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The Utility of Synthetic Biology in the Treatment of Industrial Wastewaters

Monica Joshi and Jai Gopal Sharma†
Department of Biotechnology, Delhi Technological University, Delhi-110042, India
†Corresponding author: Jai Gopal Sharma; sharmajaigopal@dce.ac.in

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ABSTRACT

Effective treatment of industrial wastewater effluents before discharging them to the soil and water bodies has always been one of the paramount environmental concerns. The pollutants in untreated wastewater effluents have hazardous implications for human health and the ecosystem. Conventional physical and chemical processes of industrial wastewater treatment have many complications and they often fall short in the treatment of new and diverse varieties of pollutants. Several microbial strains in nature have shown their remediation property, but they possess limited efficiency in breaking down pollutants into non-toxic components. Synthetic biology is a perfect amalgam of two fields – biological science and engineering, and it has transformed our ways of understanding the functioning of complex biological systems. Researchers have reported that some engineered microbes can achieve remediation efficiency of up to 100% in specific pollutants such as heavy metals and hydrocarbons. For example, microbes like *Pseudomonas veronii* have been shown to reduce cadmium concentrations by up to 100%, and *Pseudomonas putida* has been shown to reduce phenol concentrations by 92%. Synthetic biology-based biosensors are also being developed for pollution monitoring and control of industrial wastewater. In this review, we discuss these advancements of synthetically engineered microorganisms in the treatment of industrial wastewater.

INTRODUCTION

Industries are the major driving force behind the growth and development of a country, but they also produce toxic effluents that are the leading cause of water and soil pollution (Saxena 2020). Effluents released from the manufacturing processes of petrochemical, textile, electroplating, pharmaceutical, and food industries constitute industrial wastewater (Nasr 2022). This effluent consists of a variety of organic and inorganic matter, dyes, and metal ions (chromium, cadmium, lead, copper, zinc, and nickel) (Nasr 2022). These contaminants cause the degradation of the natural biota of water, air, and soil by changing their physiochemical properties (Saravanan et al. 2023). Furthermore, untreated wastewater discharged into the river can cause several severe diseases like chronic dermatoses and skin cancer, lung infection, and eye irritation (Kesari et al. 2021). Hence, the wastewater discharged from these industries is the prominent cause of toxicity to human health and the environment (Singh et al. 2023). It has been reported that around 3-10 billion gallons of untreated wastewater is released into the environment, and an estimated 80 percent of wastewater is reused globally without any intervention of physical or chemical treatment (Saddique et al. 2023). The rise in the global population will also lead to the global rise of the concertation of discharged industrial wastewater. Untreated industrial wastewater pollutants contain harmful pollutants which, if entered into the water supply, would lead to deterioration of water quality (Jones et al. 2021) or, if entered into the food chain, would lead to severe allergies and other diseases. Hence, effective wastewater treatment processes are needed.

For the treatment of industrial wastewater, many chemical and physical techniques have been in application, like physical sedimentation, physical filtration, ozonation, chemical precipitation, adsorption, and ion exchange (Saravanan et al. 2021). However, they have many limitations, like high costs needed for optimum working and maintenance, low resource recovery from industrial wastewater, and utilization of high energy for the treatment processes to happen (Dutta et al. 2021, Yaashikaa et al. 2022). Biological methods that are utilized for the treatment of industrial wastewater are microbial remediation, aerobic treatment, anaerobic treatment, oxidation ponds, and activated sludge. These methodologies also have many limitations, such as the sludge disposable problem in the case of activated sludge methodology, the need for very large space to be successfully operational in the case of oxidation ponds, high capital cost, and odor nuisance in anaerobic treatment, cost expensive in aerobic treatment (Saravanan et al. 2021). Hence, we need a microbial wastewater system over a traditional wastewater system that would be green, sustainable (Sharma et al. 2023), and highly efficient. It has been long established that the intrinsic degradation property of microbes can be utilized in the degradation of environmental pollutants. This intrinsic property is applied in the bioremediation process where microbes, via their metabolic processes, degrade and convert the pollutants into less toxic substances. Despite this impressive ability of microbes, bioremediation has many limitations, like incomplete breakdown of organic pollutants, low efficiency of microbial remediation, and remediation process only effective for biodegradable contaminants (Jabbar et al. 2022). These limitations of microbial bioremediation processes can be improved by synthetic biology.

