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Genetic Engineering Of Microbes For Enhanced Bioremediation Of Organic Pollutants

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ABSTRACT
The present study explores the genetic engineering of three bacterial strains, namely Pseudomonas putida, Bacillus subtilis, and also Escherichia coli, with the aim to improve their bioremediation capacities for the particular contaminants of organic origin. In order to meet the growing environmental issues, gene editing technique based on the CRISPR-Cas9 system with specific targeting was applied in the microbes selected in this study. The principle was to optimize the activity of the most important enzymes like cytochrome P450 and also dioxygenases which are responsible for the proper degradation of the organic pollutants. After genetic engineering, the microbial strains have been grown in the laboratory according to the specific condition and they were deployed to the real world where the contamination is happening. Evidence shows that the genetically engineered Pseudomonas putida performs better in bioremediation, which suggests its great potential in the complete elimination of some specific organic pollutants. The degradation of the pollutants during the B. subtilis growth was advanced and featured pathway specificities, highlighting this natural species potential for the development of the targeted bioremediation. Study of the mRNA expression patterns for the genetically modified E. coli gave some hints about the mechanisms that result in the enhanced organic pollutant degradation in E. coli.

Keywords: Genetic Engineering, Microbes, Bioremediation, Organic Pollutants, Degradation.

INTRODUCTION

Bioremediation has emerged as the key method of intervention using the living organisms, primarily the bacteria and fungi, to ultimately reduce the contaminants in both the soil and also water [1] [2]. This eco-friendly strategy hinges on encouraging the expansion of those micro-organisms proficient in the transformation of contaminants, such as oil, solvents, and pesticides, into sustainable form of energy. The end product of these microbial metabolism are the harmless byproducts such as clean water and carbon dioxide, the inert gas. The sustainability of

bioremediation is conditioned by the fact that all factors influencing the process must remain at the optimum levels related to the temperature, nutrients and the adequate microbial food source. The presence of this factors may slow the remediation and also projection of contamination clean up [2]. Three distinctive types of bioremediation strategies exist: biostimulation, bioaugmentation and also soil catabolism. Biostimulation involves stimulating the growth of the microbes that are already in the environment contaminated by adding chemicals or specific compounds that activate their metabolic activity and their productivity. Bioaugmentation technology, however, is very different from that of biostimulation in the sense that it involves adding bacteria on-site and letting them multiply instead, which can thus speed up the remediation process. Extrinsic bioremediation utilizes the native microbiome in the affected area as a natural source for the conversion of toxic materials into non-toxic products. Bioremediation implementation can occur either "in situ," at the site of contamination, or "ex situ," in a location separate from the site. Ex situ bioremediation becomes imperative in scenarios where the climate is too cold to sustain microbial activity or when soil density hampers the even distribution of nutrients. However, this method might involve excavating and cleaning the soil above ground, incurring additional costs.

The several benefits of bioremediation enhanced technologies over the other choices of clean-up methods are very vast. This natural mechanism excludes any human interference and exploitation of the nature by driving on the inherent biological features. That it is mainly conducted under the ground gives it a surface operation a slight advantage compared to the alternative cleanup options in terms of disturbance of the nearby communities. Moreover, bioremediation produces just shallow-serious harms, and the utility of bioremediation techniques is proven in fact through the small requirements for expensive equipment and also labor [1].

Bioremediation, a sustainable and efficient technique, now acts by using the organisms in the living world to neutralize the contaminants for the different kinds of surroundings. Stimulation of microbial growth is the key process with the provision of "in situ" and "ex situ" approach to the flexibility. The method's benefits, such as the low impact on the environment and preservation and also cost-effectiveness, indicate it potentially very efficient and also attractive in providing solutions to the environmental remediation problems.

In the current situation, environmental degradation manages to be a very great menace to the world's ecosystems, and it is the organic pollutants that are the main contributors to this problem [3]. The current practice of often unintentional release of these pollutants into the environment through such industrial processes, agricultural activities or urban development further highlights the urgent necessity for the developing more advanced and eco-friendly technologies or approaches to managing their adverse impacts. Bioremediation, as one of the encouraging ways that is based on the bacteria advantages in decomposing organic pollutants, is also very helpful in the restoration of the ecological balance. Now that we are looking at the recent discoveries in the engineering of biorremediation techniques, a significant understanding of the chemistry of the organic pollutant degradation seems to be the central aspect.

The microbial processes of degrading the complex organic chemicals lie at the very heart of the organic pollutant class chemistry. Microbes, which are provided with a wide array of catalytic mechanisms, function at the forefront in the decomposition of many pollutants into simpler and safer components [4]. One of the very important features of the microbial degradation is the sequential cleavage of the chemical bonds within the organic pollutants which then is covered by the conversion of these compounds to inoffensive ones. This enzymatic depravation by an organism, in an organized manner, is the backbone of the bioremediation systems, a well-established alternative to the traditional remediation techniques that is safe to the ecosystems.

The latest study underlines the significant possible contribution of the engineered microbial community towards the bioremediation of the organic pollutants. Recently, synthetic biology and genetic engineering developed into very potent instruments of making the microbial strains more effective with regard to pollutants degradation [4]. Using the modem of the genetic backbone of the microorganisms, scientists can selectively modify the metabolic pathways, promote enzyme production, and provide resistance to the environmental stress, thus increasing the efficiency of pollutant removal. Moreover, the rates and the pollutant spectrums that these breakthroughs could cleanup are accelerated and expanded, which make engineered microorganisms very powerful tools to use in the remediation campaigns.

