Genetic Innovations in Environmental Restoration: The power of rDNA Technology

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Declaration

It is hereby declared that

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2. The paper does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

The thesis titled Review of Genetic Innovations in Environmental Restoration: The power of rDNA technology submitted by umma hany of fall ,2024 has been accepted as satisfactory in partial fulfillment of the requirement for the degree in MS.c. un Biotechnology on 8th may 2025.

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Abstract:

Innovative approaches in bioremediation have become necessary due to recent environmental difficulties, and recombinant DNA technology is developing as a game-changing alternative for environmental restoration. By improving the genetic capacity of microorganisms to more effectively break down environmental contaminants, genetic engineering approaches have transformed conventional bioremediation procedures.

Researchers have successfully altered microbes to express particular genes that target different environmental toxins using rDNA technology. Increased tolerance to environmental stresses, improved substrate selectivity, and improved degradation pathways are the outcomes of these alterations. When it comes to decomposing harmful substances including heavy metals, herbicides, industrial waste products, and persistent organic pollutants (POPs), the modified microorganisms work exceptionally well.

The creation of specialised bacterial strains with various degradative capacities is very important. These strains can handle many contaminants at once because they have recombinant plasmids that include genes for different catabolic enzymes. These organisms' ability to survive and function in harsh environments has also been enhanced by the introduction of stress- tolerance genes, which increases their suitability for in-situ bioremediation applications.

Recent developments have concentrated on creating durable genetic constructs that sustain their improved capabilities throughout several generations and optimising gene expression systems. Monitoring capabilities have been further enhanced by the incorporation of biosensor genes, which enable real-time evaluation of biodegradation processes. Along with cutting treatment durations and expenses, these advancements have greatly improved the bioremediation procedures' dependability and efficiency.

There are still issues with the release of genetically modified organisms into the environment and the possible ecological effects of doing so. Suicide

genes and containment tactics that restrict bacterial survival outside of specified locations are being developed as part of ongoing research to solve these issues. In the future, new gene combinations will be investigated, and synthetic biology concepts will be used to develop more complex and regulated bioremediation systems.

This review paper investigates the role of recombinant DNA (rDNA) technology in advancing bioremediation techniques. It explores the genetic modification of microorganisms to improve their efficiency in degrading or detoxifying environmental pollutants, focusing on applications, challenges, and future prospects.

Keywords: Bioremediation, Recombinant DNA Technology, Genetic Engineering, Environmental Biotechnology, Pollution Control, Microbial Enhancement

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Content

Introduction

Environmental pollution is now a major worldwide concern that endangers human health, ecosystem stability, and biodiversity. It has reached critical levels, posing severe threats to public health, biodiversity, and the global climate. Rapid industrialization, urbanisation, and increased consumption of natural resources have led to widespread contamination of air, water, and soil. Conventional pollution mitigation methods—such as filtration systems, chemical treatments, and waste management strategies—are often limited in effectiveness due to high costs, the generation of secondary pollutants, and an inability to address pollution at the source. These methods also struggle to keep pace with the scale and complexity of modern environmental challenges, underscoring the urgent need for more sustainable, innovative solutions.

A key component of contemporary biotechnology is recombinant DNA technology, which modifies genetic material to produce organisms with novel traits. Although this technology has transformed many industries, such as agriculture and health, its potential to reduce environmental pollution is especially exciting. Scientists are able to create new approaches for the breakdown and detoxification of contaminants by genetically modifying microbes to have particular characteristics. These organisms that have been bioengineered can be adapted to target various pollutants, such as industrial waste, plastic debris, heavy metals, and oil spills. This introduction examines the fundamentals and practical uses of recombinant DNA technology in environmental pollution mitigation, emphasising the technology's contribution to the development of effective and long-lasting bioremediation techniques.

An innovative method of combating environmental pollution is provided by recombinant DNA (rDNA) technology, which entails the modification of genetic material to produce creatures with improved or novel capabilities.

Scientists can create specialised jobs for microbes, plants, and even higher organisms that help reduce pollution thanks to recombinant DNA technology. Its application in environmental research is garnering more and more attention. This technology has already proven its potential in a number of industries, including agriculture and medicine. Researchers are able to create creatures with the ability to degrade harmful pollutants, absorb heavy metals, and monitor environmental toxins with a level of precision never before possible by using genetic engineering.

The bioremediation of polluted settings is one of the exciting uses of rDNA technology. Hazardous substances including heavy metals, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), which are frequently resistant to natural degradation processes, can be metabolized and detoxified by engineered microorganisms. For example, oil spills can now be broken down more efficiently by genetically modified bacteria than by their naturally occurring equivalents, providing a potent weapon for controlling marine pollution.

Researchers are also investigating the phytoremediation potential of transgenic plants in addition to microbes. Genetic modification of these plants is possible to make them metabolize organic contaminants or hyperaccumulate heavy metals from contaminated soils. These developments improve pollution cleaning effectiveness while also offering a sustainable and environmentally beneficial substitute for conventional techniques. In addition, biosensors that can identify and track environmental contaminants in real time are made possible by recombinant DNA technology. Early warning systems and more successful environmental management techniques are made possible by these biosensors, which are frequently based on genetically modified microorganisms or enzymes. They offer extremely sensitive and specific impurity detection.

Although rDNA technology holds great promise, there are obstacles and moral dilemmas associated with using it to reduce environmental pollution. It is important to carefully consider the ecological dangers and long-term effects of releasing genetically modified organisms into the ecosystem.

A successful implementation of these biotechnological solutions will depend on a number of elements, including public acceptance and regulatory frameworks. An innovative method for combating environmental contamination is provided by recombinant DNA technology. This technology offers the possibility of more accurate, long-lasting, and efficient pollution management methods through the creation of customized biological solutions. In order to fully utilize rDNA technology in the pursuit of a better and cleaner environment, further research must be done. Strong regulatory measures and public involvement are also necessary.

This introduction offers a concise and comprehensive summary of the ways in which recombinant DNA technology might be applied to mitigate environmental contamination, emphasising the obstacles that must be overcome as well as the technology's potential uses. This brief section describes the ways that recombinant DNA technology lowers pollution in the environment.

Bioremediation

Bioremediation refers to any process that uses biological systems—either living organisms or their byproducts—to remove or neutralize pollutants from the environment. This technique is employed in various settings, including air, water, soil, industrial effluents, and flue gases. The organisms typically involved in bioremediation include:

- Bacteria: Microorganisms that can metabolize a wide range of organic and inorganic pollutants.
- Fungi: Particularly useful in mycoremediation, where fungi break down complex organic compounds.
- Microalgae: Capable of absorbing heavy metals and other contaminants from water.
- Plants: In phytoremediation, plants are used to absorb, accumulate, and detoxify pollutants from soil and water.

Mechanisms of Bioremediation

Bioremediation operates through several biological processes:

1. **Biodegradation**: The breakdown of organic contaminants into simpler, less harmful substances (e.g., carbon dioxide and water). This process often involves microbial metabolism.
2. **Bioaccumulation**: The uptake of contaminants by living organisms from their environment. This is particularly relevant for heavy metals, which can be absorbed by plants or microorganisms.
3. **Oxidation and Hydrolysis:** These chemical processes can enhance the solubility of organic pollutants, making them more accessible for microbial degradation.
4. **Phytostabilization**: Involves the use of plants to stabilize contaminants in the soil and prevent their migration to groundwater.

Types of Bioremediation

- Bioremediation strategies can be classified into two main categories:
- In Situ Treatments: These methods treat contaminated materials directly at the site without excavation. Examples include:
    - Bioventing: Enhancing oxygen delivery to promote aerobic degradation of contaminants in soil.
    - Biosparging: Injecting air into groundwater to increase oxygen levels and stimulate
    - Bioattenuation: natural process to reduce contaminated concentration over time without Human intervention , through monitoring is essential to ensure effectiveness.

- Ex Situ Treatments: Contaminated materials are removed from the site for treatment. Example include:
- **Composting**: Using controlled biological processes to degrade organic waste.
- **Landfarming**: Spreading contaminated soil over a large area to promote natural degradation.

Historical Context and Advances

The field of bioremediation gained momentum in the 1980s with advancements in genetic engineering. One of the most significant milestones was the development of Pseudomonas putida, a genetically engineered microorganism (GEM) created by Ananda Chakrabarty. This "superbug" was designed specifically to degrade hydrocarbons found in oil spills. Patented in 1980, it represented a breakthrough in applying recombinant DNA technology to environmental remediation.

Examples of Bioremediation Using rDNA Technology

Bioremediation using recombinant DNA (rDNA) technology has made significant strides in addressing environmental pollution through the engineering of microorganisms. Here are some notable examples:

Oil Spill Cleanup

Genetically modified bacteria, such as certain strains of Pseudomonas and Alcanivorax, have been engineered to enhance their ability to degrade hydrocarbons found in crude oil. Research following the Deepwater Horizon oil spill revealed that specific bacteria possess genetic pathways that allow them to break down complex oil compounds, including alkanes and aromatic hydrocarbons, more efficiently.

By sequencing the DNA of these oil-eating bacteria, scientists identified genes responsible for their degradation capabilities, paving the way for targeted applications in future oil spill response.

Heavy Metal Degradation

Engineered organisms can be designed to express metal-binding proteins that facilitate the accumulation and neutralization of heavy metals such as mercury, cadmium, and lead. By introducing specific genes into native bacteria, researchers enhance their capacity to sequester these toxic metals from contaminated environments.

This approach not only helps in detoxifying polluted sites but also offers a sustainable method for managing heavy metal contamination.

Plastic Waste Decomposition

Recent advancements have led to the engineering of microorganisms capable of producing enzymes that degrade plastics more efficiently. For instance, researchers have modified certain bacteria to express enzymes like PETase and MHETase, which can break down polyethylene terephthalate (PET), a common plastic found in bottles and packaging.

This biotechnological approach addresses the growing issue of plastic pollution in landfills and aquatic environments by converting plastics into biodegradable by products.

Bioremediation Advantages Over Traditional Methods

Bioremediation offers several advantages compared to traditional physicochemical treatment methods:

- **Sustainability:** Utilizes natural processes and organisms, reducing reliance on harsh chemicals.
- **Environmental Friendliness:** Minimizes secondary pollution by using biological systems that integrate into ecosystems.
- **Cost-effectiveness:** Often less expensive than conventional methods due to lower energy requirements and reduced material costs.
- **Scalability:** Can be applied at various scales, from small sites to large contaminated areas.