Using synthetic biology, we can modify metabolic pathways or/and create new metabolic pathways in new microbial cells that would be able to degrade not only biodegradable pollutants but also synthetic pollutants. The main synthetic pollutants occurring in the environment are pesticides, pharmaceuticals, polycyclic aromatic hydrocarbons (PAHs), phthalates, chlorinated phenol compounds, absorbable organic halides, and inorganic metal ions (Bhatt et al. 2021). Synthetic biology is a field that utilizes the principles of both biology and engineering field to create novel biological systems having modified or new functions. This is achieved by using molecular and computational tools that create the new genetic architecture of a microbe (Jiménez-Díaz et al. 2022). This new genetic architecture consists of a series of new components (gene promoters, transcription factors, enzymes, etc.) that together build new metabolic pathways with end products that could be studied and even re-build (Rylott & Bruce 2020). Hence, in the field of environmental remediation, advances in synthetic biology led to the construction of microorganisms that can scavenge and biodegrade a variety of toxic wastes like aromatic compounds, pesticides, microplastics, greenhouse gases, etc (Thai et al. 2023).

The integration of the field of synthetic biology and remediation (synthetic bioremediation) using microbes for the treatment of industrial wastewater has shown immense advancements in recent times. Many engineered microbial strains have shown their capability to degrade contaminants. Recently, Tomijiro Hara et al., in their experiment, engineered an *E. coli* strain capable of degrading a variant of Polychlorinated biphenyls (organohalide pollutants) in a natural landscape at a level that is sufficient for clean-up of PCB pollution (Hara et al. 2021). Sharma et al. reported that the genetically engineered *Pseudomonas* strain shows an enhanced ability of metal remediation in aquatic and terrestrial ecosystems near the industrial sites of pulp and paper (Sharma et al. 2021). Enzyme engineering within the microorganisms not only speeds up the remediation process but also solves the key limitations of chemical treatments and minimizes over-reliance on them. Additionally, microorganisms, with the help of synthetic biology tools, are created that can function as biological sensors (Aminian-Dehkordi et al. 2023). These biological sensors help in the real-time monitoring of pollutant levels and, hence, are an effective technique for monitoring water quality (Aminian-Dehkordi et al. 2023).

Synthetic biology can also be used to study the microbiota of industrial wastewater treatment plants using tools such as metagenomics, metatranscriptomics, metaproteomics, and metabolomics. These tools can help identify the key genes, enzymes, pathways, and microorganisms involved in pollutant degradation and reveal the relationship between running parameters and the keystone microorganisms (Jiang et al. 2022). In the world of bioremediation, synthetic biology could be used to design biosensors, enzymes with unique activities towards persistent organic xenobiotics, organisms that are resistant to challenging environmental conditions, robust biopolymers, artificial storage organelles for toxic metals, and much more (Rylott & Bruce 2020). Here in this review, we present synthetic biology approaches applied in the treatment of industrial wastewater. We begin with describing important components used in bioengineering including chassis, genetic circuits, transcription factors, and gene editing tools. Then, we move to specific synthetic biology ways for the treatment of industrial wastewater, where we describe strategies like metabolic pathway modifications, enzyme engineering, and synthetic microbial consortia for pollutant degradation. Furthermore, we discuss

biosensors for the detection of pollutants in industrial wastewater. Finally, the role of biofilms in enhancing the efficiency of biodegradation is discussed.

SYNTHETIC BIOLOGY COMPONENTS USED IN BIOENGINEERING

Sometimes, the term engineering biology is used as an alternative to the term synthetic biology (Zhang et al. 2023). The ultimate goal of synthetic biology is to bioengineer microorganisms having specific functionalities (Kim et al. 2016a). This goal is achieved by following the iterative "design-build-test-learn" (DBTL) cycle (Kitano et al. 2023, Garner 2021). In the first stage, the design of a biological system with pre-defined functionalities is created, followed by the build step, where simulations of that system will be conducted (Garner 2021). Assessment of differences between the performances of the simulation and the actual biological system in a real-life situation is conducted (test stage), and new insights are driven from it (learn stage) (Garner 2021).

Chassis

Despite the advancements in microbes-assisted remediation, bioremediation when using native microbes is not able to effectively degrade calcitrant pollutants because of genetic instability in microbes (Yaashikaa et al. 2022) and minor fluctuations in environmental like changes in pH, temperature, etc., could interfere with their ability needed for the degradation of the pollutants (Singh et al. 2021). Synthetic biology can overcome these challenges by building microbial systems as chassis for the bioremediation of these pollutants. In synthetic biology, chassis is referred to as a genetically modified model cell (McCarty & Ledesma-Amaro 2019). In a chassis, computational and molecular tools are used to engineer the genetic circuits and metabolic pathways to express desired results (Kim et al. 2016b). Most commonly, chassis organisms used in synthetic biology are the bacteria *Escherichia coli* and *Bacillus subtilis* and the yeast *Saccharomyces cerevisiae* (Tang et al. 2020, Kim et al. 2016b). These organisms have properties that make them a suitable chassis. These organisms are characterized by fast growth, supported by a wide variety of molecular tools. This led to an easy understanding of synthetic biology circuits and metabolic pathways (Kim et al. 2016b). Chassis can be constructed in two ways: top-down approach and bottom-up approach (Chi et al. 2019).

