al., 2006).

### *7.4 DNA Microarray Technique*

Non-recombinant cells and GEMs are identified and counted using DNA microarrays, which use both DNA and rRNA as probes. The sensitivity and specificity of this approach limit the degree of quantification (Cho & Tiedje, 2002). A new detection approach can be used to identify GEMs from indigenous people. By comparing the gene sequences of GEM and the 5S rRNA gene of *Vibrio proteolyticus*, the engineering of *E. coli* with the 5S rRNA gene of *V. proteolyticus* may be easily confirmed (Hedenstierna et al., 1993). Single cells interacting with monoclonal antibodies can identify GEMs with unique surface protein genes *phoE-caa* (Zaat et al., 1994).

## 8 Molecular Techniques for Generating Genetically Modified Microorganisms for Bioremediation

### *8.1 Molecular Cloning*

Cloning is a technique for making multiple copies of a gene, investigating gene function and making multiple copies of a gene. For molecular cloning, a plasmid vector is required, as is the copying or synthesis of a DNA fragment with a defined purpose. Plasmids are small circular DNA molecules that proliferate autonomously of their host bacteria's chromosomal DNA. Plasmids that have been redesigned are inserted into the host species and permitted to multiply. The inserted DNA fragment is replicated along with the majority of the bacterial genome during cell division. The vector is made up of a lot of small DNA sequences that restriction endonucleases can digest. Endonucleases that recognize and cleave a specific region in DNA sequences to produce sticky DNA are known as restriction endonucleases. Palindrome sequences, which are four to eight nucleotide sequences, are recognized by the majority of restriction enzymes in plasmid DNA. This means that the complementary nucleotide sequences in the forward and reverse directions are nearly identical. Using the enzyme DNA ligase, digested DNA fragments with sticky ends of both foreign and host DNA are annealed together to generate double-strand DNA. As a result, plasmids with a foreign gene are referred to as recombinant DNA, and the proteins they create are referred to as recombinant proteins. Certain environmental conditions can boost or stifle protein production, giving scientists more control over how a protein is expressed.

### 8.2 Electroporation

Electroporation is a quick and easy way to get a foreign gene into a bacterial host. To take the DNA in, high-voltage electric pulses are employed, inducing transient penetration of the plasma membranes. In a tank containing an appropriate buffer, foreign DNA and the protoplast of the host cells are held between two electrodes. The protoplasts are positioned using a 1 MHz electric current in di-electrophoresis. The electrostatic force causes membranes to disintegrate and openings to form, allowing DNA to pass through efficiently. Dc power pulses of 1–3 kV are being used to induce fusion after the DNA has been delivered into the host organism. Electroporation can be used to electroporate foreign DNA with a molecular size of up to 240 kb. Because genome sequencing demands long DNA segments, this characteristic gives this technique an advantage.

Field strength is determined by several factors:

(i) Electric pulse voltage, resistance and capacitance
(ii) Temperature and pH
(iii) Density and protoplast size
(iv) Host cell and genetic features
(v) post-pulse therapy impact

### 8.3 Protoplast Transfusion

The protoplast transformation process involves PEG-induced DNA absorption in protoplasts and subsequent cell wall rebuilding. This method can change up to 80% of plasmids and is better suited and efficient for even the most esoteric plasmids. Protoplast transformation involves the following steps: hypertonic DNA therapy and PEG treatment. The following are the essential elements that govern protoplast transformation mediated by PEG:

(a) Culture conditions and cell density – late log phase cells are ideal for transformation.
(b) DNA concentrations of 0.1–1 g trigger swift transformation.
(c) Tonicity – sucrose, sorbitol, potassium and sodium chloride, lithium and ammonium chloride all induce transformation at particular doses.
(d) pH of 3.5–5 was shown to be optimum for PEG mediation protoplast transformation.
(e) The influence of temperature and reaction time – effective DNA uptake in protoplast is caused by a 10-minute reaction period and a temperature of 22 °C.

### 8.4 Biolistic Transformation

The gene of interest is encapsulated in amorphous tungsten or gold beads with a diameter of 0.36–6 m and transferred to recipient bacterial cells through helium gas stimulation through a halting screen in biolistic transformation. The foreign DNA is maintained inside the bacterial cells after the pellet DNA molecule passes through them. When the helium pulse sweeps the microcarrier-coated DNA in the specimen cartridge through the barrel, the target is retained, enabling it to reach the host cell properly. This method uses a simple transformation mechanism and does not require the use of a binary vector. The method's significant shortcomings include the complexity of establishing single-copy mutant events, ridiculous prices of apparatus and microcarriers, random intracellular localization and the inability to fulfil single-copy transgenic events.

