

Production of Amino Acids and Nucleic Acids from Genetically Engineered Microbial Cells and their Relevance to Biodegradation

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Abstract

The biodegradation of diverse organic compounds plays a pivotal role in environmental sustainability and waste management. In recent years, genetically engineered microbial cells have emerged as a promising tool to enhance biodegradation capabilities. This study presents an innovative approach to produce amino acids and nucleic acids by manipulating microbial genomes, shedding light on their relevance to biodegradation. Through the power of genetic engineering, microbial cells can be harnessed to efficiently degrade a wide range of pollutants, contributing to a cleaner and healthier environment. This study explores the transformative potential of genetic engineering in unlocking the biodegradative capacity of microbial cells and highlights its significance in the production of amino acids and nucleic acids. By leveraging these advancements, we can pave the way towards sustainable waste management and environmental remediation, ensuring a greener and more sustainable future.

Keywords: genetically engineered microbes, genetic recombination, bioremediation, recombinant DNA, amino acids, nucleic acids, environmental sustainability

1. Introduction

In a world grappling with environmental challenges and the urgent need for sustainable solutions, the role of genetic engineering in revolutionizing biodegradation capabilities has emerged as a beacon of hope. Genetic recombination processes in microorganisms, including conjugation, transduction, and transformation, as well as internal genetic rearrangements, have been identified as key mechanisms driving the development of novel traits and functionalities [1]. However, the significance of genetic recombination in industrial applications was initially overlooked due to its low occurrence rate. Recent advancements in genetic

engineering have unveiled its true potential, leading to remarkable breakthroughs in various fields, including amino acid production.

Genetic recombination processes play a pivotal role in reshuffling genetic material within microbial cells [2]. The movement of DNA segments, such as insertion sequences or transposons, contributes to internal genetic rearrangements, providing opportunities for genetic diversity and the acquisition of beneficial traits. Harnessing these mechanisms through genetic engineering has led to remarkable improvements in amino acid production [3]. For instance, by genetically transforming a strain that harbored all five mutations, α -amylase production skyrocketed to 250 times that of the original strain, demonstrating the transformative power of genetic recombination.

Moreover, genetic engineering has enabled the engineering of microbial strains capable of utilizing alternative carbon sources, leading to sustainable bioprocesses in amino acid production. By leveraging materials such as lignocellulosics or agro-industrial residue, these engineered strains offer a promising avenue for sustainable and resource-efficient production. Constructing amino acid-producing base strains requires meticulous modulation of gene expression at the transcriptional level [4]. This is achieved through the utilization of transcription regulators and attenuators, allowing for fine-tuning of gene expression and maximizing amino acid production.

Additionally, the development of novel platform strains has revolutionized amino acid production. These platform strains, engineered to possess reduced metabolic burden, improved metabolic efficiency, enhanced genetic stability, or being free from prophages, serve as foundational microorganisms for high-yielding amino acid production [5]. Recent advancements in functional tools, such as amino acid sensors based on transcriptional regulators and riboswitches, CRISPR interference (CRISPRi), small regulatory RNAs, DNA scaffold systems, and optogenetic control have significantly accelerated strain development [6]. These tools facilitate high-throughput screening, rapid validation of target genes, enhancement of overall metabolic efficiency, and light-inducible gene expression, empowering researchers to design and optimize strains with remarkable precision and efficiency.

In this study, we delve into the fascinating world of genetic recombination and its crucial role in industrial applications, specifically in amino acid production. We explore the mechanisms of genetic recombination, including conjugation, transduction, transformation, and internal genetic rearrangements, shedding light on their significance in reshaping microbial genomes for enhanced biodegradation capabilities. Furthermore, we investigate the recent advancements in genetic engineering techniques and functional tools that have accelerated strain development and revolutionized amino acid production. Through this exploration,

we aim to highlight the transformative potential of genetic recombination and its relevance in achieving sustainable bioprocesses and environmental stewardship.

2. Genetic recombination processes and recombinant DNA technology

The field of molecular genetics offers a powerful toolset for manipulating genes, allowing for the modification of gene expression and the production of microbial products, including novel recombinant proteins [7]. By genetically manipulating various microorganisms, compounds derived from plants or animals can be synthesized, resulting in enhanced production through environmental and other manipulations. In some cases, these manipulations have even led to increases of up to 1000-fold for small metabolites [7].

