Design Brief

Runoff remediation of atrazine and chlorobenzene via a novel *Pseudomonas putida* construct*

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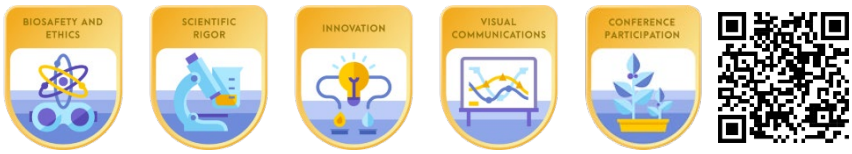
Atrazine and chlorobenzene are chemicals commonly found in pesticides and herbicides that threaten both humans and the environment as either endocrine-disrupting or organ-damaging pollutants. Our project design targets the challenge of chemical contamination in water and soil by implementing an innovative and sustainable bacterial-based remediator of these two chemicals. Utilizing Pseudomonas aeruginosa strain ADP's exceptional ability to consume atrazine as its nitrogen source, we intend to introduce the atzA, atzB, and atzC genes and the atzDEF operon responsible for atrazine breakdown into Pseudomonas putida, a compatible early colonizer in water and soil sources. P. putida strain GJ31 possesses a chlorobenzene degradation pathway, with pathway genes encoded on plasmid pKW1 and the chromosome. By introducing the synthesized atrazine catabolic plasmid pWRA-1 into P. putida GJ31, we can create a cost-effective and environmentally friendly approach to alleviate the hazards of two common toxins. Evaluating the effectiveness and safety of this design will demonstrate the effectiveness of atrazine and chlorobenzene degradation.

Keywords: Atrazine, chlorobenzene, remediation, runoff, *Pseudomonas putida*



Atrazine and chlorobenzene are chemicals commonly utilized in pesticide and herbicide formulations. In its purest form, atrazine is a white, odorless, synthetic powder. Since its introduction in the late 1950s, atrazine has been among the most heavily applied pesticides in the world. In the U.S., atrazine is the second-most widely used weed killer, with more than 70 million pounds applied across the nation each year (Conley, 2024). Atrazine is also used in Canada, Africa, and the Asia-Pacific region. In Europe, atrazine was used until 2004, when its application was banned in the European Union (Jablonowski et al., 2010).

Pure chlorobenzene is a colorless liquid. The production of chlorobenzene peaked in the 1950s when it was widely used as a compound in other chemicals serving medical and industrial purposes (Chlorobenzene, 2021). Today, it is used as a solvent in herbicides. Although the agricultural use of chlorobenzene is less frequent than atrazine, it is still prevalent in the U.S. and Asia (V., 2023).

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Both atrazine and chlorobenzene are proven to be harmful to humans and the environment. When atrazine is applied to soil, it will remain there for several days to several weeks (*Public Health*, 2011). In most cases, atrazine is broken down in soil throughout one growing season (*Public Health*, 2011). However, any atrazine that is washed from the soil into streams and other bodies of water will stay there for a long time as the breakdown of the chemical is slow in rivers and lakes (*Public Health*, 2011). Therefore, exposure to atrazine is most often contracted through contaminated bodies of water, which then impact drinking sources. Atrazine distributes itself to many parts of the body once it enters the bloodstream (*Public Health*, 2003).

The primary way atrazine can affect the body is by altering the functioning of the reproductive system. Atrazine is an endocrine-disrupting chemical that mimics, blocks, or interferes with bodily hormones, obstructing metabolism and ultimately damaging reproductive functions (Endocrine Disruptors, 2024; Mnif et al., 2011). There is an epidemiological connection between atrazine and low sperm levels in men, as well as an increase in preterm delivery (Public Health, 2003; Pathak & Dikshit, 2011, p. 1). There is no definitive consensus between scientists on whether atrazine has carcinogenic effects, but many studies point to relations between cancer and lifetime exposure to atrazine above the maximum contaminant level (3 ppb) (Pathak & Dikshit, 2011). A draft assessment from the Environmental Protection Agency found that atrazine puts 1,013 protected species at risk (Evans & Cox, 2016; Donley & Phillips, 2020).

Because atrazine predominantly contaminates bodies of water, amphibians are especially vulnerable to its exposure and health effects. Although the atrazine maximum contaminant level for humans is 3 ppb, amphibians are harmed by levels as low as 0.1 ppb (Hayes et al., 2010). Atrazine exposure in male frogs lowered testosterone levels, feminized laryngeal development, decreased fertility, and suppressed mating behavior (Hayes et al., 2010). In 2016, California recognized the pesticide as a reproductive toxin, but Hawaii is the only state to have the toxin banned (Evans & Cox, 2016; Donley & Phillips, 2020). Therefore, many Americans are still at risk of the negative side effects of atrazine exposure to this day.