## 9 Obstacles Associated with Use of GEMs in Bioremediation

While genetic engineering has resulted in a plethora of strains capable of dissolving ordinarily inaccessible pollutants in a Petri plate or bioreactor, in situ bioremediation techniques have seen little use of this expertise (Sayler & Ripp, 2000). A major cause of concern in this research is the rising recognition that the strains and bacterial species most typically used in traditional enrichment approaches do not conduct the majority of biodegradation in naturalistic conditions and may even be ineffective as bioremediation mediators. According to stable isotope probing (SIP) and analogous initiatives in microbial ecology, *Pseudomonas*, *Rhodococcus* and the typical aerobic prompt growers that are typically selected as hosts of biodegradation linked chimeric genes are substantially less pertinent under benchmark instances (Wackett, 2004). When fast-growing plants are used as biodegradation agents, excess biomass will inevitably accumulate. The best clean-up agent has the highest catalytic ability and the lowest cell mass on either hand. Biodegradation gene expression can be segregated from proliferation using stationary phase or restricted promoters (Matin, 1994). Moreover, substantial advances in recombinant DNA technology have paved the path for the development of suicidal genetically engineered microbes (S-GEMS) to eliminate such risks and allow for more secure and reliable removal of pollutants (Pandey et al. 2005).

It doesn't matter if the bacteria transplanted are recombinant or not in some cases because the concern is the implantation of foreign germs in a new environment. The insertion of bacterial biomass into a pre-existing niche may provide protozoa with a favourable environment, inhibiting bacterial overgrowth (Iwasaki et al., 1993). To get around this problem, creative solutions have been proposed, such as encasing the inoculum in plastic tubing or encapsulating it in a polymeric matrix. Its efficacy is determined by the presence of sufficient in-situ enzyme activity in the target area (Foster et al., 2002).

A field release of *P. fluorescens* HK44 for bioremediation application was successfully carried out on a reasonably large scale and in controlled field settings (Ripp et al., 2000). However, there will be concerns about the discharge of genetically modified bacteria into the atmosphere if they are employed to clean up pollution in the future. The risk of using other changed microbes in the foreseeable is still unknown. As a consequence, researchers will investigate the future opportunities of genetically modified bacterial strains in field conditions, which will aid in determining the hazards associated with utilizing genetically modified bacteria in ecological bioremediation. The microorganisms that are used in productive bioremediation technology are exposed to extreme field conditions, which is the technique's principal disadvantage. In order to create modified microorganisms, researchers must investigate other bacterial strains. The distinctive characteristics of open biotechnological applications have obviously prompted the creation of modified bacterial strains to solve new challenges.

The fundamental difficulty is to develop genetically engineered bacteria that can be employed for bioremediation in the field while being ecologically friendly. In the vast majority of situations, bacteria used in bioremediation techniques were created in the lab for a specific purpose, ignoring field conditions and other demanding scenarios. There is no indication, on the other hand, that using genetically engineered bacteria for bioremediation has any discernible negative influence on the natural microbial community. The overblown idea of risk assessment has sparked a lot of debate and inquiry in the field of environmental microbiology from the beginning. According to a new assessment, the survival of genetically modified microorganisms in complex environments is a major concern that must be dealt (Singh et al., 2011).

## 10 Advantages and Disadvantages of GEMs

The following are some of the advantages and disadvantages of using GMOs:

### *10.1 Advantages*

- Faster crop development and production, as well as larger yields, less fertilizer, less herbicides and much more micronutrients are all partly attributable to GMO technology.
- Traditional breeding includes the transformation of multiple genes at random to the generation, whereas genetic engineering entails the mobility of a block or specialized grouping of genes at a specified period.
- Even though genetically modified organisms (GMOs) are not natural, they are not always efficient. Despite the fact that lethal mushrooms exist in nature, they can be genetically modified to become edible.

### *10.2 Disadvantages*

- The majority of GMOs have not been properly evaluated, and a standard GMO test only takes 90 days.
- GMOs generated by transgenic modification are not considered natural, and their consequences remain unknown.
- Regardless of the fact that GMOs were aimed to minimize pesticide consumption, there is no assurance that the crop will be acceptable if these recombinant microorganisms are utilized.
- GMO testing frequently employs animal testing, which some claim is a violation of animal rights.
- GMO-based products are not adequately labelled, and it is still impossible to know whether products generated from genetically modified organisms are safe for human consumption.