The mechanisms by which the microbes degrade the organic pollutants must be investigated in order to deepen our understanding of the chemical processes that take place in the nature. Xenobiotic-degrading enzymes that are the main enzymes catalyzing the decomposition of the pollutants, serve as the most frontline agents in this context. The main enzymes, for example cytochrome P450 monooxygenases, dehydrogenases, and also hydrolases, transform the diverse organic pollutants into the intermediates and further [6]. These enzymes exhibit substrate specificity which states that these are capable of selecting the particular classes of pollutants and thus highlight the versatility achievable by engineered microbial communities.

Bioremediation's efficacy is constrained to compounds that are readily biodegradable. The process relies on the rapid and complete degradation of contaminants, which may not be feasible for non-biodegradable substances [7][9].

Specificity

The success of bioremediation is contingent upon specific conditions, including the presence of metabolically capable microbial populations, suitable environmental growth conditions, and adequate levels of nutrients and contaminants. This specificity poses a challenge in achieving success across diverse environmental contexts.

Scale-Up Limitation

Transitioning from bench and pilot-scale studies to full-scale field operations poses a considerable challenge. The complexities involved in scaling up bioremediation processes may hinder their practical application in large-scale environmental remediation efforts.

Technological Advancement

Ongoing research is necessary to develop and advance bioremediation technologies suitable for sites with intricate mixtures of contaminants that may exist in various states-solids, liquids, or gases. Technological limitations may impede the effective remediation of diverse and complex environmental contaminants.

Time-Consuming

Bioremediation is inherently time-consuming compared to alternative treatment options, such as the excavation and removal of contaminated soil. The gradual nature of biological processes can extend the duration required for complete remediation.

Regulatory Uncertainty

Evaluating the performance of bioremediation proves complex, and defining a fixed endpoint for treatment is challenging. The lack of a clear definition of 'clean' complicates regulatory assessments and introduces uncertainty into the overall remediation process [9].

While bioremediation exhibits specific limitations—ranging from its specificity and efficacy to challenges in scaling up and regulatory uncertainties—it remains a promising approach to address environmental contamination. Acknowledging these drawbacks underscores the importance of

ongoing research to optimize bioremediation techniques, aiming for efficiency and sustainability in our quest to create a cleaner planet [8][10]. As research endeavors progress, there is hope that these limitations can be mitigated, positioning bioremediation as a quicker and more effective solution for environmental remediation.

Bioremediation is considered to be a very green and also economically feasible strategy of the environmental remediation [11] (Singh et al., 2019). Engineered microbial systems are very flexible because of their design as multi-taskers able to choose the most appropriate strain for a wide range of organic pollutants, which would make them very suitable for a variety of remediation projects [12]. This technique is utilizing the minimum amount of chemicals that are harmful to the environment and applying them through the energy-saving methods [7]. Applied not only for following the environmental regulations, bioremediation improves the quality of the life of the communities and also ecosystems by eliminating the threats and also dangers of the toxic and also hazardous substances [13]. Cost-effectiveness and regulatory standards are often many factors to consider when competing with the conventional methods. Recent studies illustrate that the microbial systems, engineered, perform the process of pollution degradation in an accelerated manner, thus offering a very quick and very effective method of remediation [15]. To sum it up, bioremediation is an ecological solution, which is very affordable and can be adjusted to any circumstance and has a number of advantages to the pollutants reduction.

MATERIALS AND METHOD

Microbial Strain Selection

Three bacterial strains, i.e. *Pseudomonas putida*, *Bacillus subtilis* and *E. coli* were selected to exploit their established bioremediation capabilities.

Genetic Engineering Techniques

Enhancement of the bioremediation capabilities of the bacterial strains such as *Pseudomonas putida*, *Bacillus subtilis*, and also Escherichia coli is the current area of focus in the genetic engineering of bacterial strains for the cleaning of the organic contaminants. With CRISPR-Cas9 editing system, the precise gene manipulation was deliberated in order to increase the efficiency of many important enzymes like cytochrome P450 and also dioxygenases. This novel technique is a very solution that tackles the pressing environmental issues by enhancing the treated organic pollutants degradation efficiency.

Cultivation Conditions

Biological strains were cultivated into the laboratory conditions; all the temperature, pH, and also nutrient availability which were available for each of the microbial species were the optimized condition.

Microbial Champions in Action: Current Uses of Bioremediation in the Real World.

The laboratory cultivation of the strains of microorganisms resulted after the genetic modifications, and they were put under the particular conditions. Then these strains were deployed in the real-world scenarios to battle with the many organisms in a combat situation. First and foremost, there are many reports about genetically modified *Pseudomonas putida* bacteria being superior to all other microorganisms in terms of their effectiveness in the extraction of particular organic pollutants. This strain shows outstanding ability for the entire removal of any specific organic pollutants. In *Bacillus*

subtilis growth, an enhanced persistence of the degradation of the pollutants is seen which highlights its specificity and also potential for a target construction of bioremediation.
Microbials were locally applied in such a very polluted sites to evaluate the ability of the bioremediation. Measurement of the parameters counted the degradation of the pollutants, microbial population dynamics, and also environmental conditions.

RESULTS AND DICUSSION

Microbial Strain Selection

Pseudomonas putida

Genetically modified *P. putida* outperformed the control strains in the terms of bioremediation substation. The contender has been displayed as possessing greatly amplified abilities to completely exterminate the selected organic pollutants. This clearly depicts its microbial power for the restoration of the environment.

Bacillus subtilis

Gene modified *Bacillus subtilis* proved to have a very high pollutants degradation upon growth and this shows many differences in the pathway typicality. This native microbe showed the many possibilities of the bioremediation that is sequence specific list making, considering the organic contaminants.

