SJIF Impact Factor (2024): 8.675| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 9 | Issue: 8 | August 2024 - Peer Reviewed Journal

DEVELOPING GENETICALLY ENGINEERED ORGANISMS FOR BIOREMEDIATION OF POLLUTANT

Ms. Sakshi A. Ekhande , Ms.Anjali Shinde, Ms.Dr.Rani Mhetre , Mr.Dr Vijay Sable

ABSTACT

Worldwide, environmental pollution is a serious issue that has an impact on ecosystems, public health, and biodiversity. At the moment, traditional technology cannot resolve this problem because the necessary treatments are costly, time-consuming, and unsuccessful [4] . The method of removing toxic waste from the environment by means of biological agents is now referred to as bioremediation[1].The dangers involved in releasing genetically modified microorganisms (GEMs) into the environment have limited the use of GEMs for pollution abatement [2].The usual engineering approaches based on physicochemical methods for the remediation of these polluted sites present both technical and financial challenges. [The most promising, reasonably efficient, and economical approach is bioremediation, which uses microorganisms to remove contaminants[8]. Transgenic plants and genetically modified microorganisms can work together symbiotically to improve the efficiency of bioremediation at contaminated locations[4]. Due to their improved ability to degrade a broad variety of chemical pollutants, genetically modified microbes, or GEMs, have demonstrated promise for bioremediation applications in soil, groundwater, and activated sludge environments[7].

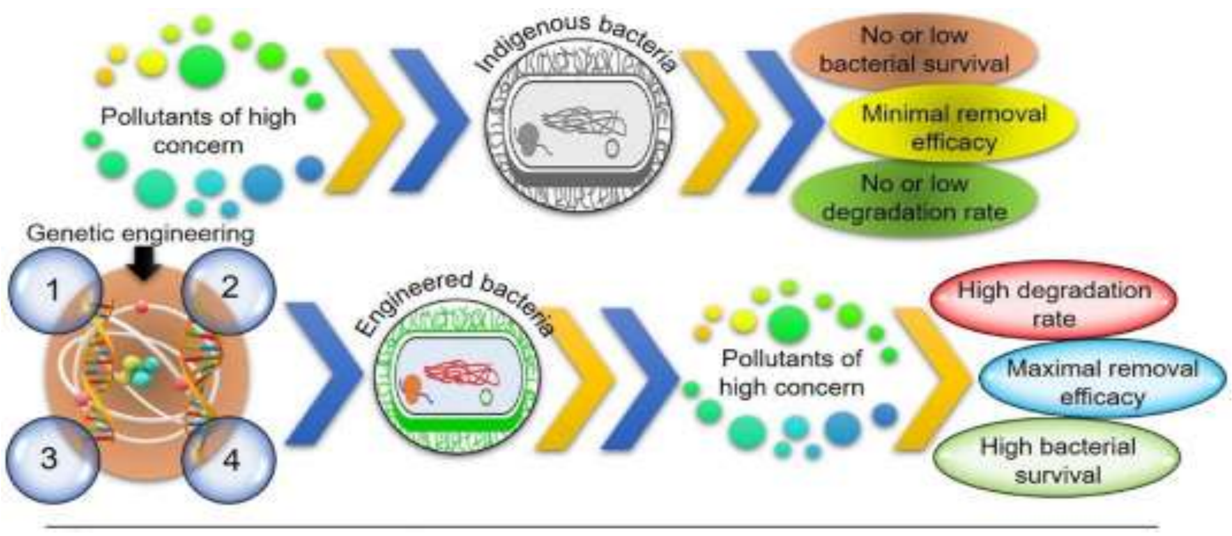
KEYWORDS : - *Bioremediation , pollutants, Recombinant DNA technology, GEMs,*