Bioremediation Limitations: Despite its benefits, bioremediation has limitations:

- **Slow Rate: The** process can take longer than traditional remediation methods.
- **Effectiveness with Heavy Metals:** While bioremediation

Advantages of Bioremediation

- **Eco-Friendly and Sustainable:** Bioremediation utilizes natural microbial processes to degrade contaminants, making it an environmentally friendly approach. It avoids the use of harmful chemicals, aligning with sustainable practices.
- **Cost-Effective:** Compared to traditional remediation methods, bioremediation is often more economical. It leverages naturally occurring organisms, reducing the need for expensive chemical treatments and extensive labor.
- **Complete Degradation of Pollutants:** Microorganisms involved in bioremediation can fully break down hazardous substances into less harmful products, such as water and carbon dioxide, ensuring thorough detoxification of contaminated sites.
- **Minimal Environmental Impact:** This method preserves the natural ecosystem by maintaining the indigenous microbial populations and avoiding the introduction of foreign substances, thus supporting the restoration of the natural habitat.

Disadvantages of Bioremediation

- **Limited to Biodegradable Substances:** Bioremediation is effective only for pollutants that microorganisms can degrade. Non-biodegradable contaminants, such as certain heavy metals, cannot be treated using this method.
- **Potential for Toxic By-products:** Sometimes, the degradation process may produce intermediate compounds more toxic than the original pollutants. For instance, the breakdown of trichloroethylene (TCE) can result in vinyl chloride, a known carcinogen.
- **Specific Environmental Requirements:** The success of bioremediation depends on optimal environmental conditions, including temperature, pH, and nutrient availability. Deviations from these conditions can hinder microbial activity and reduce effectiveness.
- **Extended Time Frames:** Compared to methods like incineration or chemical treatments, bioremediation often requires more time to achieve desired contaminant reduction levels. This extended duration can be a drawback in situations requiring rapid remediation.
- **Challenges in Scaling Up:** Translating successful laboratory or pilot-scale bioremediation processes to full-scale field applications can be complex. Factors such as heterogeneous contaminant distribution and site-specific conditions can affect performance.
- **Incomplete Degradation:** In some cases, microorganisms may not fully degrade contaminants, leading to partial breakdown and accumulation of intermediate products, which may still pose environmental risks.

Heavy Metal Pollution and Its Environmental Impact

Heavy metal pollution is a critical environmental issue caused by the excessive release of toxic metals into the environment through industrial activities, mining, agriculture, and improper waste management. Common heavy metals like lead, mercury, arsenic, cadmium, and chromium are highly toxic even at low concentrations. Unlike organic pollutants, which can naturally degrade over time, heavy metals are persistent in the environment and accumulate in ecosystems.

The impact of heavy metal pollution is severe and multi-dimensional:

1. **Soil Contamination:** Heavy metals disrupt soil structure and nutrient balance, making the land less fertile. This contamination directly affects agricultural productivity and the health of plants.
2. **Water Pollution:** When heavy metals leach into water bodies, they harm aquatic organisms by interfering with biological processes, such as reproduction and growth. This contamination can also render drinking water unsafe for human consumption.
3. **Food Chain Disruption:** As heavy metals accumulate in plants and animals, they enter the food chain, leading to bioaccumulation and biomagnification. Predators at the top of the food chain, including humans, suffer the most severe consequences.
4. **Human Health Risks:** Heavy metals are known to cause long-term health problems, including neurological damage (from lead and mercury), kidney failure (from cadmium), and even cancer (from arsenic and chromium).
5. **Air Pollution:** Emissions from industries and vehicles release heavy metal particulates into the air. Inhalation can harm both humans and wildlife.

The persistence of heavy metals in the environment makes their removal a major challenge. Traditional methods, such as chemical treatments and soil excavation, are often expensive, energy-intensive, and can create secondary pollution.

Bacteria: A Sustainable and Eco-Friendly Solution

Bacteria provide a natural and innovative solution to combat heavy metal pollution. These microorganisms, found in diverse environments, possess remarkable abilities to absorb, transform, and detoxify heavy metals. The use of bacteria in environmental cleanup is known as bioremediation.

How Bacteria Work in Heavy Metal Degradation:

- **Biosorption and Bioaccumulation:** Bacteria can adsorb heavy metals onto their cell surfaces or accumulate them intracellularly. This process involves binding metals through cell wall components or intracellular sequestration, effectively reducing metal bioavailability in the environment. **(Ali H.et.al.2019)**
- **Enzymatic Transformation:** Certain bacteria possess enzymes capable of altering the oxidation states of heavy metals, rendering them less toxic.

  For example, Thiobacillus ferrooxidans and T. thiooxidans can enzymatically oxidize uranium, facilitating its immobilization. **(Noroozi M.,et.al.2017)**
- **Siderophore Production:** Bacteria produce siderophores—iron-chelating compounds—that enhance metal mobility and reduce bioavailability.

  This process aids in the removal of metals like cadmium and zinc from contaminated soils. **(Ahemad M.et.al.2012)**
- **Biofilm Formation:** Biofilms, which are communities of bacteria embedded in a self-produced extracellular matrix, can sequester heavy metals.
- This collective behavior enhances the stability and efficiency of metal immobilization. **(Dade- Robertson, et.al.2017)**
- **Efflux Mechanisms:** Some bacteria have developed efflux systems that actively transport heavy metals out of their cells, thereby reducing intracellular toxicity and contributing to environmental detoxification.

A Step Towards Sustainable Environmental Management

Incorporating bacteria into heavy metal degradation represents a significant advancement in environmental science. It not only addresses the urgent need to manage pollution but also aligns with global efforts to promote sustainability.

By leveraging the natural capabilities of bacteria, we can reduce environmental risks, safeguard ecosystems, and protect human health while minimizing reliance on harmful industrial methods.

This eco-friendly approach not only tackles today's pollution challenges but also paves the way for innovative and sustainable solutions in environmental protection.

Heavy Metals, Their Toxic Effects, and Bacterial Degradation in Detail

Heavy metals are naturally occurring elements that have elevated concentrations, and can have significant toxic effects on living organisms and the environment.

Here's a detailed look at specific heavy metals, their impacts, and how bacteria help degrade or neutralize them:

1. **Lead (Pb) Toxic Effects:** Neurotoxin affects brain development, especially in children. Causes anemia, kidney damage, and high blood pressure in adults. Interferes with calcium metabolism, weakening bones.
   Bacterial Degradation: Bacillus subtilis and other Bacillus species precipitate lead as lead phosphate or lead sulfide. These bacteria immobilize lead, reducing its mobility in soil and water.

2. **Mercury (Hg)**
   Toxic Effects- Affects the nervous system, causing memory loss, tremors, and developmental disorders. Methylmercury accumulates in fish and enters the human food chain, leading to bioaccumulation.

   Bacterial Degradation: Pseudomonas and other mercury-resistant bacteria use mercury reductase enzymes to convert toxic Hg^{2+} into elemental mercury (Hg^0), which is less toxic and volatile.

   These bacteria can operate in aquatic environments to mitigate mercury contamination.

3. **Cadmium (Cd) Toxic Effects:** Damages kidneys, weakens bones (osteomalacia), and disrupts cellular metabolic pathways. Known to cause lung cancer with chronic exposure.

   Bacterial Degradation: Sulfate-reducing bacteria (Desulfovibrio vulgaris) precipitate cadmium as insoluble cadmium sulfide. Bacteria in aquatic ecosystems help immobilize cadmium, preventing leaching into groundwater.

4. **Chromium (Cr)**
   Toxic Effects: Chromium (VI) is a potent carcinogen causing lung cancer, skin irritation, and DNA damage. Enter water systems through industrial effluents, posing risks to humans and wildlife.

- **Bacterial Degradation:**
- Shewanella reduces Cr(VI) to Cr(III), a less soluble and less toxic form.
- Chromium transformation occurs enzymatically under anaerobic conditions.

5. **Arsenic (As) Toxic Effects:** Leads to skin lesions, cardiovascular issues, neurotoxicity, and cancers of the bladder and lungs. Contaminate groundwater, affecting millions worldwide.

   Bacterial Degradation: Arsenic-oxidizing bacteria like Pseudomonas aeruginosa convert toxic arsenite (As^{3+}) to less toxic arsenate (As^{5+}).
   Sulfate-reducing bacteria immobilize arsenic by forming stable arsenic sulfide compounds.

6. **Nickel (Ni) Toxic Effects:** Prolonged exposure causes dermatitis, lung cancer, and organ damage. Toxic in aquatic environments, harming fish and amphibians.

   Bacterial Degradation: Ralstonia bioaccumulates nickel, storing it in intracellular compartments. Nickel-tolerant bacteria reduce its mobility and toxicity in contaminated soils.

7. **Zinc (Zn) Toxic Effects:** Excess zinc interferes with the uptake of other essential metals like iron and copper. Cause nausea, vomiting, and impaired immune functions

   Bacterial Degradation: Certain bacteria bioaccumulate zinc, reducing its bioavailability. While degradation is limited, immobilization by bacteria stabilizes zinc in the environment.

8. **Copper (Cu) Toxic Effects:** Toxic in high doses, causing liver and kidney damage. Interferes with cellular enzymes and DNA repair processes.

   Bacterial Degradation: Cupriavidus sequesters copper ions and precipitates them as copper oxides. Bacteria protect ecosystems by reducing free copper concentrations.

9. **Aluminum (Al) Toxic Effects:** Linked to neurodegenerative diseases like Alzheimer's.Affects root growth in plants, reducing agricultural productivity.

   Bacterial Degradation: Although not actively degraded, bacteria like Pseudomonas bind and immobilize aluminum, limiting its impact on plants.

10. **Cobalt (Co) Toxic Effects:** Excess cobalt leads to cardiomyopathy, thyroid dysfunction, and genotoxic effects. Toxic to aquatic life and disrupts cellular redox balance.
    Bacteria degradation: Acinetobacter species bioaccumulate cobalt, storing it within their cells. These bacteria help stabilize cobalt in contaminated ecosystems.