Genetic Circuits

To get a desired expression from the host cell, it is important to achieve precise control of gene expression, which is achieved by using synthetic genetic circuits. Synthetic genetic circuits are made up of networks of multiple interconnected gene switches that regulate cellular function by controlling the initiation and termination of a target gene expression (Xie & Fussenegger 2018). The synthetic biology field is primarily based on the construction of genetic circuits that could work either independently within cells or together by combining with a cell's biological network (MacDonald & Deans 2016).

Transcription Factor

Transcription is a crucial process as it is the fundamental step in the molecular processes that determine cell behavior and health (MacDonald & Deans 2016). Gene expression in a cell is initiated by the transcription process (Tietze & Lale 2021). The promoter situated upstream region of the gene's coding sequence determines the rate of transcription in a cell (Eisenhut et al. 2024). Therefore, scientists often alter the promoter region to regulate the strength of gene expression (Eisenhut et al. 2024). Hence, the most important regulatory point in a genetic circuit is transcription (Bradley et al. 2016). Scientists are aiming for better control of the genetic circuits by studying transcriptional networks in the cell.

Moreover, they are looking for endogenous trigger systems that turn on genetic circuits. With the help of these endogenous trigger systems, they can fine-tune the genetic circuits to find disease, bio-engineer new cell functions, and program cells with the ability to make independent decisions (MacDonald & Deans 2016). Another aspect of transcriptional regulation that makes it crucial for synthetic biology applications is that transcriptional regulation paves the way for a cell to regulate its enzymatic levels and stoichiometry, thereby preventing harmful metabolite accumulation, underutilized enzymes and the formation of unnecessary macromolecular compounds (Engstrom & Pfleger 2017).

Transcription in cells is regulated by transcription factors that activate or repress promoter activities (Tietze & Lale 2021). Synthetically engineered transcription factors are composed of a binding domain that identifies and then binds to a specific target DNA sequence and an effector domain that can activate or inhibit transcription (Eisenhut et al. 2024). Using in-silico technology, synthetic transcription factors can be created. Synthetic transcription factors for the regulation of gene expression are derived from proteins like zinc fingers (ZFs), transcription activator-like effectors (TALEs), and clustered regularly interspaced short palindromic repeats associated protein (CRISPR-Cas) (Chen et al. 2022). Synthetic zinc fingers are constructed by combining several zinc protein domains, where each zinc finger protein is typically made of 30 amino acids that are organized in a $\beta\beta\alpha$

motif. These engineered zinc fingers can identify 9-18 base pairs of specific DNA sequences, facilitating the targeting of desired sequences within the genome and thereby regulating gene expression (MacDonald & Deans 2016, Dai et al. 2018). On the other hand, TALEs are huge DNA-binding proteins having a size greater than 120 kilodaltons. These proteins have secretion signals on their N-termini end and nuclear localization signals on their C-termini end. The central helical domain region constitutes the bulk of these proteins as it is made of 15.5-19.5 near-perfect repeats of 34 amino acids and is responsible for the majority of DNA sequence-specific binding activity (Engstrom & Pfleger 2017). Recently, CRISPR-based tools have gained immense popularity for metabolism regulation, either independently or as part of a genetic circuit (Lv et al. 2022). CRISPRi (CRISPR-interference), a modification of CRISPR system, is used for sequence-specific transcriptional regulation. CRISPRi is made of two units – Cas9 endonuclease, which is catalytically inactive, and a single guide RNA (sgRNA), which precisely binds to targeted DNA at specific locations in the genome. Inactive Cas9, when targeted to promoter regions, acts as an RNA-guided repressor protein, which stops transcription by blocking polymerase movement through steric hindrance. With the help of this tool, multiple gene regulation can happen simultaneously by changing either the expression of Cas9 or the level of complementarity between the synthetic guide RNA (sgRNA) and the target sequence (Kent & Dixon 2020).