INTRODUCTION

Environmental biotechnology's basic research and field application emphasis are found in the bioremediation and natural attenuation areas.The amount of contaminated land and water has increased as a result of the global population boom[1]. number of genetically modified microbes (GEMs) have been successfully created recently. However, in order to use bioremediation, environmental Their in situ applicability is limited by regulatory restrictions and considerations[2]. The traditional methods of remediation have involved either excavating contaminated soil and disposing of it in a landfill or capping and enclosing the polluted parts of a site[3].The rise in mining and heavy industry has led to xenobiotic chemical contamination of the environment. Xenobiotic substances' intrinsic toxicity can cause direct harm to environmental ecosystems[4].These days, anthropogenic organic pollutants are widely distributed in the environment and can be quite resistant to the biodegradation mechanisms exhibited by naturally existing microorganisms[5].Due to the carcinogenic properties of certain of the more persistent hydrocarbons, such as PCBs and PAHs, hydrocarbons can become harmful if they get into the food chain . Organic contaminants in the sediments have the potential to momentarily bind with particle matter, creating equilibrium relationships at the water-sediment interface[6].The use of genetically modified microorganisms (GEMs) in bioremediation has not advanced much in the last ten years[7].The demand for energy, the need to detoxify the contaminants, or chance could all be factors in the microbial transformation (cometabolism) .Pollution is the process by which a resource (natural or man-made) is, more often than not, rendered unfit for use by people due to harmful effects or damages caused by the contaminants. Since the beginning of time, pollutants have existed, and life as we know it now has always developed alongside them. The earth will always be a filthy planet because to pollution mimics from comets, space dust (about 100 t of organic dust per day), and geothermal and volcanic activity[8].

Mechanism of Bioremediation by Genetically Engineered Microbes

High concentrations of metal ions can either completely prevent the microbial population from carrying out any of its various metabolic functions or they can make the organisms resistant to the high metal concentrations. Heavy metals are more difficult to remove from contaminated ecosystems than many other pollutants, despite the fact that they can biodegrade and create less hazardous, less mobile, and/or less bioavailable compounds. Though their speciation and bioavailability may vary depending on environmental conditions, these metals are ultimately eternal and cannot be broken down physiologically. Certain metals, such zinc, copper, nickel, and chromium, are essential or useful micronutrients for microbes, plants, and animals.[15].



1.Screening & clobing of highly effective degraning genes. 2.increasing the expression of enzyme with degradation function of microorganisms 3.Protoplast fusion by blending the advantages of bothparent for pollution degradation 4. Expression degradation gene for different pollutants to construct super engineered bacteria[28].

Development of recombinant DNA technology for bioremediation

In prokaryotes, post-transcriptional processing of a multi-cistronic mRNA containing the coding regions for all of an enzyme's subunits is frequently used to regulate the expression of genes encoding for enzyme subunits . With this kind of regulation, the requirement for several promoters of varying intensities for each subunit's gene is removed.Through degradative genes, DNA enables an organism to acquire the capacity to digest a foreign substance. Genetically modified microbes and recombinant microorganisms have been employed as an efficient method for the breakdown of pollutants[12].The generated mRNA hairpin was intended to be positioned one nucleotide from the 5/-end using a synthetic DNA cassette. The DNA cassette's sequence was selected to give the resultant hairpin a specific secondary structure and dG of formation[11].Using 2 ll of each DNA fraction and their decimal dilution as templates, a PCR amplification of 16S rDNA was performed to assess the purity of the DNA recovered following treatment with Sepharose 4B.Following the PCR amplificability tests, fractions 2, 3, 4, and 5 from the purification of Sepharose 4B were combined for every soil sample. For PCR tests, DNA was resuspended in TE pH 8 after being precipitated with ethanol[14].By definition, plasmids with a broad host range are able to replicate in multiple host organisms. The benefit of employing these vectors is that they can be replicated only once, with the resultant clone being utilised by numerous bacteria.If a gene is being cloned for use in numerous hosts, narrow-host range plasmids have the drawback of being able to replicate in a smaller number of hosts than broad-host range plasmids.A transposon is a particular DNA sequence that moves randomly within a chromosome on its own[11].This study set out to both establish the applicability of DNA-based technologies in a full-scale bioremediation strategy and to choose a candidate location for bioremediation. In order to accomplish these goals, we created, produced, and applied DNA chips for the diagnosis of contaminated soil; furthered our investigation of a site with a favourable prognosis for biological degradation by using genomic data; and bioslurped the contaminated locations that were chosen[13].Using a DNA thermal cycler, nested PCR was performed in a total volume of 50 ~1 plus an overlay of mineral oil. After the tubes were heated to 80°C, the inner and outer reactions were set up using "hot-start," which involved introducing the dNTPs. DNA electrophoresis was carried out utilising accepted methods. Terminal deoxynucleotidyl transferase (TdT) was used to tail capture probe B in order to facilitate reverse dot blot (RDB) detection. After the electrophoretic separation, DNA was denatured within the gel and the entire gel content was transferred (blotted) onto a flexible membrane, typically a nitrocellulose filter, so that the DNA fragments would bind to this support without changing their relative positions within the gel. The final reaction concentrations were 50 units of TdT, 250 nmol of TTP, and 100 pmol of probe in a 100 ~1 volume of the buffer suggested by the manufacturer. Washing in TE stopped the reaction after IO–30 minutes. Probe D was end-labeled in a reaction using I nmol of probe, 2 nmol of digoxigenin-I I-ddUTP, and 50 units TdT in a 100 ~1 volume of the manufacturer's suggested buffer as a direct RDB control. The reaction was incubated for two hours at 37°C and then for ten minutes at 70°C[16]. It only takes one nucleotide alteration at a restriction cutting site to change the pattern of digestion and influence cleavage. The Southern blot method, which was first introduced in 1976, marked a breakthrough in the physical mapping of DNA by restriction enzyme digestion.The various heuristic and practical methods that use restriction enzymes have shown to be essential for physical DNA mapping . The ability to insert targeted deletions of genes or promoter sub-regions has been made possible by restriction enzyme-mediated DNA editing. This allows researchers to compare the behaviour of deleted templates with wild-type counterparts in terms of substrates for RNA transcription/processing and translation .The field of restriction enzyme characterisation has advanced quickly, leading to the identification of four distinct groups of enzymes [17].