Chlorobenzene also poses severe human and environmental health risks. Unlike atrazine, which is primarily concentrated in aquatic areas, water degrades chlorobenzene in less than 24 hours; however, it persists in soils for months (Pravasi, 2014). Humans may be exposed to chlorobenzene by drinking water or eating contaminated food, or by contacting chlorobenzene-contaminated soil on their skin. Such exposure is most likely to occur in the workplace or the vicinity of chemical waste sites; exposure to chlorobenzene is primarily occupational (Chlorobenzene, 1992). Proximity to chlorobenzene may cause damage to the lungs, kidneys, and liver (Chlorobenzene, 1992). Humans occupationally exposed to chlorobenzene exhibit signs of neurotoxicity such as numbness, muscle spasms, hyperesthesia, and decreased oxygen in the bloodstream (ToxFAQs™ for Chlorobenzene, 2020). Direct contact with skin caused burning and erythema at the site (Chlorobenzene, 2004). Tremors and restlessness were observed in animals under exposure to high concentrations of chlorobenzene (ToxFAQs™ for Chlorobenzene, 2020).

There exists no conclusive proof that chlorobenzene induces cancer; however, studies in animals have indicated that chlorobenzene produces liver nodules (Agency for Toxic Substances and Disease Registry U.S. Public Health Service, 1990). This may be a testament to its carcinogenic nature as certain cancer types start as small nodules dispersed throughout the liver.

Granular activated carbon filtration treatment plants treat wastewater and can reduce atrazine below 3 ppb. Yet, these plants require a significant capital investment (Albanito et al., 2015). Our synthetic biology design offers a more sustainable and cost-effective solution to the pervasive issue of chemical contamination in environmental settings. By harvesting the natural capabilities of *Pseudomonas* sp. ADP and *P. putida* GJ31, our construct facilitates the breakdown of atrazine and chlorobenzene.

Utilizing bacteria as a remediator promotes a cyclic, self-sustaining cleanup process, effectively leveraging the biochemical pathways that are enhanced to address these toxic contaminants. This innovative approach reduces reliance on industrial methods and ensures an enduring measure of detoxifying contaminated water and soil.

Systems level

This project aims to degrade chlorobenzene and atrazine in soil and water, thus preventing the dangerous ingestion of the two chemicals by humans and wildlife. In this design, we propose an atrazine and chlorobenzene super remediator bacteria. *P. putida* is an ideal chassis, as it can grow in soil that faces long-term temperature changes without any effect on its growth and survival (Fonseca et al., 2011). Although the ideal temperature for growth is 30°C, the bacterium can still grow in temperatures as low as 4°C (Fonseca et al., 2011). *P. putida* strain GJ31 contains genes from pKW1 and chromosomal genes that aid in chlorobenzene degradation (Kunze et al., 2009). The cultured bacteria will grow on a mineral salt medium supplemented with chlorobenzene to collect baseline data on the chlorobenzene degradation rates (Oldenhuis et al., 1989). We will synthesize a new plasmid, pWRA-1 (Figure 1), utilizing the polycistronic system, containing atrazine-degrading related genes from plasmid pADP-1–an atrazine degradation plasmid from *Pseudomonas* sp. ADP—and a GFP-selectable marker. The decision not to include pADP-1's open reading frames, transposase genes, insertion sequences, and transferrin genes into our constructed plasmid is essential to prevent unwanted recombination and instability within the host genome. This ensures the maintenance of essential genes and the reliability of expression of the gene of interest without interference from undesirable elements. As outlined in the devices section, the newly synthesized plasmid containing the atrazine-degrading genes will be transformed into the chassis, *P. putida* GJ31, via electroporation utilizing the Coleman lab protocol (Coleman Lab, 2021) (Figure 2). Selection for transformants relies

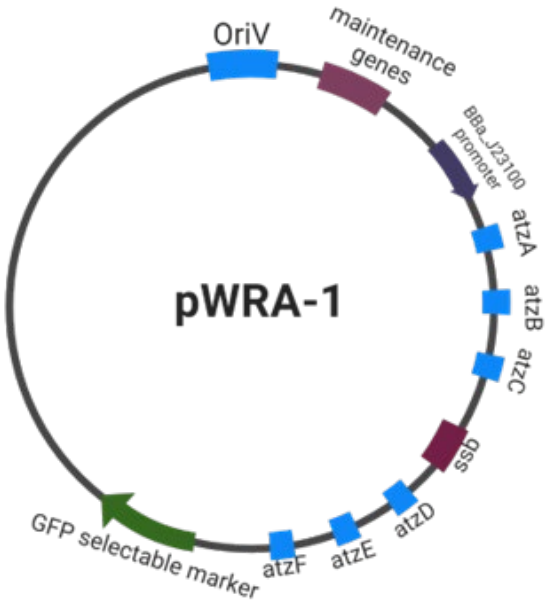

Figure 1. The pWRA-1 plasmid. The plasmid was designed to enhance the remediation of chlorobenzene and atrazine contamination, containing atrazine-degrading genes from pADP-1 and a GFP-selectable marker for transformant encoding.

on fluorescence, as coded by the GFP protein on the plasmid. Fluorescent transformants will be tested for atrazine remediation using an atrazine-supplemented mineral salt medium (Zhu et al., 2019).