Gene Editing Tools

We can rationally engineer microbial cells at a global (genome) or local (gene) level by precisely modifying DNA sequences with the help of gene editing tools (Rafeeq et al. 2023). It has been shown in many types of research that genetically modified organisms are better at bioremediation processes than native microorganisms across various environments such as soil, groundwater, and activated sludge, exhibiting enhanced capabilities in degrading diverse organic pollutants as they show greater resistance to heavy metals, has greater substrate range, increase enzyme activity, and binding affinity (Haripriyan et al. 2022). The gene editing process involves the use of engineered enzymes (nucleases) that are able to create double-strand breaks in the DNA at desired locations. These double-strand breaks are then repaired by nonhomologous end joining (NHEJ) or homology-directed repair (HDR) (Barreiro & García-Estrada 2022). Key genetic tools employed for this are CRISPR-Cas, ZFN, and TALEN. Among these, CRISPR is the most effective and productive gene editing tool. CRISPR-Cas system has three main types – I, II, and III (Jaiswal et al. 2019). The Cas9 cleavage protein binds to the target sequence that is determined by the RNA guide molecules, which is the sequence after the protospacer adjacent motif (PAM) sequence in the genome. Following this, scientists can delete, correct, or insert genes into the break. Deletion occurs with the aid of non-homologous end-joining (NHEJ), and correction and insertion occur with the aid of homology-directed repair (HDR) (Gao et al. 2020). CRISPR-Cas system can modify the metabolic degradation pathway of specific pollutants in a microorganism by inserting or deleting the genes at multiple regions, thereby enhancing the bioremediation process (Ahmad et al. 2023). In a study, it was found that the biotransformation efficiency in *Bacillus licheniformis* reached a hundred percent after the deletion of the *yvmC* gene by employing the CRISPR-Cas9 technique (Rafeeq et al. 2023). When compared with the CRISPR-Cas system, both ZFN and TALEN cannot modify multiple genomic regions and can randomly bind to DNA sequences, leading to a much higher probability of off-target mutation (Jaiswal et al. 2019). The limitation of CRISPR-Cas is large Cas protein size leads to difficulty in cell delivery, the requirement of more whole genome data, and some chances of off-target mutation (Ranjbar & Malcata 2022). Hence, gene editing tools aim to achieve a better microbial bioremediation process by introducing diverse genes into the microbial cell to target specific pollutants (Hassan & Ganai 2023).

SYNTHETIC BIOLOGY WAYS FOR TREATMENT OF INDUSTRIAL WASTEWATERS

Microbial enzymes are responsible for the breakdown of toxic pollutants. There are four steps in the mechanism of microbial degradation of pollutants: 1. Microorganisms produce surfactants for the emulsion of pollutants; 2. The outer layer of the microorganism then adsorbs emulsified pollutants; 3. The adsorbed pollutant enters from the outer cell film layer to the cell layer via passive or active transport; 4. Upon entering the cell, the pollutant undergoes an enzymatic reaction with the corresponding enzymes to complete its degradation process (Yaashikaa et al. 2022).

In this new era of advancements in multi-omics techniques and genetic engineering, we can now select the most suitable microorganism host for the remediation process. Various tools based on genomic, proteomics, and metabolic data are employed to identify catabolic genes, novel pathways, or proteins involved in the biodegradation process so that we can engineer efficient metabolic degradation pathways for a target pollutant. Additional analysis of transcriptomic data can equip us with important information like cellular responses, post-exposure to toxic pollutants, and their influence on the metabolic state (Jiménez-Díaz et al.

2022). Synthetic biology synthesizes its components (gene promoters, transcription factors, enzymes, etc.) with the help of molecular tools and system biology, that together construct the metabolic degradation pathways with outputs that can be tested, reconstructed, and fine-tuned (Rylott & Bruce 2020).

CONSTRUCTION OF MODIFICATIONS IN METABOLIC DEGRADATION PATHWAYS

A series of general steps can be followed for the construction of modifications in metabolic degradation pathways: 1. Select your target pollutant and determine its physical and chemical properties; 2. Study the existing metabolic pathways in microorganisms that can degrade the target pollutants. Investigate the information of metabolites and enzymes that serve as the building blocks of the degradation pathway; 3. Select a suitable chassis based on multi-omics data analysis. Utilize molecular bio tools to introduce gene-encoding enzymes responsible for pollutant degradation into the chassis; 4. Optimize the synthetic metabolic degradation pathway. This can involve adjustments in the expression level of pathway genes and balancing metabolic flux. The pathway is shown in Fig. 1. For instance, *Pseudomonas sp. The B13* degradation pathway was modified, which led to increased efficiency in the degradation of methyl phenols and methyl benzoates (Ahmad et al. 2023). However, the process of screening the host for suitable enzymes that are capable of acting on the target pollutant is time-consuming. Additionally, conducting multiple biodegradation assays simultaneously in the laboratory poses significant challenges (Adetunji et al. 2023). Thanks to the advancements in computational techniques, in-silico bioremediation methods have emerged to cater to this problem. This in-silico approach relies on various scientific fields, including genomics, computational biology, proteomics, bioinformatics, molecular modeling, molecular dynamics simulation (MDS), and specialized algorithms for pathway prediction. Additionally, this approach also utilizes the dataset from several microbial databases. Furthermore, progress in sequencing technologies and bioinformatic algorithms has helped us in improving the bioremediation efficiency as these advancements led us to the further in-depth analysis of genomic sequences, which helped us in finding the innate metabolic enzymes and pathways for bioremediation (Tran et al. 2021).