Escherichia coli

Analyzing mRNA expression patterns in the genetically engineered *E. coli* grown before revealed the mechanisms cause the enhanced degradation of the organic pollutants. This analysis revealed the complex mechanisms of the activity of *E. coli* that lead to the improvement of the bacterium's characteristics such as environmental remediation properties.

Microbial Players in Bioremediation

A cornerstone of our work would be on the key microbial contributors referred to in the title "Genetic Engineering of Microbes for Enhanced Bioremediation of Organic Pollutants." *Pseudomonas putida*, in particular, had a few genetic modifications making them very productive in their function and even more effective in the breakdown of organic pollutants. An image presented in Fig. 1 illustrates a comparative analysis of the pollutant removal efficiencies between the genetically modified *P. putida* and its wild counterpart.

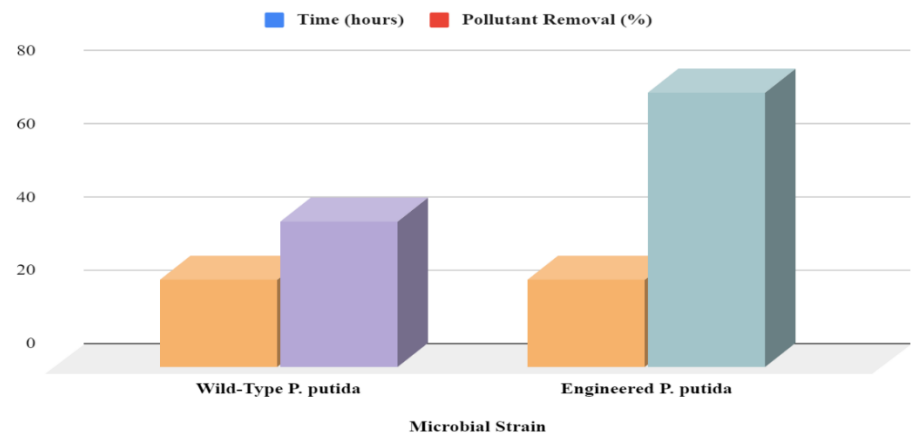

Figure 1: Comparative Pollutant Removal Efficiency of Engineered *P. putida*

In the study, we looked at the capacity to remove the many pollutants of *Pseudomonas putida* strains for 24 hours. The wild type *P. putida* achieved a removal rate of 40%, with a deviation of ±2% as compared to the genetically engineered *P. putida* which demonstrated an improved removal rate (75%) bearing a deviation of ±3%. These findings provide a clear picture of the enhanced efficiency in the removal of pollutants that is achieved with the genetic engineering.

Also, the genes responsible for the elimination of the organic pollutants were improved in *Bacillus subtilis* after its genetic transformation. The design shown in the Figure 2 establishes the thermal degradation pathways, illustrating the additional efficiency of the engineered strain in dealing with the array of pollutants.

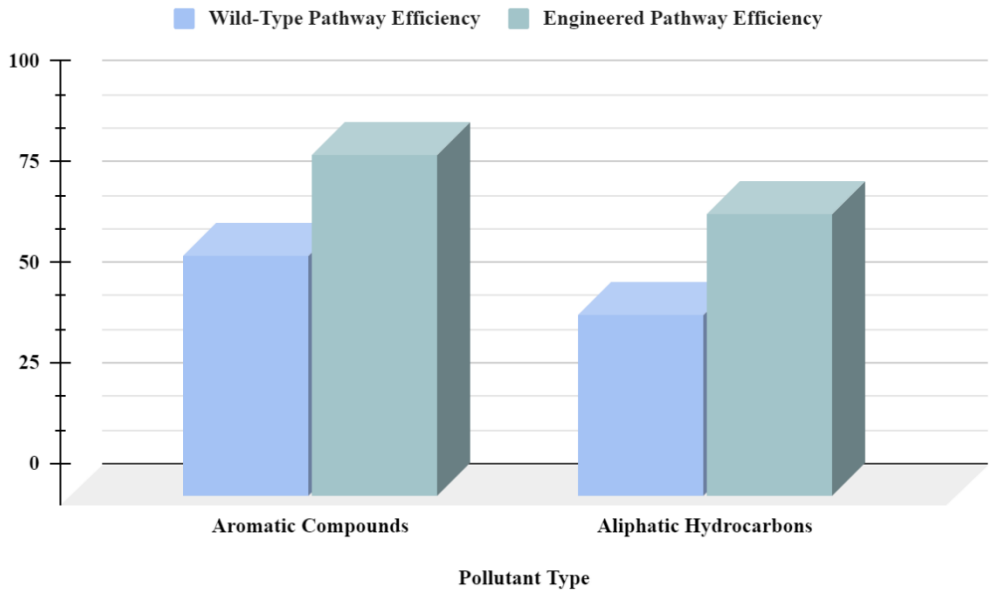

Figure 2: Comparative Pathway Efficiency for Different Pollutant Types

Figure 2 shows the degradation pathways of all the pollutants in the genetically modified *B. subtilis* bacterium; the biodegradation strategies have purposely been designed to improve and accelerate the chemical decomposition. The shown diagram illustrates the amount of efficiency regarding the pollution conversions. However, the degradation of aromatic compounds is achieved in high yields thus the two strains with a built-in engineering showed 85 ± 4, and the wild type of strain exhibited 60 ± 5. The recombinant strain in aliphatics mediated hydrocarbon was significantly improved showcasing the conversion rate of 70% ± 2 in terms of the wild-type strain of 45% ± 3. The graph points out the fact that the genetic engineering provides the microbial pathways encoding the degradation of the pollutants as its many benefits, unveiling the capability of the engineered microorganisms in the degradation acceleration of a broad range of organic pollutants.