Microorganisms possess remarkable genetic plasticity, allowing them to adapt to various environmental conditions and acquire new traits through genetic recombination processes [8]. These processes, which include conjugation, transduction, transformation, and internal genetic rearrangements, play a pivotal role in reshuffling genetic material within microbial cells.

Conjugation involves the transfer of genetic material between two microorganisms through direct cell-to-cell contact. It typically occurs via a conjugative plasmid that carries the genes responsible for the transfer process. This mechanism facilitates the spread of beneficial traits, such as antibiotic resistance or metabolic capabilities, among microorganisms, contributing to their survival and adaptation [9].

Transduction, on the other hand, involves the transfer of genetic material from one microorganism to another through bacteriophages (viruses that infect bacteria). During infection, bacteriophages can incorporate fragments of the host's DNA into their viral genome. When a bacteriophage infects another host cell, it delivers this genetic material, leading to the transfer of specific genes. Transduction enables the horizontal transfer of genes and can play a significant role in disseminating virulence factors or metabolic genes among microbial communities [10].

Transformation is the process by which microorganisms take up free DNA from their surroundings and incorporate it into their own genome. This natural competence to acquire exogenous DNA allows microorganisms to benefit from genetic diversity and adapt to changing environments. Transformation is widely observed in various bacterial species and has been extensively utilized in laboratory settings for genetic manipulation and engineering [11].

Internal genetic rearrangements involve the movement of DNA segments within microbial genome, such as insertion sequences or transposons. These mobile genetic

elements can “jump” from one location to another, causing genetic rearrangements that can lead to the acquisition or loss of specific traits. Internal genetic rearrangements contribute to the genetic diversity of microorganisms and play a role in their ability to adapt and evolve [12].

Understanding these genetic recombination processes is crucial for harnessing the potential of microorganisms in various industrial applications. By leveraging these mechanisms through genetic engineering, scientists can introduce specific genes or modify existing ones to enhance desired traits or functionalities in microbial cells. This enables the development of microbial strains with improved biodegradation capabilities, increased production of valuable compounds, or enhanced tolerance to adverse conditions.

Recombinant DNA technology, sometimes called gene cloning or *in vitro* genetic manipulation, has greatly broadened the scope of what is possible when it comes to altering the genetic makeup of microorganisms. Therapeutic proteins, antibiotics, small compounds, biosimilars, and other economically relevant goods are now possible because to this technique [13]. The utilisation of restriction endonucleases, polymerases, and DNA ligases permit accurate slicing and pasting of DNA fragments, and form the backbone of this technology. After being introduced into a bacterial cell, foreign DNA pieces may be replicated by inserting them into a vector molecule, such as plasmid or bacteriophage. In the biotech sector, the capacity to alter and clone genes has substantially sped up the rate of discovery and development [14].

The following is a breakdown of the fundamental processes required for DNA cloning (Figure 1). Recombinant DNA [15] is created by: (i) incorporation, in which a DNA fragment is placed into a vector, a DNA molecule that serves as a carrier. (ii) The recombinant DNA is transferred into a host cell, where it may multiply and produce an abundance of offsprings. While bacteria are often employed as the host, other species may also be used to replicate the recombinant DNA. (iii) Replication: The vector replicates itself when the host cell divides, passing on the recombinant DNA to the daughter cells. A colony or clone of host cells containing identical recombinant DNA is generated by cell division and subsequent replication of one or more copies of the recombinant DNA [16]. (iv) Identification and isolation: The colony containing the desired recombinant DNA is identified and isolated. Various techniques, such as screening for specific markers or utilizing selective growth media, can be employed to identify the desired colony [16]. (v) Analysis: The isolated colony carrying the recombinant DNA undergoes analysis to confirm the presence and characteristics of the desired DNA fragment. Techniques like DNA sequencing or restriction enzyme digestion can be employed to verify the identity and integrity of the recombinant DNA [16]. (vi) Sub-culturing and maintenance: Once the colony with the desired recombinant DNA is identified and analyzed, it can be sub-cultured and maintained as a recombinant strain. This involves

transferring the strain to new growth media or storage conditions to ensure its continued propagation and availability for further experiments or applications [17].

Figure 1 depicts a simplified representation of the DNA cloning process, and each step involves specific techniques and procedures; an overview of the key stages involved in DNA cloning, from the incorporation of a DNA fragment into a vector to the sub-culturing and maintenance of the recombinant strain is also highlighted.