The EAWAG Biocatalysis/Biodegradation Database, which contains the EAWAG-BBD pathway prediction system, can be used for the in-silico construction of metabolic degradation pathways. This EAWAG biodegradation database contains comprehensive information on microbial biochemical catalysis reactions that include biodegradation pathways for various chemical compounds and their microbial enzymes. By accessing this database, users can predict the degradation pathway of the target pollutant (Singh et al. 2020). Kelly et al. proposed a mechanism based on computational methods to find a suitable enzyme candidate for the degradation of toxic personal care products present in wastewater that are not effectively eliminated by conventional wastewater treatment. Computational analysis was done to study the biodegradability of the specific pollutant, and a pathway prediction system was utilized to find the metabolic pathways and enzymes that would react with the pollutant. They were successfully able to identify the enzyme with a degradation rate 40 times higher than previously reported rates (Aukema et al. 2017).

In addition to the design of the degradation pathway, the choice of chassis is also very important. For this, several microbial cells have been considered over the years, but none of them satisfies all the desired characteristics that are required in an ideal microbial degrader host. *Pseudomonas Putida,* which is soil bacteria, has emerged as a potential host degrader as it fulfills many criteria for growing and surviving in adverse environmental conditions and the ability to degrade recalcitrant pollutants (Dvořák et al. 2017). With the help of next-generation sequencing and omics data analysis, we can study biochemical catalysis taking place inside the microbial host as well as between the microbial host and the contaminant environment (Yaashikaa et al. 2022). The incorporation of synthetic metabolic pathways into actual producer strain has been

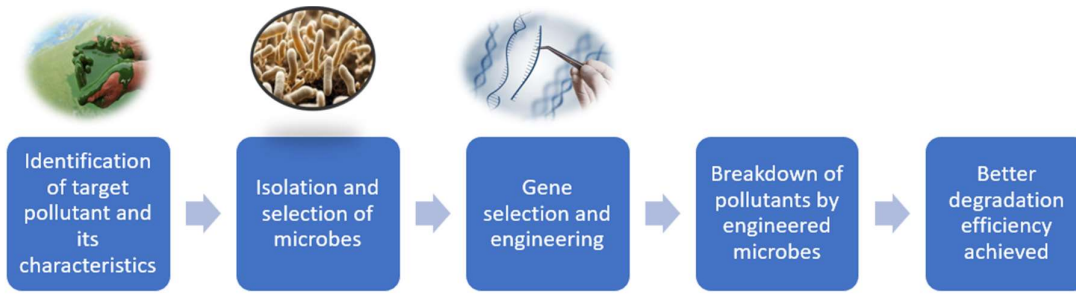

Fig. 1: Workflow of pollutant degradation using synthetic biology.

made easier by the new technological advancements in genetic engineering. DNA assembly tools, such as BioBrick assembly, Gibson assembly, Golden Gate assembly, ligase cycling reaction, single strand assembly, transformation-associated recombination (TAR) cloning, and uracil-specific excision reagent (USER) cloning, are examples. These tools have simplified the assembling process of multicomponent and large-sized gene clusters. DNA assembly tools have enabled the expression of assembled metabolic pathway genes using plasmids. Additionally, we can construct optimized metabolic pathway genes for expression in specific host strains (e.g., codon-optimized) using oligonucleotide and gene synthesis technologies (Choi et al. 2019). By engineering metabolic degradation pathways, we can prevent the production of toxic intermediates (Yaashikaa et al. 2022).