Device level

Plasmid pKW1 is already present in *P. putida*, containing genes responsible for converting chlorobenzene to acetyl-CoA. We plan to synthesize genes from the pADP-1 plasmid and insert them into our chassis. This plasmid comes from *Pseudomonas* sp. strain ADP, which aids atrazine degradation in the surrounding environment. *Atz* genes, which degrade atrazine, convert atrazine to ammonia and carbon dioxide. For our design, we aim to synthesize relevant genes in the atrazine degradation pathway and genes necessary for successful translation, which includes the *atzA*, *atzB*, *atzC* genes, the operon *atzDEF*, the plasmid replication and maintenance genes near the origin of replication.

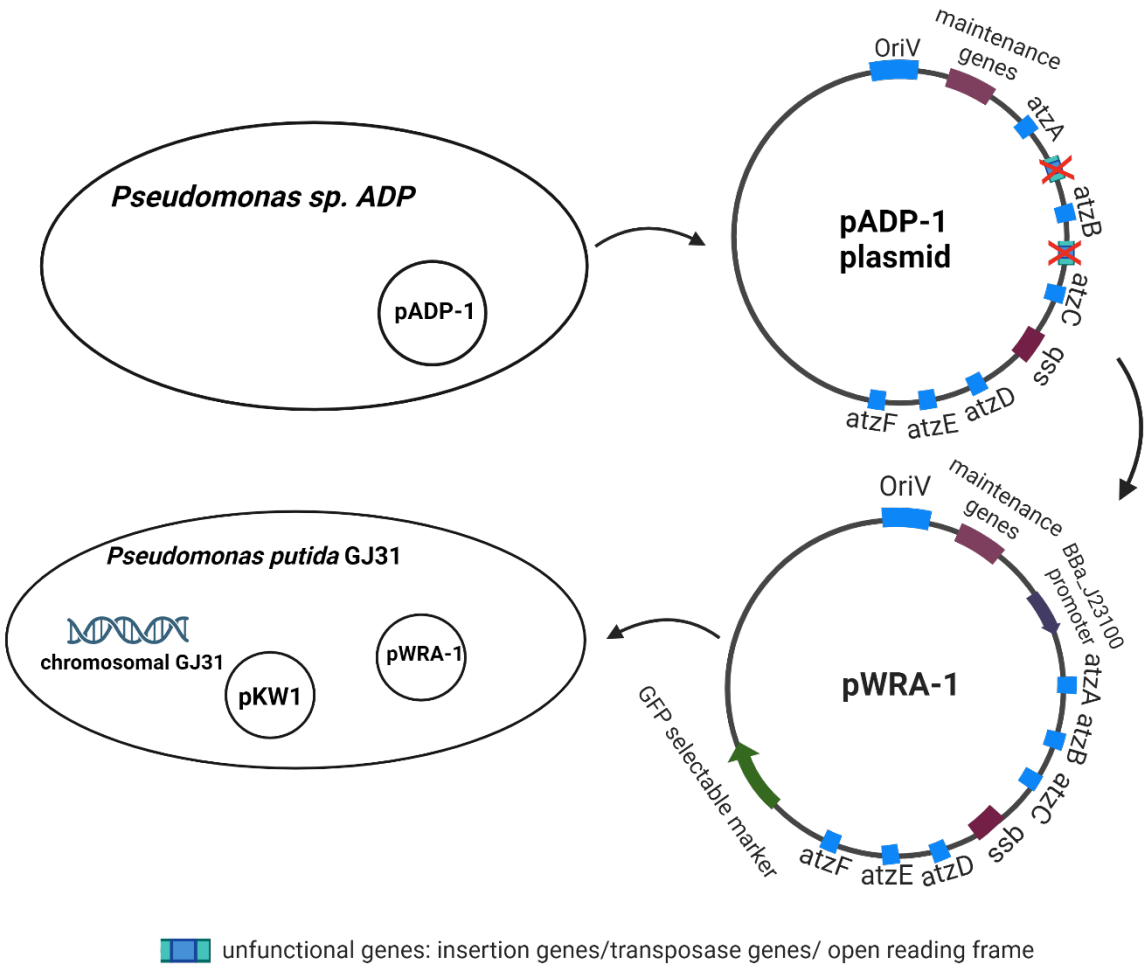

Figure 2. pWRA-1 construction and transformation in P. putida GJ3.

We aim to construct a polycistronic expression system, a typical structure in bacteria, where the genes *AtzA*, *B*, and *C* are all transcribed together (Tan et al., 2001). To achieve this purpose, we aim to synthesize *AtzA*, *B*, and *C* and operon *AtzDEF* under a constitutive promoter BBa_J23100 (*Part: BBa_J23100*, 2006). Spacer sequences and ribosome binding site sequences BBa_J61100 are placed between each *AtzA*, *B*, and *C* to ensure enzyme products are expressed as separate enzyme products (*Ribosome Binding*, n.d.). Since we have the DNA sequence of each of the needed genes for atrazine degradation, we are choosing to utilize a plasmid design company to build a synthetic plasmid. No cloning is needed, and it should speed up the process considerably. Lastly, we will insert a GFP selectable marker after *AtzF* to ensure transformation efficiency, enabling the discrimination between successfully transformed/ untransformed *P. putida*. We prefer GFP-selectable markers over antibiotic resistance due to the safety concern of releasing antibiotic-resistant bacteria into the environment. We will purchase part GFP selectable marker BBa_K608011 from the Registry of Standard Biological Parts, which includes constitutive promoter J23110 and genes from plasmid J61002. We will add the restriction enzyme to both ends corresponding to the vector's cloning sites, finishing plasmid construction.