Genetically Engineered Microbes for Remediation

According to environmental biotechnology, heavy metals can be extracted from aqueous solutions by microorganisms like filamentous fungi, yeast, and bacteria[4].Bacteria and pollutants need to come into touch for degradation to occur. This is difficult to accomplish since the pollutants and bacteria are not distributed evenly throughout the soil. Certain bacteria are chemotactic, meaning they go towards contaminants they sense. These bacteria are mobile. Fungi and other such bacteria grow filamentously in the direction of the contamination. Although a wide variety of organisms, including plants, can be employed in bioremediation, microbes have the most promise. The first recyclers in nature were microorganisms, namely bacteria and fung[i][1].It is possible to separate microorganisms from practically any kind of environment. Microbes are adaptable and can develop in a variety of environments, including subzero temperatures, intense heat, desert heat, water, an abundance of oxygen, anaerobic circumstances, the presence of dangerous substances, and waste streams. An energy source and a carbon source are the two primary needs.

Aerobic : When oxygen is present.It has frequently been reported that these bacteria break down hydrocarbons, including polyaromatic chemicals and alkanes, as well as pesticides. For many of these bacteria, the pollutant serves as their only source of energy and carbon .
Anaerobic : - the absence of oxygen is called anaerobic. Compared to aerobic bacteria, anaerobic bacteria are not used as much[3].

In aquatic ecosystems, algae are essential for the biomonitoring of organic contaminants. In certain instances, adding the bacterial culture in the form of activated sludge to the Chlorella vulgaris microalgal consortia greatly accelerates the hydrocarbon biodegradation process at the oil-polluted location. It has been demonstrated that choosing suitable yeast and bacterial strains to employ in combinations during biodegradation investigations is a highly successful process. These ideas have recently been applied by some researchers to assess the possibility of a microbial consortium consisting of varying proportions of bacteria and yeast for the biodegradation of diesel oil[19].

Bioremediation by Genetically Engineered Plant

Various plant species are environmentally benign and economically viable ways to clean up water and soil. Certain possible cellular and molecular processes found in plants may be used to aid in the detoxification of heavy metals. On contaminated areas, plants can break down pollutants in a way that is less harmful to the environment and profitable. Metals can be dissolved in soil by plants, which then absorbs them into their roots and transports them to the shoots[4]. For all living things, mercury and its derivatives pose a risk. Bacteria have developed strategies for settling in environments contaminated with mercury, and it has been discovered that an operon of mercury resistance (mer) genes codes for transporters and enzymes necessary for biochemical detoxification [20]. Mercury finds its way into water streams through industrial processes such the manufacturing of chemicals, paper, and textiles, as well as as a byproduct of gold mining . The distinctive characteristic of elemental mercury is its liquid state at normal temperature, which makes it susceptible to volatilisation[29]. The following qualities are desirable in a plant for phytoremediation:1) the capacity to accumulate metals, preferably in the aboveground portions; (2) tolerance to the concentration of metals accumulated;(3) rapid growth and high biomass;(4) a widely distributed, highly branched root system; and (5) ease of harvesting. Trees are good candidates for genetic engineering because they are high biomass plants that can survive for a long time and have the innate potential to do phytoremediation.