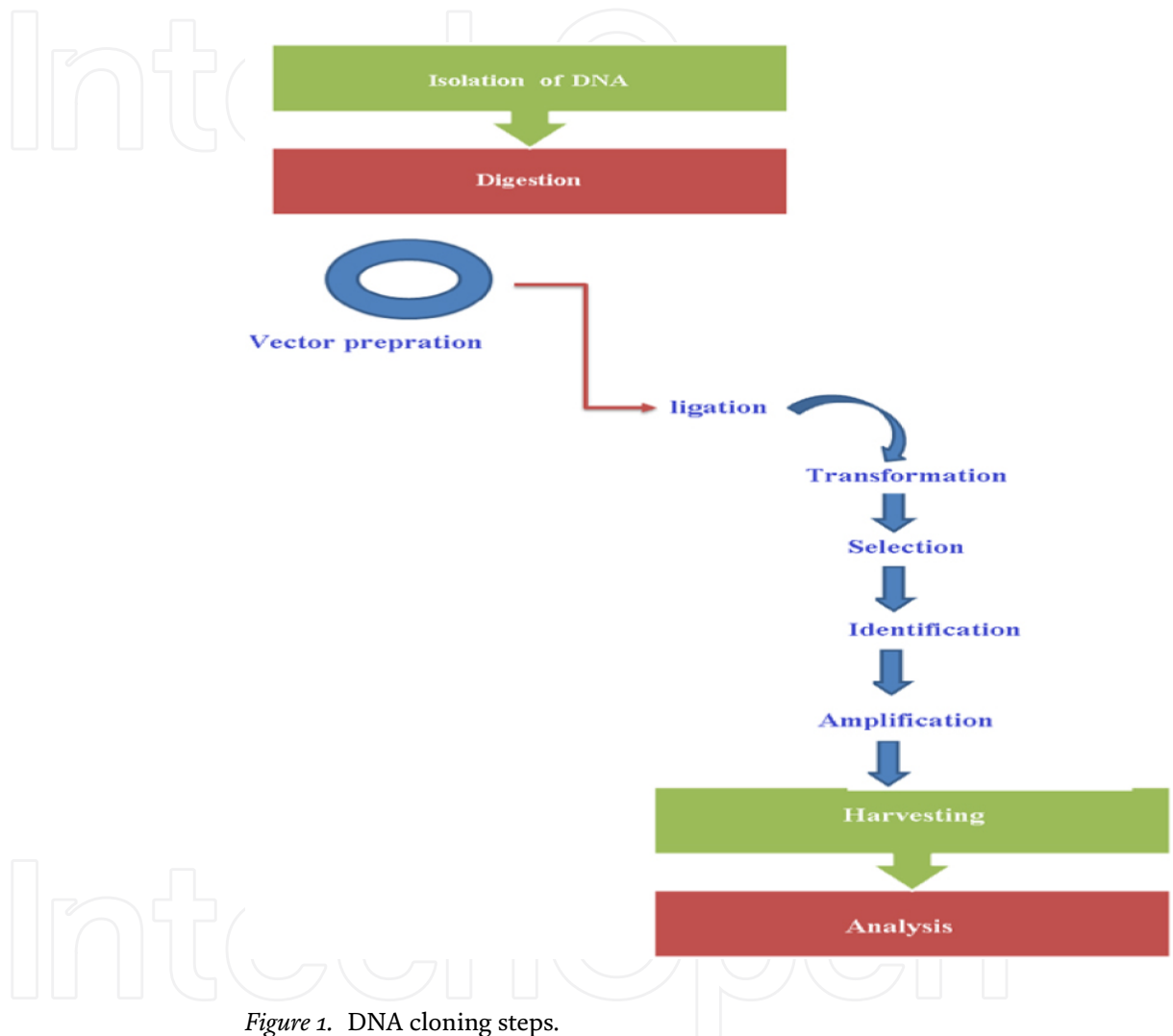


Figure 1. DNA cloning steps.