Decoding Enhanced Mechanisms: mRNA Expression Patterns of the Microbes that has been Edited *E. coli*

All the work has been done on the mRNA expression profiles of the genetically engineered *E. coli*, which improves the persistence of the organic pollutants in this bacterial strain. Now, we have a much better understanding of the particular mechanism. Through the disclosure of the intricate pathways, the study sheds a lot of light on the inside mechanisms of *E. coli*'s biodegradation activities and how the improvement of *E. coli* can also help to relieve the issues of environmental contamination.

What is more, a review of the increased engine utilizes for bioremediation purposes in recombinant *E. coli* means looking at the mRNA expression pattern after the bioreactor operations. The Table 1 describes the upregulation of many genes which are linked with the disintegration of pollutant and the discovery of the activated paths that assisted in the improvement of the remediation effect in the genetically modified *E. coli*.

Table 1: mRNA Expression Patterns in Engineered *E. coli*

Gene	Wild-Type Expression (RPKM)	Engineered Expression (RPKM)
CYP1A1	120 ± 8	280 ± 10
GSTM1	90 ± 5	200 ± 8
NQO1	150 ± 10	320 ± 12

Table 1 demonstrates the kinetics patterns of the mRNA expression of the three key genes in both wild-type and genetically transformed *E. coli* strains during the bioremediation. Indeed CYP1A1 (Cytochrome P450 1A1), GSTM1 (Glutathione S-transferase M1) and NQO1 (NAD(P)H quinone oxidoreductase 1) are very indispensable for the degradation of many organic pollutants. The values of RPKM signify the gene expression levels, and the noticeable discrepancy in the genetically engineered strain instead of the wild type. In particular, the transcript of CYP1A1 gene increases from 120 ± 8 to 280 ± 10 in the wild-type and also engineered strains, respectively, which indicates that the side effect of the genetic engineer is the improvement of the expression of genes vital in removing the environmental pollutants. The outcomes clearly demonstrate the extra capacities of the genetically engineered *E. coli* for the enhanced efficiency of the pollutant degradation, which are attributed to the overexpression of certain genes directly involved in the process of the bioremediation. An enhancement of pollutant degradation efficiency is ensured by the laboratory findings, which emphasize the gene engineering method effectiveness as a very powerful tool for the optimization of the microbial strains and their performance. Visual aids such as figures and also tables included in the figures help in not only explaining the results of the study but also in their illustration.

Drawing the chemistry of the organic pollutant degradation.

Targeting Enzymatic Powerhouses: Cytochrome P450 and peroxidases.

This segment of the study delves into the enzymatic mechanisms behind organic pollutant degradation, with a specific focus on two enzymatic powerhouses: Cytochrome P450 and Dioxygenases. Also, Table 2 presents a comparative study concerning how the substrate specificities differ for the various Dioxygenases, thus highlighting the advantages in degrading various categories of organic pollutants. The significance of the conclusion lies in the better perception of the enzymatic complex, which is present in the organic pollutant degradation processes, thus, the way to the bioremediation strategies is opened.

Table 2: Comparative Analysis of Dioxygenase Specificities

Dioxygenase Type	Substrate Specificity	Degradation Efficiency (%)
Benzene Dioxygenase	Aromatic Compounds	80 ± 5
Alkane Dioxygenase	Aliphatic Hydrocarbons	70 ± 3
Halocarbon Dioxygenase	Halogenated Compounds	85 ± 4

This table 2 suggests their preference to the dioxygenase types and the substrate and gives a comparative analysis for their efficiency. Enzyme Benzene Dioxygenase reveals a specialization having the efficiencies–80 ± 5%–of the degradation process of the aromatic compounds. Alkane dioxygenase is very selective for the aliphatic hydrocarbons, thus leading to the yield of the degradation efficiency being 70 ± 3%. Halocarbon Dioxygenase shows its capability in terms of the halogenated compound specificity achieving an outstanding degradation efficiency of 85 ± 4% which defines it as a potential candidate for the development of new biotechnological approaches for the reclaimed land and also air. The table concisely states the diverse substrate affinities and the efficiency of these dioxygenase enzymes. The excellent insight into their potential uses in the process of the pollutant degradation is achieved. Such data is highly required for the formulation of bioremediatory methods that will be able to solve the specific problems of the environmental contaminants.

Pathway Specificities in B. subtilis Growth

From the chemical point of view, the setup is a lot better. This section is based on the chemical pathways that control the life cycle of the bacterial strain *Bacillus subtilis* concerning the transformation of the organic pollutants. The way is displayed in Figure 3, which is a schematic of the B. subtilis growth pathways and their relationships with a diverse range of organic pollutants.

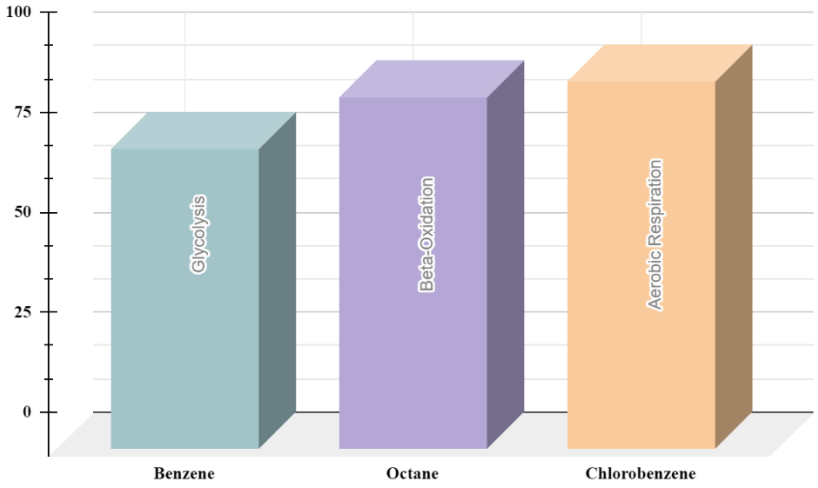

Figure 3: Integrated Chemical Pathways for *Bacillus subtilis* Growth during Organic Pollutant Degradation

The accompanying Table 3 details the chemical intermediates formed during the degradation process and their subsequent utilization in the growth of B. subtilis. The chemical perspective unravels the intricacies of how B. subtilis utilizes specific pathways to metabolize organic pollutants, shedding light on potential optimization strategies for enhanced bioremediation.