Enzyme Engineering

Over the past few decades, it has been reported that several bacterial species have the ability to produce enzymes. Many of these bacterial enzymes have shown their role in the mitigation or elimination of complex environmental pollutants (Singh et al. 2020). Enzymes are highly efficient biological catalysts, and they can transform toxic compounds into simpler, non-toxic compounds. Thereby, bacteria utilize these enzymes in their metabolic cycle for the effective removal of pollutants from the contaminant sites. Among the major classes of enzymes, oxidoreductases, and hydrolases are the classes that have been most extensively researched for their property of degrading toxic compounds into environmentally safer compounds. This is due to the fact that these enzyme classes possess high catalytic activity and the ability to target wide ranges of substrates, including xenobiotic pollutant compounds (Zhu et al. 2019). Over the past few years, researchers have been working on isolating microbial enzymes to use in biodegradation pathways instead of using the whole organism. This is because enzymes exhibit greater substrate specificity and a higher motility rate owing to their smaller size. However, we are still not able to fully utilize the potential of this technique because of some limitations like lower productivity, activity, and stability of enzymes, as they are very sensitive to the changing environmental factors, and that will have an adverse effect on their activity (Ahmad et al. 2023).

Also, for the enzymatic degradation of complex environmental pollutants like lignin in paper and pulp effluents, pesticides, aromatic hydrocarbons, and plastics, large quantities of enzymes need to be produced. However, these enzymes are produced in insufficient quantities (Aminian-Dehkordi et al. 2023). However, recently, some studies have shown how, by using recombinant DNA technology technique, we can produce large quantities of required enzymes. For example, according to a recent study, recombinant enzyme laccase was produced by various host organisms like bacteria, yeast, and filamentous fungi (Zhu et al. 2019). In another study, it has been shown that the production of recombinant hydrolases, such as lipases, carboxylesterases, and cutinases, has a high potential for pollutant remediation (Zhu et al. 2019). Furthermore, enzyme engineering can modify such properties in enzymes, making them have better catalytic activity and stability than native enzymes (Yang et al. 2021), so that they will be useful for onsite bioremediation of contaminated sites (Bhatt et al. 2021). Enzymatic properties of the rate-limiting enzymes of the important degradation pathways are extensively researched, and from that, important enzymatic data are analyzed (Yang et al. 2021).

The steps involved in enzyme engineering are as follows: 1. Protein engineering; 2. Modification of the enzyme; and 3. Screening of the modified enzyme (Bhatt et al. 2021). Protein engineering can be done in three ways – rational design (site-directed mutagenesis), random methods like random mutagenesis, and directed evolution. Rational design is a useful approach if we already know the structure and mechanism of the target protein. In a recent experiment, a novel fungal polyphenol oxidase was engineered by site-directed mutagenesis to produce different enzyme variants with increased catalytic activity and varying specificities for bioremediation of chlorophenols (Zhu et al. 2019). Another study reported that after the rational modification of the carboxylase enzyme, the biodegradation of a harmful phenol derivative was increased by forty percent, and the stereoselectivity property of that modified enzyme was increased by thirty-nine-fold (Aminian-Dehkordi et al. 2023). On the other hand, the directed evolution approach can yield enzymes having enhanced properties even in the absence of information regarding protein structure or catalytic mechanism (Zhu et al. 2019). For instance, using this approach, ligninolytic enzymes in fungi were modified with properties such as extreme pH resistance (Zhu et al. 2019).

Synthetic Microbial Consortia

Microbial cells rationally engineer their metabolic pathways by using metabolic engineering. The overall efficiency and functioning of these reconstructed pathways depend on various factors, including precursors, cofactor demands, and the optimal expression of pathway enzymes. However, sometimes, these reconstructed metabolic degradation pathways do not function as expected because of a metabolic burden on the microbial cell. To improve this limitation of metabolic burden in a cell, microbial co-cultures have been rationally designed to distribute the metabolic burden of complex and lengthy biosynthetic pathways across

different strains or species, thereby enhancing bio-production performance. This co-cultivation technique can successfully reduce the metabolic stress of engineered microbial cells caused by the overexpression of long and complicated metabolic pathways (Jawed et al. 2019).

When the population of two or more microbial species live together in a symbiotic relationship, this relationship is known as microbial consortia. The microbial consortia can perform complex metabolic processes that would be impossible for a single strain to do. A single strain of a microorganism is not able to produce different varieties of enzymes that are required for the complete degradation of the xenobiotic pollutants found in the environment. It has been observed that naturally occurring microbial consortia can degrade complex compounds but their degradation efficiency is less. They can degrade compounds like plastics, petroleum, antibiotics, azo dyes, and some pollutants found in sewage. We can enhance the degradation efficiency by constructing synthetic microbial consortia by adding new genetic materials and modules (Sharma & Shukla 2020).