Factors Influenzing Bioremediation of Contaminated Site

Numerous biotic and abiotic elements influence the development and behaviour of microbial cells, which in turn influences a range of biological processes taking place inside a microbial community. The multiphasic heterogeneous environment that the bioremediation process is subjected to (Boopathy 2000) affects the rate of reactions. Implementing a process with inadequate knowledge of the elements driving it frequently results in reduced process efficacy[21].The availability and capacity of reduced organic materials as energy sources is one of the main factors influencing the activity of bacteria . The average oxidation state of the carbon in the material determines whether a pollutant will be an efficient source of energy for an aerobic heterotrophic cell[22]. Redox potential (Eh), pH, ionic strength, solubility, presence or absence of electron acceptors and donors, temperature, and the age of organometalic ions are examples of physicochemical factors. Many intrinsic features of microbes influence how quickly substrates degrade. For example, plasmid-encoded genes give specificity for substrates and encode particular enzymes (proteins). However, microbes, particularly bacterial cells, have been shown to have diverse specificity for various substrates in the natural world[21]. Several plant species have been employed in the phytoremediation of soil contaminated by petroleum. Since petroleum is poisonous to plants, TPH concentrations above a certain point prevent plants from growing. Fertiliser addition has a significant impact on both plant growth and microbial activity, making it a crucial factor in determining the bioremediation process's efficiency[23].

Containment Strategies After Bioremediation

Regarding GEO's continued presence in the environment during bioremediation, people are worried. Pieper and Reineke found that plasmids can spread to other bacteria through both recombinant genes and selection markers, as well as the chemical and physical stability of the plasmids. It may be possible to address this issue by substituting selected markers that focus on antibiotic resistance with selective markers that do not [4].The three treatment approaches were (a) biostimulation with inorganic nutrient addition (O-N), (b) organic remediation (oiled control; O-C), and (c) biostimulation with inorganic nutrient addition plus nitrate (O-NN), an alternative electron acceptor. To assess the effectiveness of the treatment, a randomised complete-block design with repeated measures was employed .The inorganic nutrient supply was commercial-grade fertiliser (diammonium phosphate), with a target dose of 40 mg N/ kg dry sediment weight (ppm N) at each application event.The baseline samples were taken two days before the petroleum was applied. Four days following the petroleum application, on Day 0, the experiment started with the first treatment application[24] .The most popular use of the bioremediation approach is the treatment of soil contaminated by coal, petrol, crude oil, petrol, etc.When used to vast areas with low pollutant concentrations, this approach works well. Because it is widely accessible and has minimal negative effects on the environment and ecosystem, it is well accepted by the general people. In addition, it is less expensive and disrupts the soil structure less[25.]

Current Challenges and Perspective

The long-term impacts of environmental pollution on human health are a major global concern. The body's ability to use mitochondria to produce energy may be altered by Pb accumulation, leading to neurological disorders, paralysis, and finally death. Plants, animals, and humans are all at risk from some heavy metals. Certain plants that are edible have the potential to absorb dangerous metals from the soil and become ingested by people and other animals, leading to negative consequences. Bureaucratic obstacles and the political climate provide challenges to the application of genetically modified bacteria in bioremediation[4].Microorganisms face significant challenges when dealing with polyethylene because of its hydrophobicity, massive molecular impurities, and absence of reactive functional groups in the polymer backbone. Long chain aliphatic compound consumption by microorganisms is well-documented, and n-alkane consumption by bacteria and fungus has been demonstrated .Plastics have the advantage over many of the materials they have replaced in various applications, such as extending the shelf life of food, lowering the risk of infection in medical equipment, and creating robust yet lightweight building materials.The biodegradation investigations show that a variety of fungal species can cause modifications to the physical and chemical characteristics of LDPE and HDPE, as well as the polymer chains' fragmentation and, to a lesser degree, the polymer's absorption and mineralisation[26].

CONCLUSION

Many organic pollutants pose a persistent toxicological risk to humans and wildlife alike, including pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls. These pollutants are not easily broken down[5] ·Using microbial activity, bioremediation is a very effective and appealing option for cleaning, managing, and returning contaminated river water to its original state[27]. Although there are a few present obstacles to overcome, including the spread of transgenic pollen, the horizontal transfer of plasmids among bacteria, and the low survival rate of GEO and transgenic plants, GEO may be a viable and sustainable strategy for the bioremediation of contaminated sites[4].Bioremediation can be carried out in relation to process validation, optimisation, and its impact on the ecosystem. Bioremediation can now be turned from a mere practice into a science through the prudent use of models that can predict the activity of microorganisms involved in bioremediation[15] .

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