***Bacillus cereus*:** Heavy metals are a group of metals and semi-metals that can be identified by their high density and typically harmful characteristics.
The most significant environmental worry among all the heavy metals is the dangerously elevated levels of Cd, Pb ,Cr which are trash elements that are totally useless for living things.
Microorganisms have a variety of methods such as Biosorption, Bioaccumulation, Bioprecipitation, Extracellular polymeric substances, Enzymes reduction for eliminating environmental heavy metals.
Bacillus uses three main methods to remove heavy metal: **Biosorption, Bioaccumulation and Bioprecipitation.**
Bacillus cereus is a versatile soil bacterium, has garnered attention for its ability to remediate heavy metal- contaminated environments.

- **Mechanism of *Bacillus cereus* :**
- **Biosorption: *Bacillus cereus*** can adsorb heavy metal ions onto its cell surface through interactions with functional groups like carboxyl, hydroxyl, and amino groups. This passive process reduces metal bioavailability and toxicity.

Below are the detailed steps of the biosorption mechanism:

- **Adsorption to Cell Surface:**

The bacterial cell wall of B. cereus is rich in functional groups such as carboxyl (-COOH), hydroxyl (-OH), amino (-NH2). These groups interact with metal ions through:

- **Ion exchange:** Metal ions replace lighter ions like H^+ or Na^+ already bound to these groups.

Electrostatic attraction: Negatively charged groups on the cell surface attract positively charged metal ions.

- **Metal Speciation:**

The type of metal ion and its speciation (e.g., Pb^{2+}, Cd^{2+}, or Cr^{6+}) influences the adsorption capacity.

Chromium, for instance, may undergo reduction to a less toxic form (Cr^{3+}), which precipitates on the surface.

- **Role of Cell Wall Polymers:**

The peptidoglycan layer of Gram-positive bacteria like B. cereus is a key component in biosorption.

Exopolysaccharides (EPS) produced by B. cereus enhance biosorption by providing additional binding sites.

- **Non-Metabolic Binding:**

Biosorption is primarily a passive process independent of the bacterium's metabolic activity.

It depends on the physicochemical properties of the cell surface and environmental factors such as pH, temperature, and ionic strength.

- **Multilayer Adsorption:**

At higher metal concentrations, biosorption may involve multilayer adsorption, where ions bind to previously adsorbed metal ions, forming layers.

Bioaccumulation mechanism used by Bacillus cereus to remove heavy metals like lead (Pb), cadmium (Cd), and chromium (Cr)

- **Initial Contact:**

Heavy metal ions in the environment come into contact with the bacterial cell surface. Passive diffusion or facilitated transport brings metal ions near the cell membrane.

- **Metal Ion Uptake via Transport Systems:**

Specific metal transport proteins or channels in the bacterial membrane actively or passively transport metal ions into the cell.

Active transport: Uses energy (ATP or ion gradients) to bring metal ions into the cell against their concentration gradient.

Passive transport: Facilitated diffusion occurs when metal ions move along their concentration gradient.

- **Intracellular Binding:**Once inside the cell, metal ions are bound to intracellular components to reduce their toxicity.

  Metallothioneins: Small proteins with high affinity for heavy metals bind and sequester these ions.

  Polyphosphates: Serve as storage molecules, binding metal ions to limit their free concentration.

- **Detoxification through Reduction:**Some metals, like chromium, undergo enzymatic reduction. For example, Cr^{6+} (toxic) is reduced to Cr^{3+} (less toxic), which is less reactive and easier to manage intracellularly.

- **Compartmentalization:**
  The bacterium may sequester metals into specific organelles or vesicles to isolate them from vital cellular processes, thereby minimizing damage.
- **Bioprecipitation is the process by which microorganisms convert soluble heavy metal ions into insoluble forms through metabolic or enzymatic activities, resulting in the removal of these metals from contaminated environments.**

Here's how Bacillus cereus achieves bioprecipitation:

- **Metal Ion Contact:**

Soluble heavy metal ions (e.g., Pb^{2+}, Cd^{2+}, Cr^{6+}) interact with the bacterial cell surface.

The bacterium initiates specific biochemical processes to convert these ions into less soluble or non - soluble forms.

- **Enzymatic Reduction (for Redox-Active Metals):**

Bacillus cereus produces enzymes such as reductases to transform metal ions into a less toxic and less soluble state:

Example: Hexavalent chromium (Cr^{6+}), a highly toxic and soluble form, is reduced to trivalent chromium (Cr^{3+}), which precipitates as insoluble chromium hydroxide [$Cr(OH)_3$].

- **pH Modulation:**

The bacterium can modify the local pH by secreting metabolites like ammonia or organic acids.

Changes in pH promote the precipitation of metals as hydroxides, carbonates:

Lead (Pb^{2+}): Forms insoluble lead carbonate ($PbCO_3$). Cadmium (Cd^{2+}): Precipitates as cadmium sulfide (CdS).

- **Interaction with Extracellular Polymeric Substances (EPSON):** Bacillus cereus secretes extracellular polymeric substances, which provide nucleation sites for metal ion aggregation and precipitation.
- **Formation of Metal Precipitates:**

The combined effects of enzymatic activity, pH alteration, and EPS production lead to the conversion of soluble metal ions into insoluble precipitates.

These precipitates settle in the environment, effectively removing heavy metals from the aqueous phase.

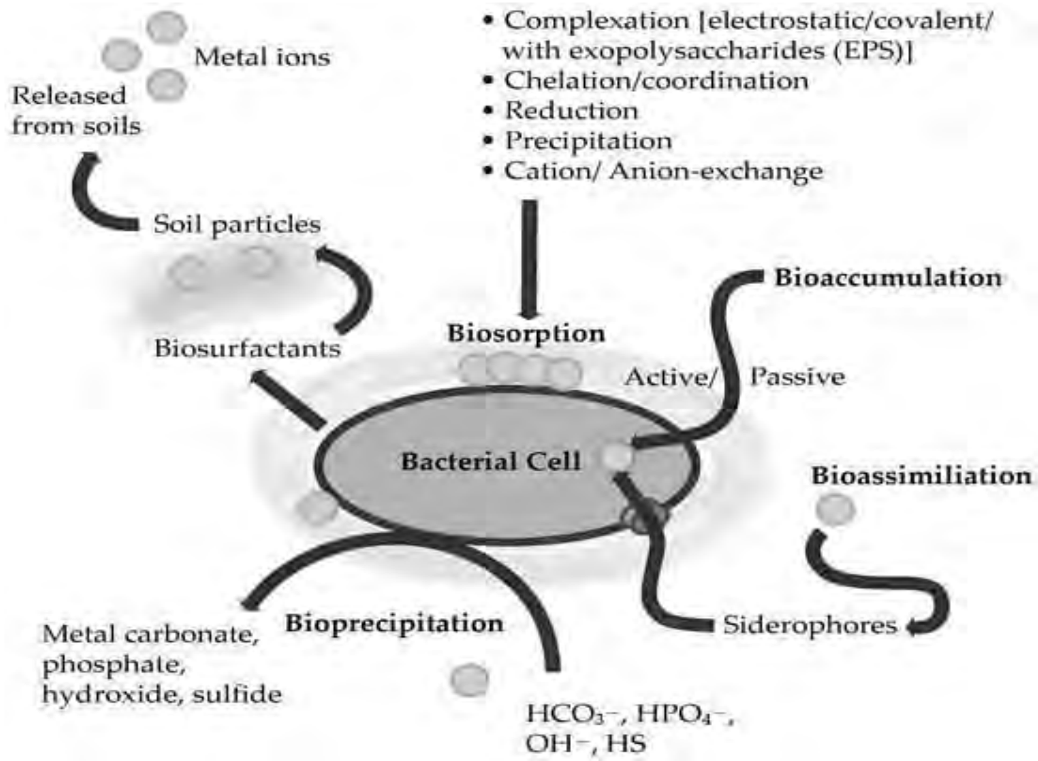

Figure1: Remove heavy metal by bacteria (*Bacillus cereus*)

Advantages

- **High Efficiency in Metal Removal Broad Spectrum:** B. cereus can remove various heavy metals, including lead (Pb^{2+}), cadmium (Cd^{2+}), mercury (Hg^{2+}), chromium (Cr^{6+}), arsenic (As^{3+}), and nickel (Ni^{2+}).
- **Multiple Mechanisms:** It employs biosorption, extracellular polymeric substance (EPS)-mediated biosorption, bioaccumulation, and bioprecipitation to effectively reduce metal concentrations in the environment.
- **Cost-Effectiveness:** B. cereus can be cultivated using simple and inexpensive nutrients, making it a cost-effective option for bioremediation efforts.
- **Environmental Friendliness:** Utilizing B. cereus for heavy metal removal avoids the introduction of harmful chemicals, reducing the risk of secondary pollution.
- Biodegradability: The by-products of B. cereus activity are generally non-toxic and biodegradable, contributing to environmental sustainability.
- **Adaptability to Diverse Environments:** Wide pH and Temperature Range: B. cereus can function across various environmental conditions, enhancing its suitability for field applications.
- Resilience: Certain strains of B. cereus demonstrate resistance to high metal concentrations, maintaining effectiveness in contaminated areas.
- **Potential for Genetic Improvement:** Genetic Engineering: Advancements in recombinant DNA technology can enhance B. cereus's metal-binding capacity, tolerance to extreme conditions, and production of biosurfactants or EPS, improving its bioremediation efficiency.
- **Soil and Plant Health Improvement:** Nutrient Mobilization: B. cereus improves soil quality by enhancing nutrient availability, aiding plant growth in contaminated areas.
- Reduction in Metal Uptake by Plants: It helps prevent heavy metal toxicity in crops by reducing metal bioavailability in the soil.
- **Wastewater and Industrial Effluent Treatment:** Effective Treatment: B. cereus strains have been employed to treat industrial effluents containing heavy metals, effectively reducing metal concentrations in wastewater.

Limitations

- **Metal-Specific Efficacy:** B.cereus shows proficiency in reducing certain metals, its effectiveness varies across different heavy metals.
- B. cereus strains achieved up to 99.9% removal of Cr(VI) in 24 hours under optimal conditions, indicating high efficiency for chromium.
- However, the removal rates for other metals like lead and cadmium may not be as high, suggesting metal-specific limitations.
- **Environmental Condition Sensitivity:** The bacterium's metal removal efficiency is influenced by environmental factors such as pH and temperature.
- Optimal chromium reduction by B. cereus occurs at neutral pH and temperatures around 37°C.
- Deviations from these conditions can significantly reduce its bioremediation capabilities, limiting its application in diverse environmental settings.
- **Toxicity Thresholds:** High concentrations of heavy metals can be toxic to B. cereus, inhibiting its growth and metabolic functions.
- Although some strains tolerate elevated metal concentrations, there exists a threshold beyond which the bacterium's viability and remediation efficiency decline, posing a challenge in heavily contaminated sites.
- **Potential Pathogenicity:** Certain strains of B. cereus are opportunistic pathogens, raising concerns about their safe application in environmental bioremediation.
- The risk of introducing pathogenic strains into the environment necessitates careful selection and assessment of B. cereus strains used for bioremediation purposes.