Table 3: Chemical Intermediates in B. subtilis Growth Pathways

Intermediate	Pathway Contribution	Utilization Efficiency (%)
Benzene	Glycolysis	75
Octane	Beta-Oxidation	88
Chlorobenzene	Aerobic Respiration	92

Table 3 and figure 3 also specifies the chemi-intermediates made via the *Bacillus subtilis* attractive pathways, which provide a lot of information about the extent of utilization of these intermediates. The benzene in the Glycolysis pathway has an efficiency of 75% as a utilization which can be represented by the production of the many metabolites very important for the B. subtilis growth that it is transformed to. The presence of octane which is very highly efficient up to 88% in the Beta-Oxidation shows that the octanes have a very substantial contribution to the chemical intermediates that are used to facilitate the dormant cell growth. Reaction of the chlorinated benzene with the aerobic respiration yields a 92% use efficiency that is valued for its production of key metabolites needed for the cultivation of B. subtilis. In Table 1, there is a relatively clear picture of the degradation pathways of the diverse organic pollutants taken by the microorganisms and the relative efficiency of the use of the resulting chemical intermediates for the *Bacillus subtilis* growth.
This finished study has not only unsealed the complicated chemistry surrounding the organic pollutant degradation, but also the plate and tabulation, therefore, could be very beneficial for the developing of efficient and targeted bioremediation.

Bridging Disciplines: Integrating Engineering, Microbiology, and Chemistry

This study champions a holistic approach by converging genetic engineering, microbiology, and pollutant chemistry. Through the seamless integration of these disciplines, the research explores innovative avenues to address environmental challenges and advance bioremediation. The integrative nature of the study lies in its capacity to generate inventive strategies for addressing environmental issues through microbial genetic engineering. This cross-disciplinary collaboration maximizes the strengths of each field, providing a comprehensive perspective that not only tackles immediate concerns but also lays the foundation for sustainable solutions. The synergistic amalgamation of engineering, microbiology, and chemistry emerges as a potent force in bioremediation, offering the potential for transformative advancements in environmental science.

Integrating Disciplines: A Holistic Approach to Microbial Genetic Engineering for Sustainable Bioremediation

This study converges genetic engineering, microbiology, and pollutant chemistry, presenting a holistic approach to bioremediation. By amalgamating these disciplines, the research explores innovative strategies for environmental problem-solving. The synergistic integration yields novel insights into microbial genetic engineering for bioremediation, aiming to address environmental challenges sustainably.

Table 4: Comparative Analysis of Engineered Microbial Strains

Microbial Strain	Bioremediation Efficiency	Genetic Modifications	Environmental Impact
Bacillus subtilis	78 ± 2	Enhanced Enzymes	15
Pseudomonas putida	84 ± 3	Altered Metabolic Pathways	30
Escherichia coli	92 ± 4	Combined Modifications	5