Parts level

Plasmid pKW1, along with its functional chromosomal genes, contains four functional

genetic groups responsible for chlorobenzene degradation: a cluster chromosome codes for *CbzAaAbAcAd* and *CbzB* genes; a *CbzTEXGS* cluster, which codes for the *CbzE* and *CbzJ* genes; a *Nah* cluster which codes for *NahK*, *NahO*, *NahM*, and *NahL*; and an *Mhp* cluster which codes for *MhpF*, *MhpE*, and *MhpD* (Kunze et al., 2009). Those clusters function together to degrade chlorobenzene to acetyl-CoA (Kunze et al., 2009) (Figure 3).

We utilize plasmid pADP-1 derived from strain *Pseudomonas* sp. ADP to degrade atrazine in this design, and the functional genes in the atrazine degradation pathway are specifically *AtzA*, *B*, *C*, *D*, *E*, and *F*. *AtzA* encodes the enzyme atrazine chlorohydrolase, which removes chlorine from atrazine, converting it into hydroxyatrazine; *AtzB* encodes for enzyme hydroxydechloroatrazine ethylaminohydrolase that removes aminoalkyl side chains to form *N*-Isopropyammelide; *AtzC* encodes for enzyme *N*-Isopropyammelide Isopropyl amidohydrolase, which converts N-Isopropyammelide to cyanuric acid (Govantes et al., 2010). *AtzDEF* forms an operon that separately functions to degrade atrazine even further: *AtzD* codes cyanuric acid amidohydrolase, which converts cyanuric acid to Biuret, then 1-Carboxybiurethydrolase, which is encoded by *AtzE*, converts biuret to Allophanic acid; last, *AtzF*, which codes for Allophanate hydrolase, degrades Allophanic acid to ammonia and carbon dioxide (Govantes et al., 2010) (Figure 4).

Safety

While the Biosafety Level of our chassis, strain GJ31, is unspecified in previous research and ATCC, nearly all strains of *P. putida* fall under biosafety level 1 (Espinosa et al., 2023), and strain GJ31 has been used in experimentation beforehand (Kunze et al., 2009). Thus, GJ31 should not pose risks to healthy humans. Since *P. putida* strain GJ31 is naturally found in soil and water and can metabolize chlorobenzene naturally, the introduction of our bioengineered *P. putida* is not expected to impact the microbial community.

This design synthesizes *Pseudomonas* sp. ADP's atrazine degradation genes with a backbone to construct the pWRA-1 plasmid. Following pWRA-1 construction, we will transform the plasmid into the chassis, *P. putida* GJ31, via electroporation. Several safety and ethical considerations include gene pathogenicity to the recipient GJ31, specifically potential cellular component-degrading enzymes and genes that grant survival advantages. To address the risks of pathogenicity introduction, the genes from *Pseudomonas* sp. ADP will be thoroughly

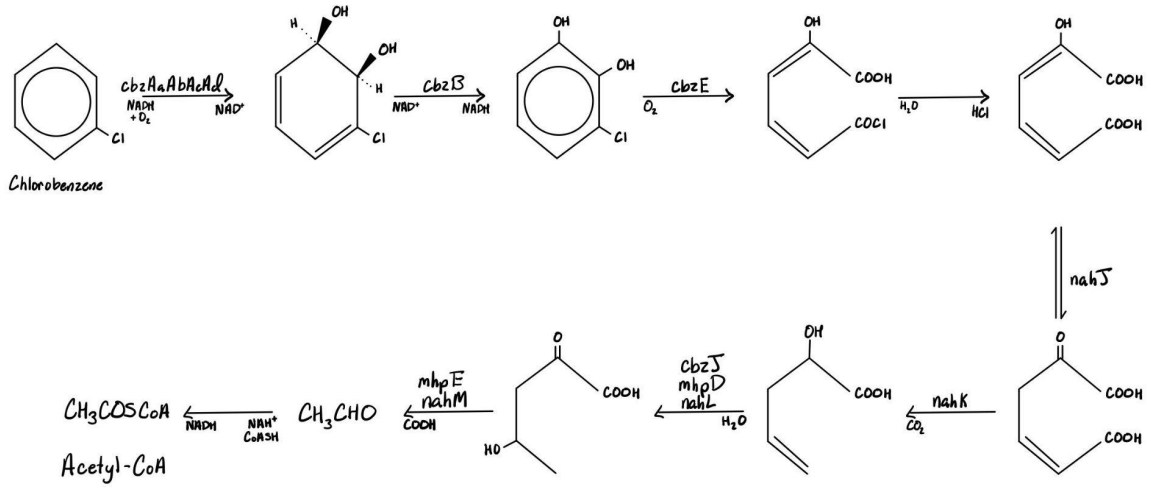

Figure 3. Chlorobenzene degradation pathway. Chlorobenzene degradation pathway and the genes involved. Each gene allows for the step-by-step breakdown of chlorobenzene into acetyl-CoA.