Future Prospects with rDNA Technology

- **Genetic Engineering:** Introducing genes responsible for metal resistance and accumulation from other organisms could bolster the bacterium's ability to withstand and sequester higher metal concentrations.
- **Pathway Optimization:** Modifying metabolic pathways to increase the production of EPS or other metal-binding compounds can improve metal sequestration efficiency.
- **Regulatory Considerations:** While genetic modifications hold promise, considerations regarding the release of genetically engineered microorganisms into the environment must be addressed to ensure ecological safety.

Pseudomonas aeruginosa **:**

Heavy metals are widely distributed elements with a large atomic weight, density, and numerous uses in various industries. Environmental issues affecting humans, animals, plants, and ecosystems are caused by heavy metal contamination.

Various heavy metals accumulated in the soil and water as a result of this contamination. Following their release into natural sources and excessive accumulation, several heavy metals, including copper, cadmium, nickel, lead, chromium, mercury, and others, have detrimental effects on human health and the biological system.

Biomineralisation, biosorption, phytostabilization, hyper- accumulation, bio-stimulation, mycoremediation, cyano-remediation, and geno-remediation are some of the biotechnological techniques used to eliminate these heavy metals.

In the natural world, microbes have the ability to recycle and break down accumulated heavy metals, reducing their toxicity.

Microorganisms such as bacteria, fungus, and algae were identified. Because of its high efficacy, low cost, and environmentally friendly nature, microorganism- based bioremediation of heavy metals is also a cost-effective and efficient method.

Furthermore, the buildup and detoxification of heavy metals is accomplished through microbial bioremediation, which involves the interaction of microbes and metals.

Different bacterial strains have distinct ways of adapting to and eliminating heavy metals from contaminated settings.

Bacterial strains have adapted to grow at high concentrations of heavy metals through a variety of ways. Additional mechanisms include metal sorption, enzymatic reduction or oxidation, accumulation and absorption, and extracellular precipitation.

Additionally, resistant genes were used to achieve metal tolerance through molecular methods.

Bacterial strains resistant to heavy metals can thrive in environments with high concentrations of these metals, and they may be useful in bioremediation of soils with high concentrations of heavy metals.

Lastly, when compared to other bacterial strains, ***Pseudomonas aeruginosa*** was able to tolerate and eliminate the largest amount of heavy metals.

***Pseudomonas aeruginosa* employs several mechanisms to remove and tolerate heavy metals, including:**

- **Biofilm formation:** P. aeruginosa forms biofilms—structured communities of bacteria encased in extracellular polymeric substances (EPS).
- These biofilms can sequester heavy metals, reducing their toxicity. The EPS matrix binds metal ions, preventing them from penetrating and harming bacterial cells.
- Studies have shown that biofilm-associated P. aeruginosa exhibits increased resistance to heavy metals like copper, lead, and zinc compared to planktonic (free-swimming) cells.
- **Siderophore production:** ***P. aeruginosa*** produces siderophores— molecules that bind and sequester metal ions.
- This process reduces the availability of free metal ions in the environment, thereby decreasing their toxicity.
- Research indicates that exposure to heavy metals such as cadmium and zinc can enhance siderophore production in P. aeruginosa, contributing to its metal tolerance.
- **Antioxidant Enzyme Activity:** The bacterium increases the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) in response to heavy metal stress.
- These enzymes mitigate oxidative damage caused by metal-induced reactive oxygen species, enhancing the bacterium's survival in contaminated environments.
- **Biosorption and Bioaccumulation:** P. aeruginosa can adsorb and accumulate heavy metals from its environment.
- This capability makes it a potential candidate for bioremediation efforts aimed at removing heavy metals from contaminated water and soil.
- Studies have demonstrated its effectiveness in biosorption metals like cadmium, chromium, nickel, and zinc.

- **Efflux Systems:** The bacterium possesses efflux pumps that actively transport toxic metal ions out of the cell, thereby reducing intracellular metal concentrations and mitigating toxicity.
- These systems are crucial for maintaining metal homeostasis within the bacterial cell.

Pseudomonas aeruginosa offers several advantages in the bioremediation of heavy metals:

- **High Biosorption Capacity:** Certain strains of P. aeruginosa can tolerate lead concentrations exceeding 50 mg/mL and exhibit biosorption efficiencies ranging from approximately 80.9% to 87% when using live biomass.
- **Exopolysaccharide (EPS) Production:** The bacterium produces EPS, which plays a crucial role in heavy metal sequestration. EPS can bind metal ions, reducing their bioavailability and toxicity.
- **Siderophore Production:** P. aeruginosa produces siderophores, such as pyoverdine, which can chelate heavy metals, facilitating their removal from contaminated environments.
- **Biofilm Formation:** The bacterium's ability to form biofilms enhances its survival in contaminated environments and contributes to the immobilization and removal of heavy metals.
- **Metabolic Versatility:** P. aeruginosa can utilize a wide range of organic compounds as carbon and energy sources, enabling it to thrive in diverse environments, including those contaminated with heavy metals.
- **Genetic Adaptability:** The bacterium's genetic plasticity allows it to develop resistance to various heavy metals, enhancing its effectiveness in bioremediation efforts.

Several limitations and Challenges

- **Limited Metal Tolerance Range:** Although P. aeruginosa can tolerate certain heavy metals, its resistance levels vary depending on the metal type and concentration.
- For instance, some isolates can withstand lead concentrations exceeding 50 mg/mL, but their tolerance to other metals like mercury or cadmium may be lower.

- **Antibiotic Resistance Concerns:** Studies have indicated that heavy metal-resistant P. aeruginosa strains may also exhibit resistance to multiple antibiotics.
- This co-resistance poses a risk of transferring antibiotic resistance genes to pathogenic bacteria, complicating treatment options.
- **Environmental Adaptability:** The effectiveness of P. aeruginosa in bioremediation is influenced by environmental factors such as pH, temperature, and the presence of other contaminants.
- These variables can affect the bacterium's survival, growth, and metal uptake efficiency.

- **Incomplete Metal Removal:** P. aeruginosa may not completely remove all heavy metals from contaminated sites, especially at high concentrations.
- This limitation necessitates the integration of additional treatment methods to achieve desired remediation levels.
- **Potential Pathogenicity:** As an opportunistic pathogen, the use of P. aeruginosa in bioremediation raises biosafety concerns.
- Unintended release into the environment could pose health risks to humans and animals, necessitating stringent containment and monitoring measures.

Future prospects

- **Exploitation of Marine Resources:** Exploring marine-derived P. aeruginosa strains could yield biosurfactants with unique properties, potentially improving heavy metal removal efficiency in saline environments.
- **Economic Production Methods:** Developing cost-effective production processes, such as solid-state fermentation using agro-industrial waste, can facilitate the large-scale application of rhamnolipid biosurfactants in bioremediation efforts.
- **Broader Applications:** Beyond heavy metal removal, rhamnolipid biosurfactants have potential uses in hydrocarbon bioremediation, antibiofilm activity, and the green synthesis of nanoparticles, indicating a versatile role in environmental biotechnology.

Plastic Waste Definition

Plastic waste refers to discarded plastic materials that are no longer in use and have been disposed of by consumers or industries.

These materials, due to their synthetic composition, resist natural degradation processes, leading to their accumulation in the environment. This persistence poses significant environmental challenges.

The environmental impact of plastic waste is profound. Improper disposal and accumulation of plastic waste can lead to environmental pollution, affecting ecosystems and human health. The challenges posed by plastic waste requires comprehensive management strategies.

These include recycling, proper waste management, and the development of biodegradable alternatives.

Implementing effective plastic waste management practices is essential for mitigating environmental pollution and promoting sustainability. **(Ilyas.et.al.2018)**

Plastic waste is characterized by:

1. Plastic waste encompasses discarded plastic products that are no longer in use, such as single-use items and packaging materials. Due to their non-biodegradable nature, these plastics persist in the environment, leading to significant pollution.
2. Improper disposal of plastics into oceans and lands endangers marine species and subsequently human lives.
3. The environmental impact of plastic waste is profound. Plastics are fossil fuel-derived products, and the emissions of greenhouse gases during different processes involved in the life cycle of plastic-related products are a significant threat to the environment as they contribute to global temperature rise.
4. By 2050, a high volume of plastic production will be responsible for up to 13% of our planet's total carbon budget.
5. Furthermore, plastic waste can lead to health issues in humans. Due to their chemical structures and prolonged degradation periods, plastic waste can lead to cancer, nervous system damage, rapid genetic changes, and metabolic disorders in humans.
6. In summary, plastic waste, characterized by discarded products like single-use plastics and packaging materials, poses significant environmental and health challenges.
7. Its persistence in the environment leads to pollution, adversely affecting marine life and habitats, and contributes to climate change through greenhouse gas emissions.
8. Additionally, the degradation of plastics can result in health issues for humans, underscoring the need for effective waste management and reduction strategies.

(Shams.et.al..2021)

Proper management of plastic waste is crucial to mitigate its harmful effects on the environment and public health:

- **Sources of Plastic Pollution-**

  Single-use plastics: Items like bags, bottles, straws, and packaging are major contributors.

  Industrial waste: Factories often release plastic waste into the environment. Improper waste management: Poor systems lead to plastic entering natural ecosystems.

- **Environmental Impact-**

  Land Pollution: Plastics degrade very slowly, often taking hundreds of years, resulting in accumulation on land.

Water Pollution: Plastics end up in rivers, lakes, and oceans, affecting marine life and ecosystems.

Air Pollution: Burning plastic releases toxic chemicals like dioxins, affecting air quality.

- **Human Health Risks-**Microplastics, tiny plastic particles, enter the food chain and may pose health risks to humans, including hormone disruption and exposure to toxic substances.
- **Economic Consequences-**Costs of cleaning polluted areas are significant.Plastic pollution negatively impacts industries like tourism and fisheries.