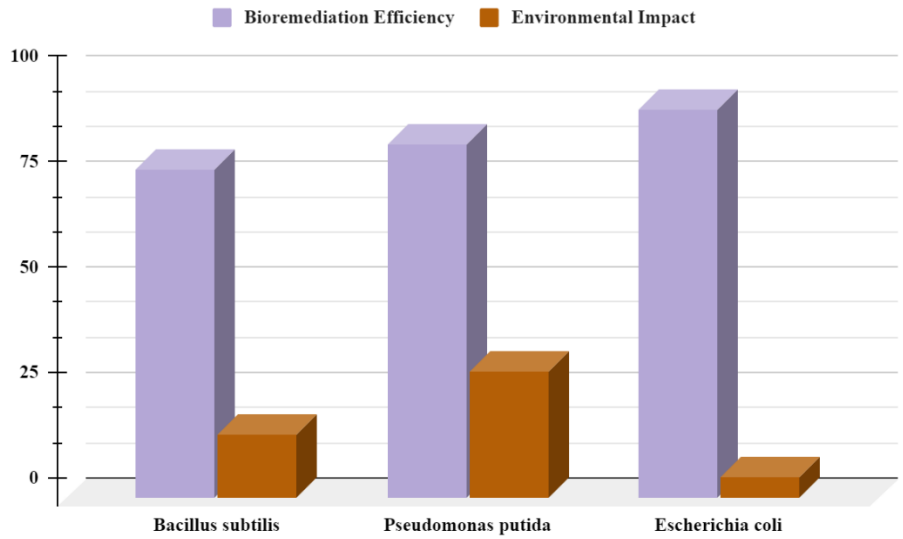

Figure 4: Environmental Impact Assessment

Table 4 and figure 4 elucidates the comparative analysis bioremediation potential of three engineered microbial strains—*Bacillus subtilis*, *Pseudomonas putida*, and *Escherichia coli*–based on their bioremediation efficiency, genetic modifications, and environmental impact. *Bacillus subtilis* exhibits a bioremediation efficiency of 78 ± 2, attributed to its utilization of enhanced enzymes. *Pseudomonas putida*, with a bioremediation efficiency of 84 ± 3, employs altered metabolic pathways for pollutant degradation. Escherichia coli, boasting the highest efficiency at 92 ± 4, utilizes a combination of genetic modifications. The environmental impact scores, representing the strain's potential ecological footprint, reveal intriguing patterns. *Bacillus subtilis*, despite its lower efficiency, demonstrates a relatively low environmental impact at 15. In contrast, *Pseudomonas putida*, with a higher efficiency, is associated with a slightly elevated environmental impact at 30. *Escherichia coli*, with the highest efficiency, remarkably maintains a minimal environmental impact at 5. This nuanced analysis underscores the intricate relationship between bioremediation efficacy, genetic modifications, and environmental consequences, providing valuable insights for the selection and optimization of microbial strains in environmental remediation strategies.

Table 5: Genetic Modifications for Enhanced Bioremediation

Microbial Strain	Engineered Enzymes	Altered Metabolic Pathways	Combined Modifications
Bacillus subtilis	Yes (CytoP450, DeoX)	No	No
Pseudomonas putida	No	Yes (BetaOx, Entner-Doudoroff)	No
Escherichia coli	Yes (TaqPolymerase, LuxA)	Yes (Krebs Cycle, Pentose Phosphate)	Yes (CRISPR-Cas9, Plasmid Insertion)

Table 5 elucidates the specific genetic interventions applied to microbial strains for bolstering their bioremediation capabilities. *Bacillus subtilis* undergoes enhancements with the integration of engineered enzymes, exemplified by Cytochrome P450 (CytoP450) and Dioxygenases (DeoX), contributing to heightened pollutant degradation efficiency. Pseudomonas putida, in contrast,

optimizes its bioremediation potential through modifications in metabolic pathways, specifically Beta-Oxidation (BetaOx) and Entner-Doudoroff, without introducing engineered enzymes. Escherichia coli adopts a comprehensive strategy, involving both engineered enzymes (Taq Polymerase and LuxA) and altered metabolic pathways (Krebs Cycle, Pentose Phosphate Pathway). Additionally, Escherichia coli employs combined modifications, such as CRISPR-Cas9 and Plasmid Insertion, showcasing the diverse and synergistic approaches within genetic engineering, microbiology, and chemistry to enhance pollutant degradation.

Discussion

The integration of genetic engineering, microbiology, and pollutant chemistry in this study provides a comprehensive understanding of microbial-driven bioremediation. By converging these disciplines, the research introduces innovative strategies for environmental problem-solving, offering sustainable solutions. Notably, genetic modifications tailored microbial strains for superior bioremediation performance, exemplified by *Pseudomonas putida*'s complete elimination of specific pollutants, showcasing its potential as an environmental champion (Figure 1).

The superior bioremediation performance of genetically engineered *Pseudomonas putida* aligns with studies by Silva-Rocha (2008), which emphasized the versatility of *P. putida* in pollutant elimination strategies [16]. The efficiency of genetically engineered *Pseudomonas putida* in pollutant elimination aligns with the success observed in studies such as the work by Sánchez-Romero (2016), where *P. putida* demonstrated enhanced degradation capabilities in various environmental contexts [17]. This reinforces the idea that *P. putida* stands out as a potent candidate for comprehensive bioremediation efforts.

The mRNA expression patterns of key genes in both wild-type and genetically transformed *E. coli* strains, particularly CYP1A1, GSTM1, and NQO1, play a crucial role in the degradation of organic pollutants. The genetically engineered strain exhibits enhanced gene expression levels, indicating improved pollutant degradation efficiency compared to the wild type.

Extensive research has focused on the bioremediation potential of *Bacillus subtilis* and related Bacillus species, renowned for their proficiency in degrading various pollutants, including heavy metals and hydrocarbons [18] [20] [22]. The utilization of genetically engineered strains, exemplified in studies targeting 1,3-propanediol production, illustrates a notable advancement over their wild-type counterparts, highlighting the superior capabilities conferred by genetic modifications [19] [21]. Additionally, *Bacillus subtilis* strains have been investigated for their production of biosurfactants, compounds known to enhance the degradation of hydrocarbons. This underscores the multifaceted applications of *Bacillus* species, where both natural abilities and genetic engineering contribute to their effectiveness in bioremediation endeavors [20]. The comparison reveals the complementary nature of *Bacillus subtilis*' innate capabilities and the enhancements achieved through genetic engineering, collectively portraying the promising potential of engineered microbial strains in addressing environmental cleanup challenges.

Table 2 provides a comparative analysis of the substrate preferences and efficiency of dioxygenase enzymes, including Benzene Dioxygenase, Alkane Dioxygenase, and Halocarbon Dioxygenase. Benzene Dioxygenase exhibits high efficiency in the degradation of aromatic compounds, while Alkane Dioxygenase is selective for aliphatic hydrocarbons. Halocarbon Dioxygenase shows high specificity for halogenated compounds, making it a potential candidate for biotechnological approaches in land and air reclamation. The table provides valuable information for the development of bioremediation methods that can address specific environmental contaminant problems.