## 11 Conclusion and Future Aspects

The eventual promise of GEMs in bioremediation may be constrained to difficulties that are simply not cost-effectively addressed by chosen field therapeutic approaches. Alternative options exist, and manipulated microbes could be used in restricted reactor technologies for bioremediation or waste treatment in the years ahead. In a wider sense, greenhouse gas reduction, carbon sequestration and waste conversion to value-added commodities are instances of these application scenarios. Pollution prevention has been proved to be more cost-effective and environmentally friendly, and demand for waste site remediation technologies is envisaged to drop significantly. Components must be reused or recycled for pollution prevention to be efficient, and this presents a new opportunity for the use of GEMs in bioremediation. Before such noticeable developments can be accomplished, however, a fundamental knowledge collection on GEM effectiveness under severe environmental conditions must be formed. Only thorough field research and a comparative life cycle analysis that considers both risk and biotechnology benefits will be able to implement this.

## References

Ang, E. L., Zhao, H., & Obbard, J. P. (2005). Recent advances in the bioremediation of persistent organic pollutants via molecular engineering. *Enzyme and Microbial Technology, 37*, 487–496.

Boye, M., Ahl, T., & Molin, S. (1995). Application of strain-specific rRNA oligonucleotide probe targeting Pseudomonas fluorescens Ag1 in a mesocosm study of bacterial release into the environment. *Applied and Environmental Microbiology, 61*, 1384–1390.

Bruins, M. R., Kapil, S., & Oehme, F. W. (2000). Pseudomonas pickettii: A common soil and groundwater aerobic bacteria with pathogenic and biodegradation properties. *Ecotoxicology and Environmental Safety, 47*, 105–111.

Bulter, T., Alcalde, M., Sieber, V., Meinhold, P., Schlachtbauer, C., & Arnold, F. H. (2003). Functional expression of a fungal laccase in Saccharomyces cerevisiae by directed evolution. *Applied and Environmental Microbiology, 69*, 987–995.

Cho, J.-C., & Tiedje, J. M. (2002). Quantitative detection of microbial genes by using DNA microarrays. *Applied and Environmental Microbiology, 68*, 1425–1430.

Crameri, A., Dawes, G., Rodriquez, E., Silver, S., & Stemmer, W. P. C. (1997). Molecular evolution of an arsenate detoxification pathway by DNA shuffling. *Nature Biotechnology, 15*, 436–438.

Dechesne, A., Pallud, C., Bertolla, F., & Grundmann, G. L. (2005). Impact of the microscale distribution of Pseudomonasstrain introduced into soil on potential contacts with indigenous bacteria. *Applied and Environmental Microbiology, 71*, 8123–8131.

DeFlaun, M. F., Paul, J. H., & Jeffrey, W. H. (1987) Distribution and molecular weight of dissolved DNA in subtropical estuarine and oceanic environments. *Mar. Ecol. Prog. Ser, 38*, 65–73.

Dejonghe, W., Goris, J., Fantroussi, S. E., Hofte, M., De Vos, P., Verstraete, W., & Top, E. M. (2000). Effect of dissemination of 2,4- dichlorophenoxyacetic acid (2,4-D) degradation plasmids on 2,4-D degradation and on bacterial community structure in two different soil horizons. *Applied and Environmental Microbiology, 66*, 3297–3304.

Dennis, P. (2005). Why the type of bacteria matter in bioremediation. *Pollution Engineering, 37*, 22–37.

Diaz-Ricci, J. C., & Hernandez, M. E. (2000). Plasmid effects on Escherichia coli metabolism. *Critical Reviews in Biotechnology, 20*, 79–108.

Ensley, B. D. (1991). Biochemical diversity of trichloroethylene metabolism. *Annual Review of Microbiology, 45*, 440–449.

Feliciano, J., Liu, Y., & Daunert, S. (2006). Novel reporter gene in a fluorescent-based whole cell sensing system. *Biotechnology and Bioengineering, 93*, 989–997.

Foster, L., John, R., Kwan Boon, N., De Gelder, L., Lievens, H., Siciliano, S. D., Top, E. M., & Ver straete W. (2002). Bioaugmenting bioreactors for the continuous removal of 3-chloroaniline by a slow release approach. *Environmental Science & Technology, 36*, 4698–46704.

Furukawa, K. (2000a). Biochemical and genetic bases of microbial degradation of polychlorinated biphenyls (PCBs). *The Journal of General and Applied Microbiology, 46*, 283–296.

Furukawa, K. (2000b). Engineering dioxygenases for efficient degradation of environmental pollutants. *Current Opinion in Biotechnology, 11*, 244–249.