The synthetic microbial consortia can be rationally constructed in two ways – top-down approach and bottom-up approach. The top-down approach involves a multi-omics study of microbial communities to understand the workings of the microbial system at the molecular level and then constructing novel communities based on this knowledge. Whereas the bottom-down approach involves rational engineering of microbial consortia based on genetic elements, modules, circuits, and metabolic pathways to obtain microbial consortia with improved efficiency, stability, and controllability. Considering the difficulty in making synthetically engineered cells, the bottom-up approach is mostly used for the construction of synthetic microbial consortia (Jia et al. 2016). Techniques that synthetic biology uses to engineer microbial cells can also be employed in engineering complex functions and behaviors of microorganisms in microbial consortia. This can be achieved by using tools such as intercellular signaling, exogenous inputs, and syntrophic interactions. All these tools, combined, can control factors like population level, task distribution, and spatial arrangements among the microorganisms in the microbial consortia (McCarty & Ledesma-Amaro 2019). In bioremediation, other than improved biodegradation efficiency, consortia also have other advantages like providing support for secondary applications of treated wastewater and the promotion of ecological sustainability (Sharma et al. 2023). In an experiment, the researcher created a consortium by combining the bio-surfactant producer *Rhodococcus erythropolis OSDS1* with petroleum hydrocarbon degraders *Serratia proteamaculans S1BD1, Alcaligenes sp. OPKDS2, Rhizobium sp. PNS1, and Pseudomonas sp. BSS9BS1*. This consortium exhibited higher efficiency and a wider range of capabilities in degrading petroleum hydrocarbons as compared to a single strain (Gao et al. 2020). Leong et al., in their experiment, showed a pollutant removal efficiency of 94% from municipal wastewater using microbial consortia involving microalgae and bacteria (Gao et al. 2020). Despite many advantages of using synthetic microbial consortia, many limitations of this technique are also there. These limitations include a limited understanding of bacteria interaction patterns and mechanisms, difficulty in rational designing efficient and functional microbial communities, and determining the roles played by these communities in different areas (Gao et al. 2020).

Another important point that should be considered when creating synthetic microbial consortia is the appropriate selection of chassis strains. It should be determined whether the chassis strains with efficient catalytic abilities would be compatible to co-exist with other strains. For this, strains with low mutation rates, no production of toxic by-products, and levels of high tolerance should be selected. Additionally, proper division of labor should be followed while dividing long degradation pathways into several strains. While synthetic microbial consortia reduce the metabolic burden on cells, excessive segmentation of metabolic pathways will also lead to confusion and disrupt efficient mass transfer among strains in consortia. Recent studies have reported an increase in the efficiency of degradation of complex compounds by microbial consortia if the strains in consortia are assembled in an orderly spatiotemporal manner. Assembly of spatial-linked microbial consortia can even involve incompatible microbial strains. This has great potential in the bioremediation field (Cao et al. 2022).

SYNTHETIC BIOLOGY APPROACH FOR DETECTION OF POLLUTANTS

The choice of method for removal of pollutants in wastewater is based on the contamination level of pollutants in the wastewater (Razzak et al. 2022). Microbes can act as very capable sensors because of some inherent qualities. Their small size enables them to be a highly sensitive small-scale detector that can sense, integrate, and react dynamically to various environmental factors. Biosensors are defined as genetically engineered microbes or independent biological components that are engineered to sense environmental signals and convert them to detectable output for us. They can also report microbial interactions in complex environments. The biosensor consists of the following modular features: 1. Sensor module – detects specific environmental conditions as input signals; 2. Processing module – calculate the input signals; 3. Output module – generates signals that can be

detected and measured. An important characteristic that a biosensor must possess to effectively study ecological processes is the capability to survive and perform in a contaminant region without getting interfered with by environmental conditions like temperature, hydration, pH, and substrate levels (Del Valle et al. 2021).