Bacteria- A Sustainable and Eco-Friendly Solution:

- The decomposition of plastic can be achieved sustainable and environmentally by using bacteria's related metabolic mechanisms to break down man - made polymers. Polyethylene is the target of Rhodococcus ruber and Kocuria palustris, whereas PET can be broken down by marine bacteria such Ideonella sakaiensis.
- After forming biofilms on plastic surfaces, these bacteria release enzymes that break down polymer chains into smaller, easier-to-manage molecules, which are then absorbed for energy.
- Comparing this biological approach to conventional disposal techniques, it not only minimises the environmental impact but also minimises plastic waste, supporting a circular economy in plastics management.

How bacteria work in degraded plastic:

The unique ability of the bacterium ***Ideonella sakaiensis*** **201-F6** degrade to metabolise polyethylene terephthalate (PET), a common type of plastic used in the production of packaging materials(food packaging, bottle packaging etc), has made it well-known.

In 2016, scientists from **Japan's Kyoto** Institute of Technology and Keio University isolated this bacteria for the first time. It was discovered in wastewater and soil at a PET recycling plant.

Since PET is a synthetic polymer that was just introduced around 70 years ago, its environment probably encouraged the creation of enzymes that can break it down.

Research has been done on the various methods for recycling or treating PET. For instance, the total PET recycling rate increased as a result of chemical or mechanical operations for PET degradation or separation**.(Sinha V,et al.2010)**

Nowadays, the majority of PET waste that cannot be recycled is burned, which releases a lot of CO2 and other air pollutants.

Attention has been focused on biological solutions for plastic waste. Multiple enzymes and metabolic pathways must be used for plastic degradation to occur.**(Kong HG.et al..2019)**

Mechanism of Polyethylene Terephthalate (PET) Degradation by (*Ideonella sakaiensis* 201-F6):

***Ideonella sakaiensis* 201-F6** is a bacterium discovered in 2016 that can degrade polyethylene terephthalate (PET) into its monomeric components.

The degradation process involves two key enzymes, PETase and MHETase, which work sequentially to break down PET.**(Yoshida, S.,et al..2016)**

Below is the step-by-step process: Adhesion to PET Surface:I. sakaiensis attaches to the PET surface using its cell surface proteinsThis adhesion is critical for positioning the bacterium to secrete enzymes directly onto the polymer.

Secretion of PETase

- The bacterium produces and secretes the enzyme PETase (polyethylene terephthalate hydrolase).
- PETase binds to the PET polymer and hydrolyzes it into smaller fragments, primarily mono(2-hydroxyethyl) terephthalate (MHET), with some terephthalic acid (TPA) and bis(2- hydroxyethyl) terephthalate (BHET) as by-products.
- **Intermediate Product Formation:**
- PETase breaks the ester bonds in PET, converting the polymer into water-soluble intermediates.
- The primary intermediate is MHET, while small amounts of TPA and ethylene glycol (EG) may also be released. **Hydrolysis by MHETase:**
- The MHETase enzyme, also secreted by I. sakaiensis, acts on MHET molecules.
- MHETase hydrolyzes MHET into terephthalic acid (TPA) and ethylene glycol (EG).

Assimilation and Metabolism:

- The bacterium absorbs TPA and EG into its cells.
- EG is metabolized via glycolysis, while TPA undergoes catabolism through specific pathways to provide energy and carbon for growth.

By-Product Utilization:

- The final products, TPA and EG, are fully metabolized, leaving no harmful residues in the environment.

- This complete degradation ensures minimal ecological impact.

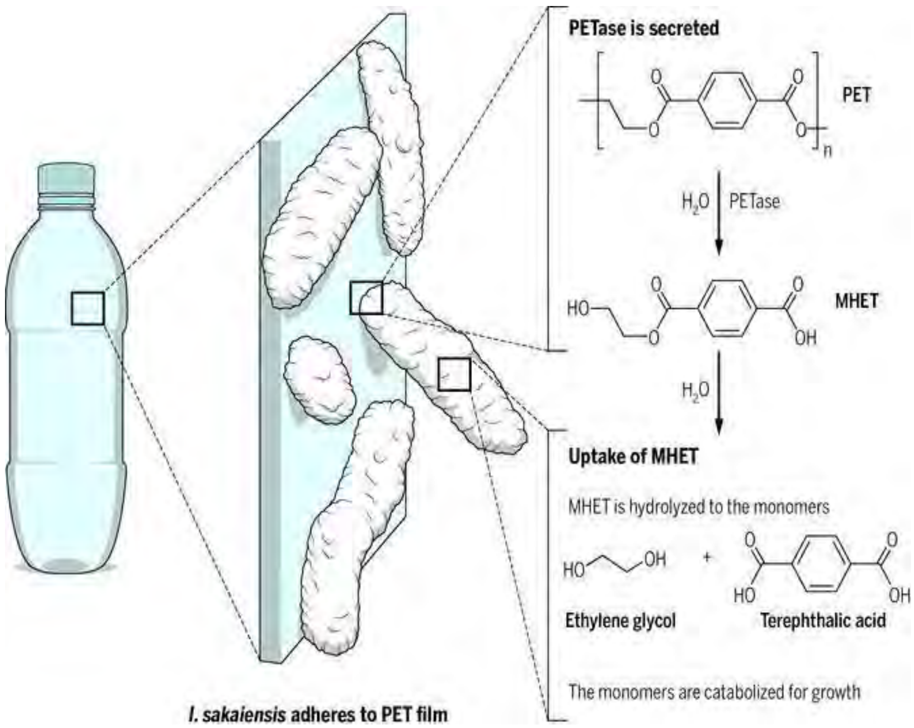

Figure2: plastic eating bacteria (Ideonella sakaiensis 201-F6)

Advantages

- Efficient PET Degradation: I. sakaiensis utilizes two enzymes, PETase and MHETase, to break down PET into environmentally benign substances, serving as its primary food source.
- Potential for Industrial Application: The bacterium's PET-degrading capabilities offer promising avenues for recycling and bioremediation, potentially reducing plastic waste in the environment.
- Genetic Engineering Opportunities: Research has shown that modifying the PETase enzyme can enhance its activity and thermostability, paving the way for more efficient PET biodegradation processes.

Limitations

- **Thermal Instability:** The PETase enzyme from I. sakaiensis exhibits low thermal stability, limiting its activity to moderate temperatures. This instability poses challenges for industrial applications that often require higher operational temperatures. **(da Costa,et.al.2021).**
- **Concentration-Dependent Inhibition:** Studies have shown that PETase experiences concentration-dependent inhibition during PET hydrolysis.
- This means that at higher enzyme concentrations, its activity diminishes, which can hinder large-scale applications. **(Avilan, L.et.al.2023).**
- **Limited Operational Stability and Reusability:** Free PETase lacks operational stability and cannot be reused effectively, which is a significant drawback for practical applications. **(Lee.et.al.2024)**
- **Sensitivity to Environmental Conditions:** The enzyme's activity is influenced by environmental factors such as pH and temperature, which can affect its overall performance in diverse settings.**(De Jesus.el.at.2023)**

Future prospects:

- **Enzyme Engineering for Enhanced PET Degradation:** Advancements in in-silico guided engineering are paving the way for the development of more efficient PET-degrading enzymes.
- By utilizing computational tools and machine learning, researchers aim to create enzyme variants with improved activity and stability, thereby enhancing the biodegradation process. **(Jayasekara.et.al.2023)**

- **Genetic Modification for Industrial Applications:** Efforts are underway to genetically modify the PETase enzyme from I. sakaiensis to increase its thermal stability and degradation efficiency.
- A combinatory approach involving structure-based rational design and machine learning has led to the creation of a PETase variant with a melting temperature of 70 °C and optimal performance at 60 °C, making it more suitable for industrial applications. **(Joho.et.al.2024)**
- **Exploration of Alternative Chassis for PET Degradation:** Researchers are investigating the use of marine microalgae as alternative hosts for PET-degrading enzymes.
- By integrating PETase into the diatom Phaeodactylum tricornutum, a promising and eco-friendly solution for biological decomposition of PET waste in saltwater environments has been demonstrated. **(Moog.et.al.2019)**

- **Biodegradation of Commercial PET Variants:** Studies have shown that I. sakaiensis can biodegrade different commercial PET materials obtained from food packaging containers.
- Over a seven-week period, significant degradation of plastic pieces with varying crystallinity was observed, indicating the bacterium's potential for practical applications in managing PET waste. **(Walter.et.al.2022)**
-

***Scientists have found another bacteria (*pseudomonas putida* KT2440) which plays a significant role in improving plastic pollution.**

***Pseudomonas putida* KT2440** : Many scientists apply this technique using the soil bacterium ***p.putida*KT2440** which is in contrast to ***Ideonella sakaiensis* 201-F6** because of its ability to grow robustly in challenging environments and its extensive genetic engineering tool and knowledge base, it has been recognised as a promising host for industrial biocatalytic processes. (**Poblete-CastroI.et.al.,2012**).It is well-known for its ability to metabolize various organic compounds, including pollutants and hydrocarbons.
This metabolic versatility makes it a prime candidate for environmental bioremediation, including plastic degradation.

Mechanism of *Pseudomonas putida* KT2440:The degradation of polyethylene terephthalate (PET) by Pseudomonas putida KT2440 involves several key steps**.**

- **PET Depolymerization:**Enzymatic Hydrolysis**:** P. putida KT2440 can be engineered to secrete PET hydrolases, such as leaf-branch compost cutinase (LCC), which hydrolyze PET into its monomers, terephthalic acid (TPA) and ethylene glycol (EG).**(Liu.et.al 2022)**

- **Monomer Uptake:** Transport into the Cell: The monomers TPA and EG are transported into the bacterial cell through specific transport systems. **(Brandenburg.et.al.2022)**
- **TPA Metabolism:** Conversion to Protocatechuic Acid: Inside the cell, TPA undergoes a series of reactions to be converted into protocatechuic acid.

  Ring Cleavage: Protocatechuic acid is then subjected to ring-cleavage pathways, leading to the formation of intermediates that enter the tricarboxylic acid (TCA) cycle.
- **EG Metabolism:** Oxidation to Glycolaldehyde: EG is oxidized to glycolaldehyde by enzymes such as alcohol dehydrogenases. **(Li.et.al.2020).** Further Oxidation to Glycolate: Glycolaldehyde is further oxidized to glycolate.