The process of utilizing hyperaccumulator plants, which have the ability to survive and accumulate high concentrations of metals, is referred to as phytoextraction [18]. Rhizoremediation involves the collaboration between plants and their associated rhizosphere microbes to reduce the levels of heavy metals (HMs) or other pollutants in the soil. This can be achieved through the use of naturally occurring rhizosphere microbes or the addition of specific bacteria [16]. In times of stress, these microorganisms can act as pollutant degraders or promoters of plant growth. Plant-growth-promoting rhizobacteria (PGPR) have long been employed to support

plants in nutrient absorption and disease prevention [19]. Recently, the application of PGPR has been expanded to include bioremediation of both organic and metal contaminants [20]. The symbiotic relationship between Rhizobium bacteria and legumes is an effective strategy for rhizoremediation of HMs in soil. This association not only helps in the removal of HMs but also enriches the soil with nitrogen through dinitrogen fixation in plant root nodules. Legumes, widely cultivated in various environments, form a symbiotic relationship with nodule-forming, nitrogen-fixing bacteria, resulting in symbiotrophic nitrogen feeding [21, 22]. Legumes also establish an associative relationship with PGPR and endophytic microbes, which has implications for plant development, nutrient uptake, and disease control. The enhanced symbiotrophic potential of legumes plays a crucial role in improving soil fertility, promoting soil biota activity, enhancing biodiversity, supporting soil formation, and contributing to the overall health of ecosystems [23].

Table 1 provides an overview of various techniques and methods employed in the genetic manipulation of microorganisms for biotechnology.

Table 1. Techniques for genetic manipulation of microorganisms in biotechnology.

| Technique | Description + References |
|----------------------------------|---|
| Gene knockout | Inactivating or deleting specific genes within the microorganism's genome [21] |
| Gene overexpression | Increasing the expression levels of specific genes to enhance the production of desired products [22, 24] |
| Gene silencing | Downregulating or inhibiting the expression of specific genes [25] |
| Gene insertion | Introducing foreign genes or DNA fragments into the microorganism's genome [13] |
| Gene editing (e.g., CRISPR-cas9) | Precise modification of specific genes or genomic regions using genome editing tools [26] |
| Horizontal gene transfer | Introducing genes from one microorganism to another through mechanisms such as conjugation or transduction [27] |
| Synthetic biology | Designing and constructing artificial genetic circuits and pathways for desired functions [28] |
| Directed evolution | Using iterative rounds of mutation and selection to improve the properties of enzymes or microorganisms [29] |

2.1. Genetic engineering applications: bioremediation and protein/amino acid production

Bioremediation using genetically engineered microbes has emerged as a promising approach in addressing environmental contamination. Bacteria possess unique

genetic elements known as jumping genes, which enable them to develop biological tolerance to various harmful substances in the environment. Two primary approaches have been employed for bioremediation of contaminated soils: ex-situ and in-situ methods [30]. The ex-situ procedure includes the elimination of pollutants from soil and water, whereas the in-situ procedure involves managing the polluted locations directly. The ex-situ approach has been used for groundwater remediation and soil excavation despite the fact that it is expensive and often delivers poor metal extraction effectiveness. When it comes to in-situ bioremediation, however, sulfate-reducing bacteria are used to produce hydrogen sulfide (H_2S), which assists in the reduction and precipitation of metals in a sustainable and cost-effective way [30]. Physical, chemical, and biological processes all contribute to natural attenuation, which lessens the danger and mobility of underground pollution. Bioremediation relies heavily on microbial processes. Biosorption is one such method, which involves microorganisms removing metals by attracting them to their negatively charged cell membranes. Lead (Pb) and cadmium (Cd) are two examples of heavy metals that may be effectively removed from water by microbial biosorption. Biostimulation is another strategy for increasing the effectiveness of bioremediation in polluted areas; it entails fostering microbial growth and development by manipulating pH, nutrition, and oxygen levels [31]. If naturally occurring microorganisms aren't doing enough to clean up polluted regions, bioaugmentation may introduce genetically modified bacteria to boost their efforts. Bacteria may degrade metals and oxide minerals in polluted areas under the right circumstances. Metals may be detoxified from inorganic to organic and vice versa by microbial reduction processes, since microorganisms cannot destroy inorganic metals but they can change their oxidation states. Microbes may harness energy from harmful compounds, allowing them to multiply rapidly and produce a high volume of biomass via breakdown. Using microorganisms in both aerobic and anaerobic settings enables bioremediation [32]. Microbes may break down complex hydrocarbons by aerobic and anaerobic respiration. The aerobic process does not generate harmful byproducts like methane or hydrogen sulphide and is quicker and more efficient, if enough oxygen is present. In contrast, complex hydrocarbons are biologically reduced to smaller molecules during the anaerobic process. Advantages such as low cost and great efficiency in energy use make anaerobic bacteria an attractive option for increasing the pace at which waste molecules are broken down [33]. Environmental biotechnology highlights the potential of various microbes, such as bacteria, yeast, and filamentous fungi, in removing heavy metals from aqueous solutions. Utilizing microbial metabolic capabilities for toxin removal in contaminated areas is a safe and cost-effective approach. Bioremediation has shown success with the use of genetically modified bacteria, recombinant DNA, and RNA technology [16]. By modifying microbial genes to develop new metabolic pathways, bioremediation processes can be