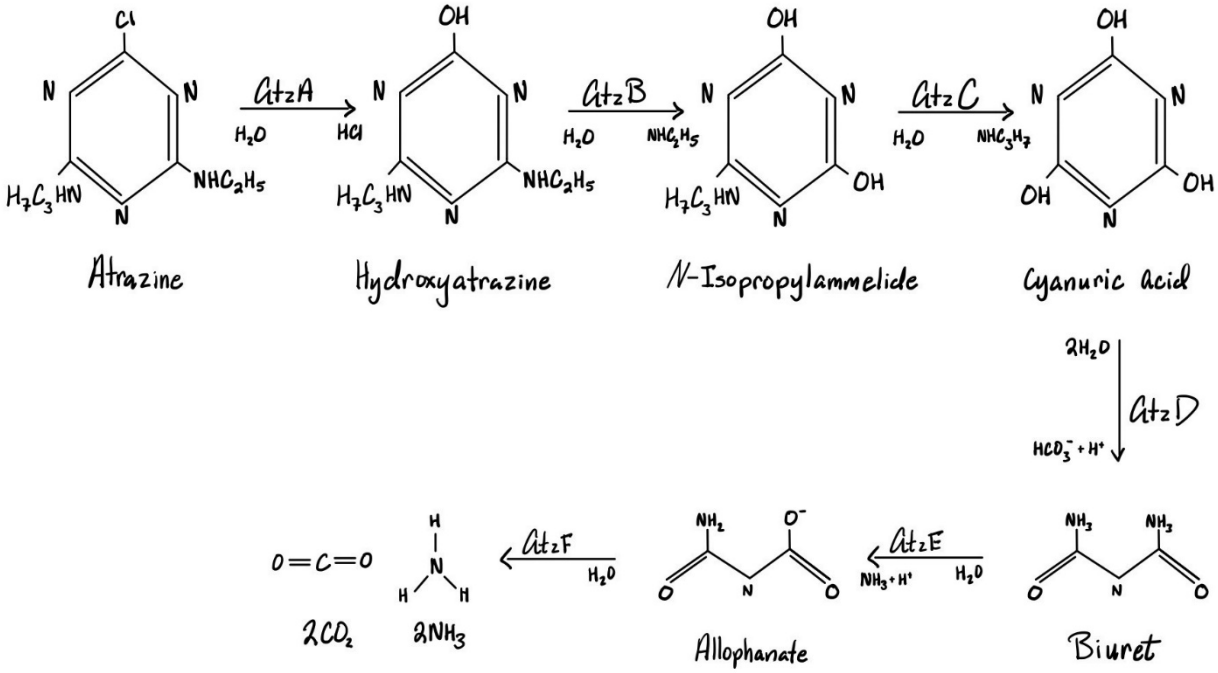

Figure 4. Atrazine degradation pathway. Atrazine degradation pathway and the genes involved. Gene AtzA encodes the enzyme atrazine chlorohydrolase, which uses H_2O to degrade atrazine into hydroxyatrazine, creating hydrochloric acid as a byproduct; AtzB codes for hydroxydechloroatrazine ethylaminohydrolase, and while converting hydroxyatrazine to N-Isopropyammelide, uses H_2O and releases a byproduct, NHC_2H_5 (Govantes et al., 2010). AtzC is the gene for N-Isopropyammelide isopropyl amidohydrolase; this enzyme uses H_2O to change N-Isopropyammelide to cyanuric acid and produces NHC_3H_7 (Govantes et al., 2010). Cyanuric acid amidohydrolase converts cyanuric acid to biuret using two molecules of H_2O, and it creates byproducts of HCO_3^- and a hydrogen ion (Govantes et al., 2010). Biuret is converted to allophanate by the enzyme 1-Carboxybiurethydrolase, found on gene AtzE, using a molecule of H_2O and producing NH_3 and a hydrogen ion (Govantes et al., 2010). The enzyme encoded by AtzF, allophanate hydrolase, uses a molecule of H_2O and produces two molecules each of ammonia and carbon dioxide (Govantes et al., 2010).

characterized and screened to minimize undesirable influences. We plan to implement bioinformatics tools to predict the constructed polycistronic system's functionality before transformation and pathogenicity tests to assess changes in chassis survival post-transformation (Naor-Hoffmann et al., 2022).

Although off-target effects were minimized through this design's approach of transforming a synthesized plasmid rather than using restriction digestion, the stability of the plasmid's genes within the chassis genome will be closely monitored. This monitoring will focus on unintended genetic modifications, including metabolic disturbances and interactions with endogenous genes. Techniques like plasmid curing–a method that inhibits plasmid replication and helps to genetically manipulate the removal of a plasmid from a bacterium (Trevors, 1986)–and retention assays, as well as regular PCR checks, will assess the plasmid stability to supervise gene loss, rearrangements, or mutations over successive generations (Trevors, 1986). Unintended genetic modifications are also subject to transcriptome analysis (Wahl et al., 2022), and metabolic profiling will assess modified GJ31's metabolic profile alteration, involving metabolites production comparison with negative control and unmodified parent strain under various experimental conditions (Mohd Kamal et al., 2022). Phenotypic characterization and fitness tests of successive generations aim to observe growth and behavior phenotypes and provide insights into functional disturbances

that might be undetectable at the genetic and molecular levels.