  Conversion to Glyoxylate: Glycolate is then converted to glyoxylate. Entry into Central Metabolism: Glyoxylate can be further metabolized, eventually feeding into the TCA cycle.

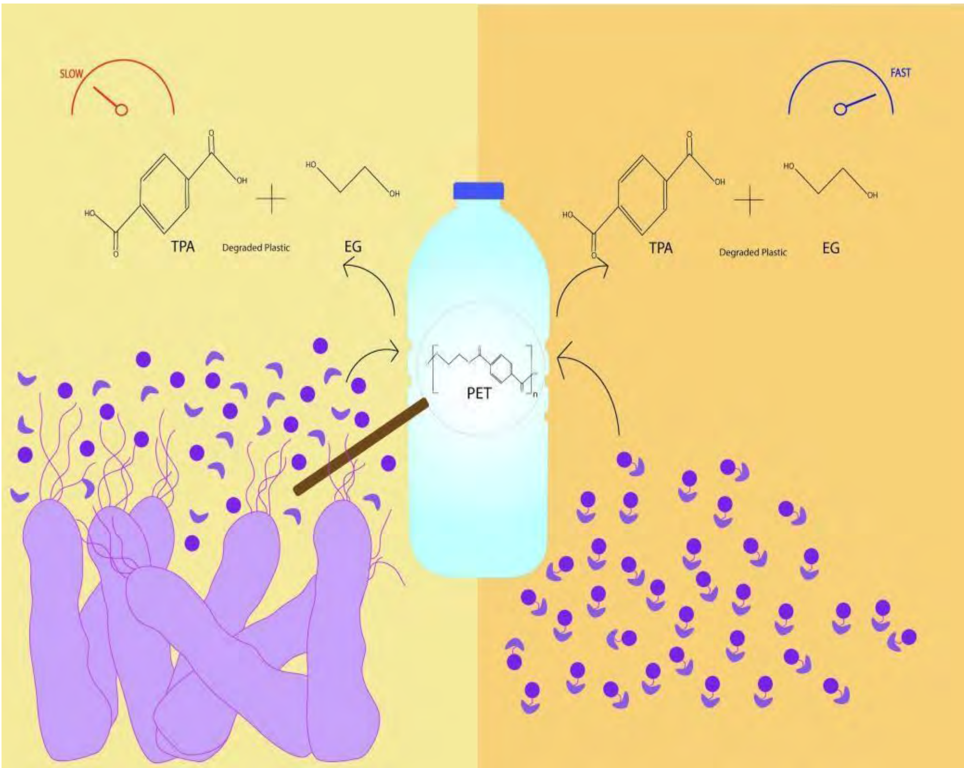

Figure3: plastic degrade by Pseudomonas putida KT2440

Advantages:

Wide Metabolic Versatility: One of the major advantages of Pseudomonas putida is its metabolic flexibility. It has an extensive range of enzymes that enable it to degrade hydrocarbons, aromatic compounds, and recalcitrant polymers.

Research shows that P. putida can break down polyethylene terephthalate (PET), polystyrene, and other plastics into simpler compounds, which can subsequently be used as carbon and energy sources.

- For example, P. putida KT2440 has been engineered to produce enzymes like monooxygenases and dioxygenases, which assist in breaking down complex polymers. **(Wilkes & Aristilde, 2017).**
- **Production of Enzymes for Plastic Breakdown:** Pseudomonas putida secretes specific enzymes that target plastic polymers.
- Enzymes such as esterases, hydrolases, and laccases act on plastics like PET, reducing their molecular weight and facilitating their biodegradation.
- A study by **(Kundungal et al. 2019)** demonstrated that P. putida effectively degraded polystyrene into intermediate compounds such as styrene oil and carbon dioxide.
- This ability is linked to the enzyme-mediated cleavage of carbon-carbon bonds within the polymer chain.
- **Adaptability to Harsh Environments:** P. putida is well- adapted to thrive in various harsh environments, including soil, wastewater, and marine ecosystems.
- Its ability to tolerate extreme pH, temperature, and oxidative stress makes it suitable for plastic degradation in polluted areas.
- For instance, Pseudomonas putida can survive and function efficiently under low-oxygen conditions, which are common in landfills and marine environments where plastic accumulation occurs. **(Vijgen et al., 2020)**

  Genetic Engineering Potential: Genetic modification has further enhanced the plastic degradation ability of Pseudomonas putida.

  Through recombinant DNA technology, scientists have introduced genes encoding plastic-degrading enzymes from other bacteria or fungi into P. putida.

- For example, Pseudomonas putida strains have been engineered to produce PETase, an enzyme derived from Ideonella sakaiensis, to degrade PET plastic more efficiently. **(Tiso et al., 2020)**
- **Bioplastic Production as a Byproduct:** Another advantage is that Pseudomonas putida can convert plastic waste into bioplastics, such as polyhydroxyalkanoates

(PHAs). PHAs are biodegradable polymers that can replace conventional plastics, thus contributing to a sustainable circular economy.

(Research by Rosenboom et al. 2021) revealed that P. putida KT2440 could metabolize styrene, a breakdown product of polystyrene, into PHAs, demonstrating its dual role in plastic degradation and bioplastic production.

Non-Pathogenic Nature: Unlike other bacterial strains used in bioremediation, Pseudomonas putida is non-pathogenic and considered safe for environmental applications.

Its status as a biosafety level 1 (BSL-1) organism makes it a preferred choice for large-scale biotechnological processes.

- **LPotential for Scale-Up and Industrial Applications:** The robust nature of Pseudomonas putida and its ability to thrive in bioreactors makes it ideal for large-scale plastic waste treatment.
- It can efficiently degrade plastics in controlled conditions, and its metabolic pathways can be optimized for industrial applications through bioengineering.

Limitations

- **Slow Degradation Rate**
- Although Pseudomonas putida exhibits the ability to degrade plastics, the rate of degradation is relatively slow compared to chemical methods.
- The enzymatic processes involved in plastic breakdown are limited by factors such as substrate accessibility, enzyme efficiency, and environmental conditions.

For example, in the case of PET degradation, the microbial activity of P. putida is significantly slower than thermal or chemical methods, which limits its large-scale applications **(Kundungal et al., 2019).**

- **Limited Range of Plastic Degradation:** Pseudomonas putida can degrade specific types of plastics, such as polyethylene terephthalate (PET) and polystyrene, but it has limited efficiency with other commonly used plastics like polyethylene (PE) and polypropylene (PP).
- These plastics are highly resistant to enzymatic hydrolysis due to their inert and crystalline structures.

This limitation restricts the broad applicability of P. putida in tackling mixed plastic waste streams**. (Wilkes & Aristilde, 2017)**

- **Dependency on Environmental Conditions:** The plastic degradation efficiency of P. putida is highly influenced by environmental factors, including temperature, pH, nutrient availability, and oxygen levels. In harsh conditions such as extreme salinity or low-oxygen environments (e.g., deep ocean ecosystems), its activity is greatly reduced.

This dependency limits its application in uncontrolled natural environments, such as marine ecosystems or landfill sites **(Vijgen et al., 2020).**

- **Energy and Resource Requirement:** The metabolic pathways required for plastic degradation often demand significant energy and nutrients, which may not be readily available in contaminated environments.
- Scaling up these processes in industrial settings could require supplementation of carbon and nitrogen sources, increasing costs **(Tiso et al., 2020).**
- **Competition with Native Microorganisms:** In natural ecosystems, Pseudomonas putida faces competition from indigenous microbial populations.
- Native microbes may outcompete P. putida for substrates or produce inhibitory compounds, which can reduce its plastic degradation efficiency.
- **Potential for Genetic Drift:** Genetically engineered strains of Pseudomonas putida designed for enhanced plastic degradation may lose their engineered traits over successive generations due to genetic drift or mutations, especially in natural environments where selective pressures differ from laboratory conditions **(Rosenboom et al., 2021)**

Future Prospects:

- **Genetic Engineering for Enhanced Efficiency:** Advances in synthetic biology and metabolic engineering hold promise for enhancing the plastic
- degradation capabilities of Pseudomonas putida.
- Future research can focus on modifying its genome to: Improve the production and activity of plastic-degrading enzymes like PETase, hydrolase, and laccase.
- Introduce genes from other efficient plastic-degrading microorganisms, such as Ideonella sakaiensis, to expand its plastic degradation range.Increase tolerance to harsh environmental conditions for better survival and activity in contaminated environments**. (Tiso et al., 2020)**

- **Development of Co-Culture Systems:**

  a) Using microbial consortia, where Pseudomonas putida works synergistically with other plastic-degrading microorganisms, can significantly enhance degradation rates.

  b) For instance, combining P. putida with fungi like Aspergillus species can facilitate faster breakdown of plastics through combined enzymatic actions **(Kundungal et al., 2019).**

- **Optimization of Environmental Conditions:**

  Developing bioreactor systems that optimize temperature, pH, and oxygen levels can improve the efficiency of Pseudomonas putida in plastic degradation.
  Controlled environments allow researchers to achieve high enzyme activity and faster degradation rates. This approach is particularly useful for industrial-scale applications.

- **Integration into Circular Bioeconomy:**

a. Future research can focus on harnessing Pseudomonas putida not only for plastic degradation but also for converting plastic waste into value-added products. For example:

b. Production of polyhydroxyalkanoates (PHAs) as biodegradable bioplastics.

c. Generation of biofuels or other industrially valuable metabolites through plastic waste bioconversion.

d. Such integration can contribute to a circular economy, reducing plastic pollution while creating sustainable solutions**. (Rosenboom et al., 2021)**

- **Field Application and Bioremediation Trials:**

a. Further field trials are required to test the performance of Pseudomonas putida in real-world conditions, such as landfills, oceans, and polluted soils.

b. Future studies should focus on strategies to enhance its survival, adaptability, and efficiency in these environments.

- **Nanotechnology-Assisted Degradation:**

a. Combining Pseudomonas putida with nanomaterials such as iron oxide nanoparticles can enhance enzymatic activity and increase plastic degradation efficiency.

b. This novel approach could pave the way for more effective microbial plastic treatment methods **(Wilkes & Aristilde, 2017).**

- **Regulatory and Safety Assessments:**

a. As genetically modified strains are developed for enhanced plastic degradation, future research must ensure their safety for the environment and ecosystems.

b. Regulatory frameworks need to be established to allow for safe large-scale deployment.