Microbial whole-cell biosensors comprise a genetic component that functions as a sensor to detect an analyte or a toxin and a reporter protein that generates a measurable output signal. These microorganisms' genomes or plasmids are genetically incorporated with the genes that are necessary for cellular response to the target analyte or toxin with the reporter protein. CFE biosensors are also known as cell-free transcription/translation systems. As the name suggests, they function independently of cellular processes; they are the isolated genetic circuits in the environment that sense and respond to the changing environmental factors. CFE biosensors have many advantages like no risk of plasmid loss and genetic instability and no need for biocontainment upon deployment. Ethical issues of using living microbes as a sensor can be avoided by using CFE. However, there is a major disadvantage to employing cell-free biosensors; at the industrial level, CFE biosensors have a high cost of production and deployment. Furthermore, with the use of bioinformatics tools, we can identify synthetic pathways to convert indetectable molecules into detectable ligands, constructing modular CFE for a wide variety of applications. Biosensor systems have modular and engineerable genetic components, which makes them highly optimizable and portable systems (Aminian-Dehkordi et al. 2023). Nowadays, we can engineer biosensors that are able to detect multiple environmental contaminants by eliciting a distinct signal for each kind of contaminant. These kinds of biosensors hold more significance in real-life situations as environmental regions are not contaminated by only one type of pollutant. Rather, they are contaminated by a wide variety of pollutants with varying physical and chemical properties (Yaashikaa et al. 2022). Whether a biosensor is suitable or not for a real-life situation is assessed by the following criteria: specificity and sensitivity to the target contaminant, range of its operation in the environment, and dynamic range. Computational tools and machine learning tools have fastened the designing and development of more advanced novel biosensors (Yu et al. 2023).

BIOFILMS IN WASTEWATER TREATMENT

Biofilm is the term coined for aggregated microbial communities that are permanently bound to the outer surface of living or non-living systems present in the environment and are encased by the self-generated layer of extracellular polymeric substance. The following steps take place in the biofilm formation: microbiome bound to the surface of the living or non-living system, synthesis of extracellular polymeric substance (EPS) around the microbiome, microbial communication via signaling, and the transition of the microbiome into planktonic cells (Chattopadhyay et al. 2022). Biofilm formation is dependent on several factors: the optimum presence of nutrients and energy required for their growth, the right environmental parameters in terms of geochemistry, the existence of inhibitors, predator microorganisms that feed on biofilms, and the effect of hydrodynamics on the transport of solutes which leads to mechanical stress on biofilms (Garg et al. 2023). Biofilms can survive harsh environmental conditions like changes in pH, occurrence of toxic chemicals and free radicals, and depletion of nutrients. Biosurfactants present in the EPS layer are involved in the formation of biofilms. These biosurfactants take part in the bioremediation process of environmental pollutants (Chattopadhyay et al. 2022). In a biofilm, pollutants are more bioavailable to the degrading organisms and microorganisms show enhanced potential in degrading pollutants. Microbial metabolism is responsible for this, and enzymes of the metabolic pathway convert environmental pollutants into non-toxic compounds (Muhammad et al. 2020).

CONCLUSIONS

With the global rise in the number of industries in the near future, there will be a tremendous rise in the volume of discharged wastewater from the industries as well. Wastewater contains various untreated harmful toxic wastes, which, when discharged into the water and soil, cause serious harm to the environment and human health. Therefore, finding novel, effective ways for the treatment of wastewater is very important. Herein, we have discussed how synthetic biology can help revolutionize the field of bioremediation of recalcitrant pollutants found in wastewater by designing effective and sustainable biological systems. Now, we can bioengineer novel microorganisms to target specific categories of pollutants and, hence, enhance the efficiency of wastewater treatment processes. Nowadays, more recalcitrant pollutants are identified in industrial wastewater, which cannot be treated by conventional bioremediation practices. Synthetically bioengineered microorganisms can solve this problem.

Moreover, the use of bioengineered microorganisms in different wastewater treatment systems, like activated sludge reactors, makes these systems highly efficient. By integrating synthetic biology techniques and these treatment systems, scientists can improve efficiency and save energy and cost. They can also design specific wastewater treatment

systems to meet the specific needs of different industries. Metabolic pathways for the degradation of target pollutants can be completely re-engineered. However, the task of optimization is difficult. Enzymatic properties can also be remodified. Synthetic biology-based biosensors are used to detect pollutant levels and also study microbial interactions with pollutants in a complex environment. Synthetic microbial consortia surpasses the limitations of the synthetic bacterium in the bioremediation of pollutants. However, many challenges limit the application of synthetic biology in the treatment of industrial wastewater pollutants. Modified microorganisms are still not able to effectively target some pollutants. We are still not able to fully comprehend the interactions between the pollutants and the microorganisms in the environment nor the interactions of the microorganisms with each other in the complex environment. It's taking time for the researchers to identify novel enzymes for bioremediation. We need to apply computational approaches to identify novel enzymes and also make them robust by necessary optimization. Furthermore, transferring synthetic microbes from the lab to the environment also leads to ethical issues. Synthetically designed microbes can disrupt the biosphere by gene transfer to other bacteria. In conclusion, many studies have shown the utility of synthetic biology in the bioremediation of pollutants but still, we need more research studies to address the challenges to harness the full potential of synthetic biology in the treatment of industrial wastewaters.

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