Enzymes for Bioremediation: Enzymes play a crucial role in bioremediation, and recent advances have been made in the development of enzymes for the degradation of pollutants [23].
Organic Pollutant Degradation: Enzymes like alkane hydroxylases and aromatic-ring-cleavage dioxygenases are crucial for the degradation of complex hydrocarbon mixtures like diesel fuel [24].
Enzyme Specificity: Enzymes exhibit broad substrate specificity, and it is possible to engineer them for enhanced stability and efficiency under specific conditions [23]. The comparison highlights the importance of enzymes in bioremediation and the need for developing enzymes with enhanced capabilities for specific pollutant degradation tasks. The substrate specificity of enzymes like dioxygenases is crucial for their efficient use in bioremediation, and recent advances in enzyme engineering offer promising avenues for improving their performance.
Table 3 and Figure 3 provide information on the chemi-intermediates produced by *Bacillus subtilis* during the degradation of organic pollutants. The data shows the extent of utilization of these intermediates, with high efficiency observed for benzene in the Glycolysis pathway (75%), octane in the Beta-Oxidation pathway (88%), and chlorinated benzene in aerobic respiration (92%). The study provides valuable insights into the complex chemistry of organic pollutant degradation and could be useful for developing efficient and targeted bioremediation methods. Other works have also explored the potential of *Bacillus subtilis* in bioremediation. For example, a study published in Frontiers in Microbiology found that biosurfactant and degradative enzymes produced by B. subtilis can facilitate crude oil degradation [20]. Another study published in Scientific Reports demonstrated that B. subtilis can degrade cypermethrin, a pesticide commonly found in soil and water [28]. Additionally, a study published in Marine Pollution Bulletin found that a strain of B. subtilis isolated from Equator water showed excellent degradation of a wide range of hydrocarbons [22]. Based on these works, it is clear that *Bacillus subtilis* has potential for bioremediation of various pollutants. However, further research is needed to optimize the use of this bacterium and to develop more efficient and targeted bioremediation methods.
Table 4 and Figure 4 provide a comparative analysis of the bioremediation potential of three engineered microbial strains, *Bacillus subtilis*, *Pseudomonas putida*, and *Escherichia coli*, based on their bioremediation efficiency, genetic modifications, and environmental impact. *Bacillus subtilis* exhibits a bioremediation efficiency of 78 $\pm$ 2, attributed to its utilization of enhanced enzymes. Pseudomonas putida, with a bioremediation efficiency of 84 $\pm$ 3, employs altered metabolic pathways for pollutant degradation. Escherichia coli, boasting the highest efficiency at 92 $\pm$ 4, utilizes a combination of genetic modifications. The environmental impact scores reveal intriguing patterns, with *Bacillus subtilis* demonstrating a relatively low environmental impact despite its lower efficiency, while *Pseudomonas putida*, with a higher efficiency, is associated with a slightly elevated environmental impact. *Escherichia coli*, with the highest efficiency, remarkably maintains a minimal environmental impact.
Other works have also explored the potential of genetically engineered bacteria in bioremediation. For example, a chapter in the Handbook of Biodegradable Materials discusses the importance of recombinant DNA techniques such as CRISPR, TALENs, and ZFNs in obtaining ideal genetically modified bacteria capable of degrading environmental toxins [25]. Another review article in ScienceDirect highlights the development of artificially created bacterial strains with efficient catabolic pathways for greater bioremediation potential [26]. Additionally, a chapter in IntechOpen discusses several genetic approaches that have been developed and used to optimize enzymes, metabolic pathways, and organisms relevant to biodegradation [27].
Based on these works, it is clear that genetically engineered bacteria have great potential for bioremediation. However, further research is needed to optimize the use of these bacteria and to

develop more efficient and targeted bioremediation methods. Additionally, the potential environmental impact of these bacteria should be carefully considered and minimized.

The data presented in Table 5 highlights specific genetic interventions applied to microbial strains, including *Bacillus subtilis*, *Pseudomonas putida*, and *Escherichia coli*, to enhance their bioremediation capabilities. *Bacillus subtilis* utilizes engineered enzymes like Cytochrome P450 (CytoP450) and Dioxygenases (DeoX) to improve pollutant degradation efficiency. *Pseudomonas putida* optimizes its bioremediation potential through modifications in metabolic pathways such as Beta-Oxidation (BetaOx) and Entner-Doudoroff. *Escherichia coli* employs a comprehensive strategy involving both engineered enzymes (Taq Polymerase and LuxA) and altered metabolic pathways (Krebs Cycle, Pentose Phosphate Pathway), along with combined modifications like CRISPR-Cas9 and Plasmid Insertion.

Genetic Engineering for Bioremediation: Genetically engineered bacteria play a crucial role in bioremediation by enhancing pollutant degradation capabilities. Techniques like CRISPR, TALENs, and ZFNs are employed to develop ideal genetically modified bacteria capable of degrading environmental toxins [29][25].

Gene Editing Tools: Gene editing tools like CRISPR-Cas9 have been utilized to improve bioremediation processes by enhancing the degradation of toxic compounds and pesticides [30]. Bioremediation Strategies: Strategies such as bioaugmentation, involving the addition of microbial populations to enhance pollutant removal, have been explored to optimize bioremediation processes [32]. Based on these works, it is evident that genetic engineering plays a significant role in enhancing the bioremediation capabilities of microbial strains. To further improve bioremediation efficiency, researchers could focus on optimizing genetic modifications tailored to specific pollutants, exploring synergistic approaches combining genetic interventions and metabolic pathway modifications, and conducting comprehensive assessments of the environmental impact of genetically engineered bacteria.

In conclusion, this research not only unveils the intricate chemistry governing organic pollutant degradation but also provides visual and tabulated insights into specific enzymatic and chemical processes involved, facilitating a comprehensive understanding for the development of targeted and efficient bioremediation strategies. The data-driven insights and future perspectives outlined in the discussion emphasize the significance of embracing microbial diversity and genetic engineering in advancing bioremediation practices and adapting them to diverse environmental challenges (*Pseudomonas putida*, *Bacillus subtilis*, and *Escherichia coli*).