***But they have some differences about their environmental conditions for degradation-**

- **Ideonella sakaiensis 201-F6:**

a. **Optimal Temperature**: 30°C to 37°C.

b. **pH**: Neutral to slightly alkaline (pH 7–8).

c. **Nutrient Requirements**: In addition to PET as a carbon source, it may require minimal organic nutrients for growth.

d. **Oxygen Requirement:** Aerobic conditions are preferred for efficient degradation.

e. Adaptability: It is highly specialized for PET degradation, using PET as the primary carbon source. **(Yoshida, S., et al.2016)**

- **Pseudomonas putida KT2440:**

a. **Optimal Temperature:** 30°C to 37°C (similar to I. sakaiensis).

b. **pH**: Typically grows in neutral pH (around 7).

c. **Nutrient Requirements:** It is a versatile strain that can degrade various carbon sources, but for PET degradation, it needs to be engineered or supplemented with enzymes like PETase and MHETase.

d. **Oxygen Requirement:** Aerobic conditions for growth and degradation are ideal.

e. **Adaptability:** P. putida has a wide range of metabolic pathways for degrading various organic compounds, but it requires genetic modification for efficient PET degradation.

(Ramos, J. L., et al. 2022)

Introduction of Oil Spill

The most common description of an oil spill is the inadvertent discharge of liquid hydrocarbons into the land and water due to human activity. Oil spills have a negative impact on the marine ecology and the shorelines that are close by because they damage and pollute aquatic habitats and the atmosphere due to the breakdown of unpredictable hydrocarbon compounds in the oil.

According to Zhang et al., over 1 billion gallons of oil were spilled globally in the past 10 years, and over 6 million tonnes of oil enter the world's oceans yearly. When spilt, these various oils have varying effects on the marine environment.

As a result, this will focus on crude oil and other products derived from hydrocarbons. Crude oil is a liquid composed of a complex mixture of hydrocarbon molecules, dissolved gases, and trace amounts of mineral salts, water, and metals like iron, nickel, vanadium, and chromium.

Each kind of crude oil or petroleum product has unique properties that are influenced by its composition. The qualities of crude oil and hydrocarbon-based products are mostly determined by their kind, which also has a direct impact on how the oil will behave in a marine environment.

When oil spills into an aqueous environment, its behaviour in the environment determines how it ends up.When oil spills into the environment, it goes through transformative processes that are referred to as "oil behaviour in an environment."

The Deepwater Horizon disaster caused more than 700,000 tonnes of oil to spill off the coast of Mexico in one instance alone. Wide-ranging effects on the ecology and economy can result from oil spills.

Over two hundred fifty thousand seabirds were killed in the **Deepwater Horizon oil leak in 1989,** which caused a $61 billion loss in damages.

Since fifty years ago, bioremediation and biodegradation technologies have been used to clean up deep-sea oil spills.

This approach is a low-tech, practical, reasonably priced process that degrades the pollutants in a short amount of time (**between one and four weeks**).

The main issues with these approaches include the loss of efficient microbial strains due to the ocean's rapid mobility and openness, as well as the existence of unpredictable and uncontrollable elements that significantly reduce remediation efficacy.

Crude oil hydrocarbon degradation has been thoroughly investigated, and several microorganisms, such as fungi and bacteria, have been identified and described for their capacity to break down crude oil hydrocarbons.

Bacteria are the main decomposers of oil spills in the environment and the most active agents in petroleum degradation. Furthermore, it is known that some bacteria only consume hydrocarbons for sustenance.

(***Alcanivorax borkumensis***):

- **Attachment to Oil Droplets:** The bacterium attaches to oil droplets, forming biofilms that facilitate the degradation process.
- **Production of Surfactants:** A. borkumensis produces biosurfactants, such as glycolipids, which emulsify oil, increasing its bioavailability for degradation.
- **Hydrocarbon Uptake and Degradation:** The bacterium absorbs hydrocarbons and metabolizes them using enzymes like alkane hydroxylases, breaking down alkanes into fatty acids and other intermediates.
- **Energy and Growth:** The metabolic breakdown of hydrocarbons provides energy and carbon sources, supporting bacterial growth and proliferation.
- **Final Mineralization:** Through further metabolic processes, hydrocarbons are fully mineralized into carbon dioxide and water, completing the degradation process.

The efficiency of these processes is influenced by environmental factors such as temperature, salinity, oxygen levels, and nutrient availability. Optimal conditions enhance the bacterium's oil-degrading capabilities, contributing significantly to the natural bioremediation of oil-contaminated marine environments.

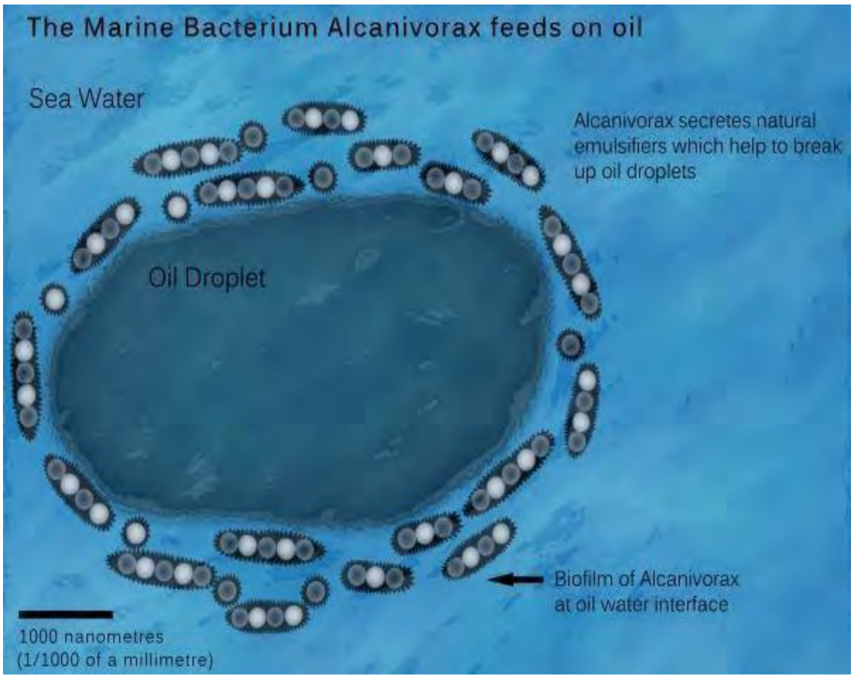

Figure4: Oil spill degrade by bacteria

Advantages

- **Specificity**: A. borkumensis specifically targets and degrades aliphatic hydrocarbons, making it highly effective in breaking down significant components of crude oil.
- **Natural Occurrence:** As an indigenous marine bacterium, its application in bioremediation is environmentally friendly and reduces the risk of introducing non-native species.
- **Biosurfactant Production:** The biosurfactants produced enhance the solubility and availability of hydrophobic oil compounds, improving the efficiency of the biodegradation process.

Limitations:

- **Nutrient Dependence:** The bacterium's activity is limited by the availability of nutrients such as nitrogen and phosphorus in the marine environment. In nutrient-poor waters, its growth and degradation capabilities can be significantly hindered.

- **Substrate Range:** While effective against alkanes, A. borkumensis is less efficient in degrading aromatic hydrocarbons, which are also toxic components of crude oil.
- **Environmental Conditions:** Factors such as temperature, salinity, and oxygen levels can influence the bacterium's degradation efficiency, potentially limiting its applicability across diverse marine settings.
-

Future Prospects and the Role of Recombinant DNA Technology:

- Advancements in recombinant DNA technology offer promising avenues to enhance the bioremediation potential of A. borkumensis:
- **Genetic Enhancement:** By introducing genes responsible for the degradation of aromatic hydrocarbons, it is possible to broaden the substrate range of A. borkumensis, enabling it to tackle a more comprehensive array of oil components.
- **Metabolic Engineering:** Modifying metabolic pathways can improve the bacterium's efficiency in utilizing hydrocarbons and increase its resilience to environmental stressors.
- **Biosurfactant Optimization:** Genetic modifications can lead to the production of more effective biosurfactants, enhancing oil emulsification and subsequent degradation.

However, the **application of genetically modified organisms (GMOs**) in open marine environments raises ecological and regulatory concerns.

Potential risks include unintended interactions with native species, horizontal gene transfer, and disruptions to existing ecosystems.

Therefore, thorough risk assessments, stringent regulatory frameworks, and the development of containment strategies are essential to ensure the safe and effective use of recombinant A. borkumensis in marine oil spill bioremediation.

***Pseudomonas aeruginosa*:**

P.aeruginosa is a common bacteria that can help with the bioremediation of oil spills in the ocean.

- **The Degradation Mechanism of *Pseudomonas aeruginosa*:**

It is a bacterium capable of degrading crude oil through a series of biochemical processes**.**

- **Biosurfactant Production: P**. aeruginosa synthesizes biosurfactants, notably rhamnolipids, which enhance the emulsification of hydrophobic hydrocarbons, increasing their bioavailability for microbial uptake. (Batista SB, et al.2006)
- **Hydrocarbon Uptake:** The bacterium absorbs the emulsified hydrocarbons into its cells, where they undergo enzymatic breakdown.
- **Enzymatic Degradation:** Specific enzymes, such as alkane hydroxylases and dioxygenases, catalyze the oxidation of hydrocarbons, converting them into intermediate compounds like alcohols, aldehydes, and fatty acids. These intermediates are further metabolized through central metabolic pathways, ultimately leading to the production of biomass, carbon dioxide, and water. (Xu X., Zhai Z., et al..2017) (Xue J., Yu Y., et al..2015).
- **Biofilm Formation:** P. aeruginosa can form biofilms on oil droplets, facilitating closer contact and more efficient degradation of hydrocarbons. (Alhede M, et al.,2009).

These processes enable P. aeruginosa to effectively degrade various components of crude oil, contributing to the bioremediation of oil- contaminated environments.

- **Advantages**

1. **Efficient Degradation:** Pseudomonas aeruginosa produces biosurfactants (e.g., rhamnolipids) that emulsify hydrocarbons, enhancing oil spill degradation.
2. **Versatility:** It can degrade a wide range of hydrocarbons, including alkanes, aromatics, and polycyclic aromatic hydrocarbons (PAHs).
3. **Survival in Harsh Environments:** This bacterium thrives in diverse conditions, including varying pH, salinity, and temperature, making it suitable for marine and terrestrial oil spill remediation.
4. **Rapid Growth:** It has a high growth rate and can adapt to nutrient- limited conditions, often found in contaminated environments.
5. **Cost-Effectiveness:** Bioremediation using Pseudomonas reduces the reliance on chemical dispersants, which are expensive and environmentally harmful.