Furukawa, K., Suenaga, H., & Goto, M. (2004). Biphenyl dioxygenases: Functional versatilities and directed evolution. *Journal of Bacteriology, 186*, 5189–5196.

Gentry, T. J., Rensing, C., & Pepper, I. L. (2004). New approaches for bioaugmentation as a remediation technology. *Critical Reviews in Environment Science and Technology, 34*, 447–494.

Gomes, K. M., Rebello, R. C., Duarte, R. S., & Rachid, C. T. (2013). Diversity of mercury resistant Escherichia coli strains isolated from aquatic systems in Rio de Janeiro, Brazil. *International Journal of Biodiversity, 6*, 1–8.

Hedenstierna, K. O. F., Lee, Y.-H., Yang, Y., & Fox, G. E. (1993). A prototype stable RNA identification cassette for monitoring plasmids of genetically engineered microorganisms. *Systematic and Applied Microbiology, 16*, 280–286.

Hrywna, Y., Tsoi, T. V., Maltseva, O. V., Quensen, J. F., III, & Tiedje, J. M. (1999). Construction and characterization of two recombinant bacteria that grow on ortho-and para–substituted chlorobiphenyls. *Applied and Environmental Microbiology, 65*, 2163–2169.

Iwasaki, K., Uchiyama, H., & Yagi, O. (1993). Survival and impact of genetically engineered Pseudomonas putida harboring mercury resistance gene in aquatic microcosms. *Bioscience Biotechnology & Biochemistry, 57*, 1264–1269.

Kang, D. G., Choi, S. S., & Cha, H. J. (2006). Enhanced biodegradation of toxic organophosphate compounds using recombinant Escherichia coli with sec pathway driven periplasmic secretion of organophos phorous hydrolase. *Biotechnology Progress, 22*, 406–441.

Koyama, A. H., Wada, C., Nagata, T., & Yura, T. (1975). Indirect selection for plasmid mutants: Isolation of colVBtrp mutants defective in self maintenance in Escherichia coli. *Journal of Bacteriology, 122*, 73–79.

Lange, C. C., Wackett, L. P., Minton, K. W., & Daly, M. J. (1998). Engineering a recombinant Deinococcus radiodurans for organopollutant degradation in radioactive mixed waste environ ments. *Nature Biotechnology, 16*, 929–933.

Matin, A. (1994). Starvation promoters of Escherichia coli. Their function, regulation, and use in bioprocessing and bioremediation. *Annals of the New York Academy of Science, 721*, 277–291.

Monti, M. R., Smania, A. M., Fabro, G., Alvarez, M. E., & Argarana, C. E. (2005). Engineering Pseudomonas fluorescens for biodegra dation of 2,4-dinitrotoluene. *Applied and Environmental Microbiology, 71*, 8864–8872.

Mulchandani, A., Kaneva, I., & Chen, W. (1999). Detoxification of organophosphorus nerve agents by immobilized Escherichia coli with surface-expressed organophosphorus hydrolase. *Biotechnology and Bioengineering, 63*, 216–223.

Nagata, T., Nakamura, A., Akizawa, T., & Pan-Hou, H. (2009). Genetic engineering of transgenic tobacco for enhanced uptake and bioaccumulation of mercury. *Biological & Pharmaceutical Bulletin, 32*, 1491–1495.

Naik, M. M., Shamim, K., & Dubey, S. K. (2012). Biological characterization of lead resistant bacteria to explore role of bacterial metallothionein in lead resistance. *Current Science, 103*, 426–429.

Pandey, G., Paul, D., & Jain, R. K. (2005). Conceptualizing "suicidal genetically engineered micro- organisms" for bioremediation applications. *Biochemical and Biophysical Research Communications, 327*, 637–639.

Parales, R. E., Bruce, N. C., Schmid, A., & Wackett, L. P. (2002). Biodegradation, biotransformation, and biocatalysis (B3). *Applied and Environmental Microbiology, 68*, 4699–4709.

Paul, D., Pandey, G., Pandey, J., & Jain, R. K. (2005). Accessing microbial diversity for bioremediation and environmental restoration. *Trends in Biotechnology, 23*, 135–142.

Peters, M., Heinauru, E., Talpsep, E. W., Stoottmeister, U., Heinaru, A., & Nurk, A. (1997). Acquisition of a deliberately introduced phenol degradation operon, pheba, by different indigenous Pseudomonas species. *Applied and Environmental Microbiology, 63*, 4899–4906.