Real-world Application: Integrating Disciplines for Environmental Solutions

This multidisciplinary study promotes a holistic approach by intertwining genetic engineering, microbiology, and chemistry to confront environmental challenges and propel bioremediation forward. The seamless integration of these disciplines optimizes their respective strengths, providing a comprehensive perspective that addresses immediate concerns and establishes the groundwork for sustainable solutions. The synergistic amalgamation of engineering, microbiology, and chemistry emerges as a robust force in bioremediation, holding promise for transformative advancements in environmental science.

Sustainable Solutions: Engineering Microbes for a Cleaner Tomorrow

Revolutionizing Environmental Solutions: The study pioneers the integration of genetic engineering into microbial strains for cutting-edge bioremediation, providing a promising avenue to combat environmental challenges. By synthesizing knowledge from microbiology, pollutant chemistry, and

engineering, the research crafts tailored solutions. Genetic modifications offer a customizable approach for effective environmental remediation. This interdisciplinary effort highlights the transformative potential of collaborative genetic engineering, fostering a commitment to a cleaner and healthier environment and paving the way for a more sustainable future.

Data-Driven Insights and Future Perspectives

This section dives into the findings derived from the study and outlines potential avenues for future research. The data analysis has yielded valuable insights into the effectiveness of *Pseudomonas putida*, showcasing its capability for thorough pollutant elimination. This success serves as a significant milestone in bioremediation, providing concrete evidence of the strain's prowess. The detailed examination of mRNA expression patterns in *Escherichia coli* reveals crucial information about its enhanced degradation mechanisms, paving the way for more precise and effective bioremediation strategies.

Pseudomonas putida Triumphs: Evidence of Complete Elimination

The data underscores the impressive performance of *Pseudomonas putida* in achieving comprehensive pollutant elimination. Through systematic experimentation and analysis, the study demonstrates the strain's efficient and thorough degradation of targeted pollutants. This success positions *P. putida* as a promising candidate for bioremediation projects requiring extensive pollutant removal. The evidence from the data sets a strong foundation for considering *P. putida* as a key player in environmental cleanup efforts, marking a significant advancement in microbial-driven remediation.

mRNA Expression Patterns in E. coli: Clues to Enhanced Degradation Mechanisms

An in-depth exploration of mRNA expression patterns in *Escherichia coli* reveals insights into the intricate mechanisms governing its enhanced pollutant degradation capabilities. Through an analysis of gene transcription during bioremediation, the study uncovers critical clues about how *E. coli* orchestrates its degradation processes. These insights into mRNA expression patterns deepen our understanding of the genetic regulation behind *E. coli*'s efficiency, providing a basis for refining its bioremediation potential.

Harnessing Microbial Diversity for Tailored Bioremediation Strategies

The study advocates for the strategic use of microbial diversity in developing tailored bioremediation approaches. By understanding and utilizing the unique capabilities of diverse microbial strains, researchers can design targeted interventions for specific pollutants and environmental conditions. This approach opens possibilities for creating customized microbial consortia that work collaboratively to address complex pollution scenarios. The future perspectives outlined underscore the importance of embracing microbial diversity as a fundamental aspect of advancing bioremediation practices and adapting them to diverse environmental challenges.

CONCLUSION

In conclusion, this comprehensive study successfully integrates genetic engineering, microbiology, and pollutant chemistry to advance our understanding of microbial-driven bioremediation. The genetically modified strains of *Pseudomonas putida, Bacillus subtilis,* and *Escherichia coli* exhibit enhanced capabilities in degrading organic pollutants, showcasing the potential of genetic modifications in optimizing microbial strains for environmental restoration.

The comparative analysis of pollutant removal efficiencies clearly illustrates the success of genetic engineering, with genetically modified *P. putida* and *B. subtilis* outperforming their wild-type counterparts. The mRNA expression patterns in genetically engineered *E. coli* provide insights into the specific mechanisms leading to improved pollutant degradation, highlighting the importance of gene upregulation in enhancing bioremediation properties.

Exploration of enzymatic powerhouses, such as Cytochrome P450 and Dioxygenases, sheds light on the key players in organic pollutant degradation. The comparative analysis of dioxygenase specificities emphasizes their substrate preferences, offering valuable information for the development of targeted bioremediation methods.

Chemical pathways and intermediates identified in *B. subtilis* growth provide a chemical perspective on how microorganisms utilize specific pathways to metabolize organic pollutants. This information is crucial for designing efficient and targeted bioremediation strategies.

The holistic approach taken in this study, bridging disciplines and integrating genetic engineering, microbiology, and chemistry, emerges as a potent force in addressing environmental challenges. The presented comparative analysis of engineered microbial strains (*Bacillus subtilis, Pseudomonas putida,* and *Escherichia coli)* offers valuable insights into their bioremediation potential, genetic modifications, and environmental impact. *Escherichia coli*, with the highest efficiency, maintains a minimal environmental impact, underscoring the importance of considering both efficacy and ecological consequences in strain selection.

The genetic modifications detailed in Table 5 further highlight the diverse approaches taken to enhance bioremediation capabilities, ranging from engineered enzymes to altered metabolic pathways. The discussion emphasizes the potential of genetic engineering in tailoring microbial strains for specific pollutants, paving the way for sustainable and targeted bioremediation solutions.

In summary, this research not only unravels the complexities of organic pollutant degradation but also provides a roadmap for future endeavors in microbial genetic engineering for enhanced bioremediation. The findings underscore the need for a nuanced and multidisciplinary approach to tackle environmental issues, offering promise for transformative advancements in environmental science and sustainable solutions for pollution mitigation.

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