Limitations:

1. **Pathogenic Nature:** Pseudomonas aeruginosa is an opportunistic pathogen, posing risks to human and animal health if introduced into certain environments.
2. **Incomplete Degradation:** In some cases, the degradation may lead to the accumulation of intermediate toxic compounds.
3. **Competition with Native Microorganisms**: In natural environments, introduced Pseudomonas strains might face competition from indigenous microbial communities, affecting efficiency.
4. **Environmental Constraints:** Its performance can be hindered by extreme environmental conditions (e.g., very low temperatures or high toxicity levels).
5. **Regulatory Concerns:** The use of genetically modified or even wild strains of Pseudomonas may face strict regulatory controls due to environmental safety concerns.

Future prospects:

1. **Genetic Engineering:** Enhancing the metabolic pathways of Pseudomonas aeruginosa through genetic modifications can improve its hydrocarbon degradation efficiency.
2. **Consortium Approaches:** Developing microbial consortia with complementary species to target a broader range of hydrocarbons.
3. **Field Applications:** More field trials to validate laboratory findings and adapt bioremediation strategies to real-world conditions.
4. **Bioaugmentation with Biosurfactant :** Increasing the use of Pseudomonas-derived biosurfactants for treating oil spills directly.
5. **Eco-friendly Strategies:** Researching ways to mitigate the pathogen- related risks by using non-pathogenic strains or deriving enzymes and metabolites for oil degradation.
6. **Policy and Awareness:** Developing policies that promote the integration of microbial bioremediation in disaster response plans.

Discussion

Recombinant DNA technology can help remove environmental pollution by creating microorganisms that can break down pollutants.

This process is called bioremediation. Recombinant DNA technology refers to the process of combining genetic material from different sources to create novel genetic combinations that do not occur naturally.

This is typically achieved through the use of molecular cloning techniques, which allow scientists to insert genes of interest into organisms (bacteria, fungi, plants, etc.) that can express them.

These genetically modified organisms (GMOs) can be tailored to perform specific tasks, such as degrading toxic substances, producing valuable compounds, or enhancing their resistance to environmental stressors.

In environmental biotechnology, rDNA technology has been used to engineer microorganisms capable of breaking down pollutants, including toxic metals, synthetic polymers, and hydrocarbons.

The underlying principle is that by altering the genetic makeup of microorganisms, scientists can enhance their natural ability to detoxify pollutants or improve their performance in specific environmental conditions.

In this discussion, we will explore the potential of rDNA technology in the context of biodegradation processes to remediate heavy metal pollution, degrade plastics, and oil spill disasters.

Heavy metals, such as mercury, lead, arsenic, and cadmium are toxic contaminants that can accumulate in the environment through industrial waste, mining activities, and agricultural runoff.

Conventional methods for removing heavy metals from contaminated sites include physical processes (e.g., excavation and landfilling) and chemical treatments, but these approaches can be costly, environmentally harmful, and inefficient.

In recent years, recombinant DNA (rDNA) technology has emerged as a promising solution for the bioremediation of heavy metals.

This technology allows for the genetic modification of microorganisms, enabling them to degrade, transform, or sequester heavy metals from contaminated environments more effectively and sustainably.

For example, bacteria such as Pseudomonas species, Bacillus species have been genetically engineered to express metal-binding proteins, enzymes, or transporters that can sequester or reduce toxic metals to less harmful forms.

Some of the engineered microorganisms can even convert toxic metals into less mobile forms, thereby preventing further contamination of the surrounding environment.

Pseudomonas aeruginosa, which has been engineered to degrade aromatic compounds and accumulate metals such as copper and zinc.

The enhanced ability of these bacteria to bind metals can be harnessed for bioremediation of contaminated water or soil.

Bacillus cereus and *Pseudomonas aeruginosa,* which play significant roles in heavy metal degradation and detoxification.

These microbes employ multiple mechanisms, including: Biosorption**,** Bioaccumulation, Enzymatic Transformation, Biofilm Formation, Bioprecipitation.

These bacterial systems demonstrate the potential for targeted remediation of specific heavy metals including Lead(Pb) Mercury(Hg) Cadmium (Cd) Chromium (Cr) Arsenic (Ar) offering site-specific solutions for contaminated environments.

Heavy metal bacterial bioremediation provides an environmentally benign, economical, and sustainable substitute for traditional remediation methods.

However, ongoing research and technical developments are required to tackle issues with environmental trust, metal specificity, and biosafety.

The use of recombinant DNA technology in connection with strong policy frameworks may enable this novel method to reach its full potential, resulting in cleaner ecosystems and lower environmental risks.

Plastic pollution is a critical environmental issue, with millions of tons of plastic waste accumulating in terrestrial and aquatic ecosystems.
Plastics like polyethylene terephthalate (PET), polyethylene (PE), and polystyrene (PS) persist in the environment for centuries due to their resistance to natural degradation processes.

Conventional disposal methods, such as incineration and landfill storage, pose further risks, including toxic emissions and limited space.

Bioremediation, particularly using bacteria capable of degrading plastics, offers a sustainable solution to this growing problem.

The degradation of plastics by bacteria like *Ideonella sakaiensis 201-F6* and *Pseudomonas putida KT2440* involves various mechanisms, such as attaching to plastic surfaces, enzymatic hydrolysis, biofilm formation, and metabolism of the by-products.

These bacteria can degrade synthetic polymers into biodegradable or innocuous by-products through attachment, enzymatic hydrolysis, biofilm formation, and metabolism of the by-products.

Bioremediation offers several advantages for plastic waste, including environmental sustainability, cost-effectiveness, scalability and adaptability, and dual benefits.

However, challenges include slow degradation rates, crystallinity of plastics, environmental dependencies, mixed plastic waste, and scaling up.

Future research directions include genetic engineering, co-culture systems development, optimization in bioreactors, introduction into the circular bioeconomy, field trials and real-world applications, and public awareness and policy support.

Genetic engineering can enhance catalytic efficiency and expand substrate specificity, while co-culture systems development can handle complex waste streams.

Optimization in bioreactors can increase the rate and efficiency of degradation, and nanotechnology innovation can enhance enzymatic activity.

Plastic bioremediation, mainly by bacteria like I. sakaiensis and P. putida, holds immense potential for solving the global plastic waste problem in a sustainable manner.

Advances in genetic engineering, bioreactor design, and microbial consortia may lead to paradigm shift in waste management, significantly reducing environmental pollution while promoting economic and ecological sustainability.

Oil spills are devastating environmental catastrophes, releasing hydrocarbons into aquatic and terrestrial environments. Conventional cleaning techniques, such as chemical dispersants, physical barriers, and burning, are expensive, inefficient, and harmful to the environment. Bioremediation employs microorganisms to degrade contaminants, making it a sustainable and environmentally friendly option. Bacteria like *Alcanivorax borkumensis* and *Pseudomonas aeruginosa* are the main contributors to oil spill bioremediation due to their ability to metabolize hydrocarbons.

These microorganisms use various mechanisms for effective degradation of oil components, including biosurfactant production, enzymatic degradation, biofilm formation, and adaptation to oil contamination.

Benefits of microbial bioremediation include environmental friendliness, cost-effectiveness, sustainability, and adaptability. However, bioremediation faces challenges such as environmental dependencies, selective substrate range, slow degradation rates, pathogenic concerns, and uncontrolled natural factors.

Future prospects and enhancements can be achieved through genetic engineering, microbial consortia, bioreactor development, biosensor technology, and policy and public engagement. Genetic engineering can enhance metabolic pathways of microbes like A. borkumensis toward hydrocarbons, while microbial consortia can improve resilience and performance. Nutrient augmentation can enhance microbial activity and degradation rates, and controlled bioreactor systems can optimize environmental conditions for microbial degradation. Biosensor technology can monitor oil contamination levels and activate specific remediation pathways, enabling precise and efficient cleanups.

Establishing regulatory frameworks and increasing public awareness about bioremediation benefits can encourage adoption and funding for advanced research.

So, Recombinant DNA technology is an important development in science that has made human life much easier.

Conclusion

Recombinant DNA technology has proven to be a highly effective bioremediation technique for tackling serious environmental contamination issues and enhancing environmental health outcomes.
Our approach to managing the three main types of pollution—oil spills, heavy metal poisoning, and the buildup of plastic waste—has been completely transformed by the development of genetically modified microbes.

Each of these threats to the environment and public health is quite serious.

Improved bacterial strains containing hydrocarbon-degrading genes have greatly increased the effectiveness of cleanup efforts in oil spill remediation.

By expressing genes that produce biosurfactants and sophisticated alkane hydroxylases, these altered organisms have shown a greater capacity to degrade hazardous petroleum chemicals that typically survive in both marine and landscapes.

The long-term health dangers linked to oil contamination, such as the release of toxic volatile organic compounds into the atmosphere and the bioaccumulation of carcinogenic chemicals in food chains, have decreased as a result of this improved degradation capacity.

The creation of microbes with improved metal-binding capacities has led to very remarkable outcomes in the use of rDNA technology in heavy metal cleanup.

The chances of these toxic substances getting into human food and water supplies is decreased by these modified strains' efficient removal of hazardous metals including lead, mercury, and cadmium from contaminated soil and water systems.

The public health effects of increased heavy metal extraction efficiency are substantial, especially in areas where mining and industrial pollutants are prevalent.

Genetically engineered organisms expressing improved forms of plastic-degrading enzymes have created new opportunities for the management of plastic waste in the fight against the worldwide plastic pollution disaster.

These modified strains demonstrate encouraging outcomes in the degradation of different kinds of plastics, which may lessen the buildup of microplastics in environmental systems and their eventual introduction into the human food chain.

This development is a critical step in addressing the endocrine disruption and other possible physiological effects of plastic pollution, which are becoming increasingly serious health problems.

As rDNA-enhanced bioremediation technologies continue to evolve, even further advancements in environmental pollution reduction and health protection are anticipated. Future studies should concentrate on making these technologies more stable and safe in a range of environmental circumstances while also optimising them for large-scale usage.

By tackling environmental pollution and its related health effects, biotechnology plays a critical role in promoting a more sustainable and healthy future for human populations and ecosystems. This is demonstrated by the success of these applications.

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