Before testing the bioengineered GJ31 in soil/water samples and simulative or testing fields, the bacterial degradation performances will first be assessed in atrazine and chlorobenzene-supplemented mineral salt medium. Our lab will comply with EPA regulations and obtain these chemicals from certified suppliers (Atrazine, 2023; Chlorobenzene, n.d.). To avoid risks associated with these toxic chemicals, chemical storage containers will be sealed and clearly labeled with hazard information, and secondary containment systems will prevent potential spills and leaks. Chlorobenzene, a flammable chemical, will also be stored in specific flammable storage cabinets to avoid associated risks. Lab personnel will be trained on evacuation, cleanup procedures, and the use of eyewash stations. We plan to place the spill kit (including absorbents, PPE, and disposable bags) near safety cabinets (Recommended Chemical, n.d.). The lab will keep rigorous documentation of chemical use, storage, disposal, and report incidents and exposures to regulatory institutions required by law.

During the entire experimentation process, we will install physical containment facilities to prevent the leakage of genetically modified organisms (GMOs) into outside environments and ensure the general safety of lab personnel. Following regulations from the MSDS, FDA, and EPA governing the use of harmful chemicals and GMOs, specific measures implemented will include wearing appropriate personal protective equipment, adhering to Biosafety Protocols, manipulating genetic modifications in biosafety cabinets, ensuring comprehensive training of lab personnel, and proper sterilization procedures for chemical and biological wastes. We also plan to handle volatile substances like chlorobenzene in fume hoods to avoid inhalation of fumes.

Potential risks with genetically modified bacteria may arise from release into the environment for actual applications (Molina et al., 2020). Though pre-release testing in simulative soil and water settings will be thoroughly implemented in varying temperatures, humidity, pH, and native bacterial settings—inadvertent consequences after environmental release remain possible. The primary concerns are contamination of soil and water ecosystems, unintended interactions with native microbial communities, and potential spread to unintended habitats. If our design is successful in degrading atrazine and chlorobenzene after testing, we will have proof-of-concept and can look into redesigning by adding initial safety features such as a kill switch. The kill switch, a model substrate-dependent suicide system, encodes a killing function and will be integrated into the Pseudomonas chromosome (Jensen et al., 1993). This will allow us to regulate *P. putida* in a controlled environment for future experiments.

Prior to any field testing, thorough environmental risk assessments will be conducted to evaluate potential ecological impacts and ensure compliance with regulatory requirements. These risk assessments will include tests for potential for horizontal gene transfer to species of the same kind; production of toxins or other harmful substances; and impacts on non-target and local organisms such as soil microbial communities, plants, and insects. The consequences of potential harm will be assessed by following the procedures illustrated in EPA's human health risk assessment sheet (*Human Health,* 2023). These assessments are necessary to study the quantity of the stressor present, prevent harmful exposure to humans, and assess its effects on the health of surrounding ecosystems (*About Risk,* 2023).

Discussions

This project aims to apply genetically engineered *P. putida* GJ31, integrated with a synthesized plasmid containing *Pseudomonas* sp. ADP genes, to break down harmful chemicals atrazine and chlorobenzene, thus creating safe and sustainable bioremediation for herbicide and pesticide-contaminated sites.

The two chemical contaminants this study aimed to degrade are atrazine and chlorobenzene, which have different typical environmental paths—atrazine is commonly found in industrial and agricultural runoff

affecting both soil and water, while chlorobenzene tends to accumulate more in soil. However, both contaminants could coexist in areas where industrial and agricultural activities overlap. As atrazine can migrate from the surface to deeper soil layers and potentially enter groundwater, it demonstrates the mobility of such contaminants between different environmental matrices. Given this, we think engineering a single microorganism capable of degrading both substances is beneficial. This approach ensures our remediation strategy is effective in diverse environmental settings, where either or both contaminants might be present.

Early consideration in chassis selection also involves constructing a microbial consortium that includes bioengineered *P. putida* for chlorobenzene degradation and bioengineered *P. aeruginosa* for atrazine degradation; nonetheless, our main concern about not using a microbial consortium is the risk of uncontrollable interspecies interactions. Engineering a single strain to degrade both pollutants can offer more stable and predictable performance. From the cultivation perspective, managing the growth conditions for one organism is simpler than maintaining optimal conditions for a consortium, thus making it easier to batch-grow the bacteria for application. *P. putida* is also known for its robustness in various environmental conditions. It can survive and function in soil and water environments where this design requires pollutant degradation. While P. aeruginosa is a human pathogen that can cause infections, *P. putida* is generally non-pathogenic, making it a safer choice for environmental applications where exposure to humans and wildlife can be a concern. *P. putida* is very commonly used in genetic modifications with accessible handling protocols, making the introduction of plasmids and other experimental approaches easier. In the meantime, from our background research, *P. putida* has a broad metabolic capacity that allows it to degrade a wide range of organic compounds, which makes it reasonable for us to utilize this versatility to include specific pathways for atrazine degradation.