Pieper, D. H., & Reineke, W. (2000). Engineering bacteria for bioremediation. *Current Opinion in Biotechnology, 11*, 262.

Qiu, Z., Tan, H., Zhou, S., & Cao, L. (2014). Enhanced phytoremediation of toxic metals by inoculating endophytic Enterobacter sp. CBSB1 expressing bifunctional glutathione synthase. *Journal of Hazardous Materials, 267*, 17–20.

Reichmuth, D. S., Blanch, H. W., & Keasling, J. D. (2004). Dibenzothiopene biodesulfurization pathway improvement using diagnostic GFP fusions. *Biotechnology and Bioengineering, 88*, 94–99.

Reineke, W. (1998). Development of hybrid strains for the mineralization of chloroaromatics by patchwork assembly. *Annual Review of Microbiology, 52*, 287–331.

Renninger, N., Knopp, R., Nitsche, H., Clark, D. S., & Keasling, J. D. (2004). Uranyl precipitation by Pseudomonas aeruginosa via controlled polyphosphate metabolism. *Applied and Environmental Microbiology, 70*, 7404–7412.

Ripp, S., Nivens, D. E., Ahn, Y., Werner, C., Jarrel, J., Easter, J. P., Cox, C. D., Burlage, R. S., & Sayler, G. S. (2000). Controlled field release of a bioluminescent genetically engineered microorganism for bioremediation process monitoring and control. *Environmental Science & Technology, 34*, 846–853.

Rittmann, B. E., Hausner, M., Loffler, F., et al. (2006). A vista for microbial ecology and environmental biotechnology. *Environmental Science & Technology, 40*, 1096–1103.

Sayler, G. S., & Ripp, S. (2000). Field applications of genetically engineered microorganisms for bioremediation processes. *Current Opinion in Biotechnology, 11*, 286–289.

Sharp, R. R., Bryers, J. D., Jones, W. G., & Shields, M. S. (1998). Activity and stability of a recombinant plasmid-borne TCE degradative pathway in suspended cultures. *Biotechnology and Bioengineering, 57*, 287–296.

Singh, J. S., Abhilash, P. C., Singh, H. B., Singh, R. P., & Singh, D. P. (2011). Genetically engineered bacteria: An emerging tool for environmental remediation and future research perspectives. *Gene, 480*, 1–9.

Suidan, M. T., Esperanza, M., Zein, M., McCauley, P., Brenner, R. C., & Venosa, A. D. (2005). Challenges in biodegradation of trace organic contaminants-gasoline oxygenates and sex hormones. *Water Environment Research, 77*, 4–11.

Top, E. M., Springael, D., & Boon, N. (2002). Catabolic mobile genetic elements and their potential use in bioaugmentation of polluted soils and waters. *FEMS Microbiology Ecology, 42*, 199–208.

Velkov, V. V. (2001). Stress-induced evolution and the biosafety of ge netically modified microorganisms released into the environment. *Journal of Biosciences, 26*, 667–683.

Wackett, L. P. (2004). Stable isotope probing in biodegradation research. *Trends in Biotechnology, 22*, 153–154.

Wang, A. A., Chen, W., & Mulchandani, A. (2005). Detoxification of organophosphate nerve agents by immobilized dual functional bio catalysts in a cellulose hollow fiber bioreactor. *Biotechnology and Bioengineering, 91*, 379–386.

Widada, J., Nojiri, H., & Omori, T. (2002). Recent developments in molecular techniques for identification and monitoring of xenobiotic degrading bacteria and their catabolic genes in bioremediation. *Applied Microbiology and Biotechnology, 60*, 45–59.

Wu, J.-F., Jiang, C., Wang, B., Liu, Z., & Liu, S. (2006). Novel partial reduc tive pathway for 4-chloronitrobenzene and nitrobenzene degrada tion in Comamonas sp. strain CNB-1. *Applied and Environmental Microbiology, 72*, 1759–1765.

Yildirim, S., Franco, T. T., Wohlgemuth, R., Kohler, H. E., Witholt, B., & Schmid, A. (2005). Recombinant chlorobenzene dioxygenase from Pseudomonas sp. P51: A biocatalyst for regioselective oxidation of aromatic nitriles. *Advanced Synthesis and Catalysis, 347*, 1060–1072.

Zaat, S. A. J., Slegtenhorst-Eegdeman, K., Tommassen, J., et al. (1994). Construction of phoE-caa, a novel PCR- and immunologically detectable marker gene for Pseudomonas putida. *Applied and Environmental Microbiology, 60*, 913–920.