Studies have shown that in non-sterilized soil, the growth of *P. putida* GJ31 was not inhibited by the indigenous microflora. Moreover, the added bacteria did not diminish rapidly when chlorobenzene was depleted; instead, they survived for at least 50 times their generation time. Considering the potential effect of easy-access carbon source on the efficiency of remediating target chemicals, we found that the addition of non-selecting carbon source peptone did not affect the chlorobenzene biodegradation rate nor the growth rate of chlorobenzene-degrading bacteria in soil suspensions (Oldenhuis et al., 1988).

However, several challenges are associated with using genetically modified bacteria for environmental applications. Limitations include potentially low degradation efficiency, which may extend over weeks or months. This prolonged process may occur at a slower rate than the accumulation of harmful chemicals, which would continue to pose environmental risks. Stresses from temperature, pH, and nutrient levels may also impact the efficiency, potentially resulting in incomplete degradation of chemicals. When applied to the environment, we will continuously monitor the remediator's performance and make ongoing improvements.

We realize that our design does not include the regulatory component AtzR, a transcriptional regulator of AtzDEF along with the original promoter for AtzDEF. In nitrogen-rich conditions, AtzR downregulates AtzDEF transcription to save cell resources (Govantes et al., 2010). If our design negatively impacts survival, reproduction, or remediation abilities in *P. putida* we will consider the incorporation of AtzR and other transcriptional elements into our design.

This project can also be applied to create a more comprehensive remediator, integrating genes from other bacteria into our modified plasmid. After modifying *P. putida* to include these genes, we will be able to broaden our design to combat additional runoff chemicals. Potential targets include glyphosate, alachlor, and per- and polyfluoroalkyl substances (PFAS) forever chemicals. Glyphosate can affect the blood-brain barrier and have short- and long-term effects on human and animal nervous systems, dysregulating important signaling

pathways (Costas-Ferreira et al., 2022). Alachlor is a "likely" human carcinogen (United States Environmental Protection Agency, 1998). PFAS may cause liver damage, thyroid disease, obesity, fertility issues, and cancer in humans (European Environment Agency, 2023). We can consider extending our project's reach to additional regions affected by different herbicides and pesticides.

Next steps

To determine optimal growth conditions for the bioengineered *P. putida*, we will culture the bioengineered strain in a CO_2 incubator with variegated pH, temperature, and humidity to observe the optimal culturing condition (Figure 5). Technicians will then collect the bacteria colonies and store them in inoculum tanks to cultivate and maintain a stable and viable population of bacteria ready for application. We will test the degradation functionality of the transformed bacteria in various soil and water samples to assess remediation efficiency under laboratory conditions. Soil tanks will be kept at a pH of 6.8 or 8.3, which is the ideal pH for *P. putida.* If the soil conditions do not fit these ideal pH levels, we could alter the pH with soil additives (Karpouzas & Walker, 2000). Sample containers will accommodate the exact weight of soil and sterile or bacterial water, and technicians will extract and test samples using chromatography or spectrometry to analyze the presence and concentration of chemical pollutants and metabolic byproducts of degradation pathways. Two controls and our modified *P. putida* will be used in soil samples with both atrazine and chlorobenzene. The first control will be *Pseudomonas sp. ADP* and our second control will be unmodified *P. putida* GJ31. This will allow us to observe plasmid compatibility and if we are hindering our strain's ability to degrade chlorobenzene or its growth rate. After exposing the bacteria to soil and water containing atrazine and chlorobenzene, we will monitor the degradation rate of both chemicals over time to outline the bioengineered bacteria's efficiency on different soil and water sources. At the same time, we will check the rate of plasmid retention by culturing the modified *P. putida* every 24 hours concurrent with lab testing. We can scale how often we test plasmid retention each week, decreasing frequency as each week passes. If the synthesized plasmid pWRA-1 is often lost, we will rethink our plasmid design and focus more on exploring ways to insert the atrazine degradation genes into the chromosome of *P. putida*. Recent research has displayed that plasmid retention in antibiotic-resistant microbes remains high despite the lack of antibiotic presence (Lau et al., 2013). We recognize that retention of the *Gfp* gene is different from retention of antibiotic resistance plasmids, but this factor is simply an unknown variable in our experimental phase. However, the research about plasmid retention in antibiotic-resistant microbes gives us confidence in our design.

If remediation is effective, we can conduct field trials in isolated real-world environments and evaluate the results in a test location compared to a negative control without the bioengineered bacteria remediator. Simultaneously, researchers

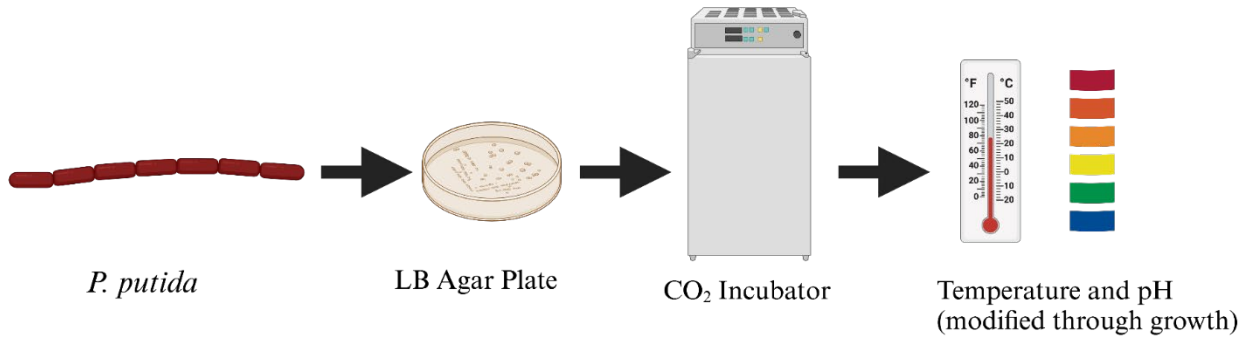

Figure 5. Optimal growth conditions for P. putida. Optimal growth conditions for P. putida, including maintaining colonies of the bacteria and stable pH levels.

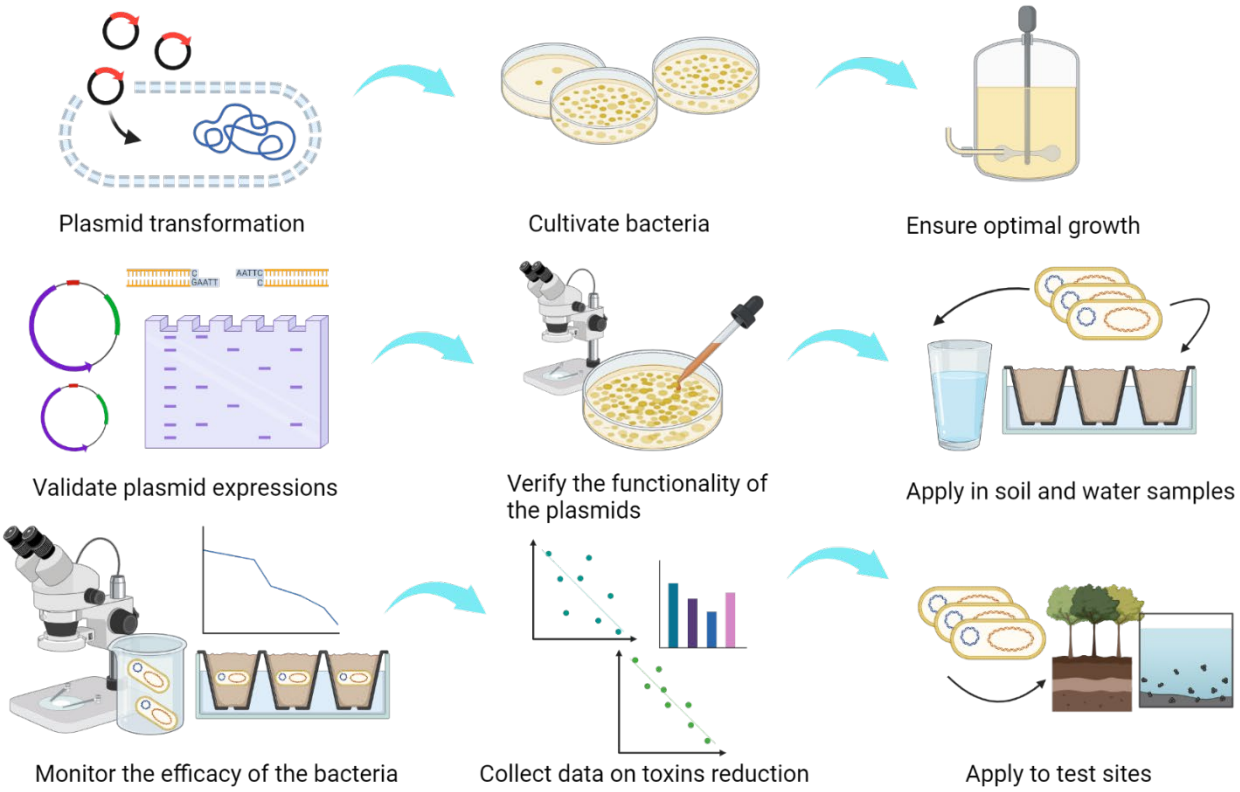

Figure 6. Process of culturing P. putida. Process of culturing P. putida, validating plasmid expression and verifying the effectiveness of the new bioengineered P. putida in both water and soil samples before being released into test sites.

must assess the environmental impact of genetically modified *P. putida* to evaluate ecological consequences (Figure 6). With successful results, we can seek approval for this project's use in atrazine and chlorobenzene remediation efforts.

Author contributions

L.W. came up with the original idea and began introductory research. Z.C., N.K., C.L., A.M., and L.W. conducted the early research process. J.F., I.H., C.H., S.K., N.K., Y.M., R.Q., C.R., B.S., A.S., L.W., C.X., and M.Z. contributed to the writing and proofreading of the paper. C.H., N.K., B.S., A.S., and C.X. animated the video. J.F., I.H., C.H., Y.M., C.R., L.W., and M.Z. designed the images and graphics for this project.

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