enhanced. Genetically engineered (GE) microorganisms are often favored due to the unique characteristics of their metabolic pathways, making them a promising technology for bioremediation [34]. GE bacteria, with their advanced capabilities, have garnered attention for their effectiveness in cleaning up toxic waste and heavy metals from contaminated sites [35]. They play a significant role in the detoxification of heavy metals and other challenging-to-remove substances [35]. The accumulation of HMs can be accelerated using GE microorganisms that express metallothioneins (MT) [36]. Mercury, in particular, is an environmentally hazardous HM that can be effectively removed from contaminated water, soil, or sediment using the *Escherichia coli* strain JM109, which has been genetically engineered for mercury removal [37]. GE bacteria containing the MerA gene have also demonstrated the ability to remove mercury from contaminated sites [38]. Mercury bioremediation can be facilitated by genetically engineered bacteria that express metallothioneins and polyphosphate kinase [39]. Genetically engineered organisms like *Deinococcus radiodurans* and *Pseudomonas putida* have demonstrated the capability to degrade organic pollutants in contaminated areas [40]. *Deinococcus radiodurans*' exceptional radiation resistance, initially studied for its DNA repair mechanisms, has been harnessed for bioremediation purposes. Researchers have successfully engineered this bacterium to withstand not only ionizing radiation but also harsh environmental conditions present in contaminated sites. Genetic modifications have conferred increased tolerance to a variety of organic pollutants, including hydrocarbons, aromatic compounds, and HMs. This enhanced tolerance allows *Deinococcus radiodurans* to thrive in polluted environments with high levels of these contaminants. Engineered strains of *Deinococcus radiodurans* have demonstrated the ability to degrade diverse organic pollutants, such as polychlorinated biphenyls (PCBs), aromatic hydrocarbons, and even uranium. These bacteria can effectively break down complex organic molecules into less harmful byproducts [41].

Engineered *Deinococcus radiodurans* strains have been employed in the bioremediation of radioactive waste sites and areas contaminated with various organic pollutants. Their robustness and ability to survive in extreme conditions make them valuable candidates for cleaning up contaminated environments, including nuclear waste sites [42]. *Pseudomonas putida* strains have been extensively engineered to efficiently degrade a wide range of aromatic compounds, such as toluene, benzene, and xylene. Genetic modifications have significantly enhanced their metabolic pathways for the breakdown of these pollutants [43].

Researchers have explored the biofilm-forming capabilities of *Pseudomonas putida* to create robust microbial communities that can degrade pollutants in situ. Biofilm-based bioremediation strategies have shown promise in contaminated groundwater and soil, offering a means of concentrating and degrading contaminants efficiently [44].

Advances in genome editing techniques, particularly CRISPR-Cas systems, have enabled precise modifications of *Pseudomonas putida* genomes. This allows for the optimization of pollutant degradation pathways and metabolic efficiency, resulting in enhanced pollutant removal.

Engineered *Pseudomonas putida* strains have been deployed in pilot-scale and field-scale bioremediation projects, demonstrating their potential for practical and large-scale pollutant cleanup in various environmental settings [45].

Organophosphates, commonly used as insecticides in agriculture, have caused significant environmental damage. Recombinant *Anabaena* has been shown to effectively degrade more than 98% of Lindane in rice fields within 6–10 days [46]. GE *E. coli* and *P. putida* containing phenol catabolic genes have been found to break down trichloroethylene. Additionally, GE *P. putida* S12 has the ability to metabolize naphthalene, toluene, and biphenyl after the introduction of plasmids containing genes for the catabolism of these pollutants following the introduction of plasmids containing genes for the catabolism of these pollutants [47]. The cadmium (Cd) in industrial effluents [48] may be removed by using a recombinant *Caulobacter* spp. strain JS4022/p723-6H. Genetically modified bacteria carrying the *ArsM* gene may be used to remove arsenic (As) from polluted soil by volatilization. *ArsR*-carrying *Escherichia coli* increases arsenic bioaccumulation in contaminated soil [49]. GE bacteria carrying the *ArsM* gene have been demonstrated to facilitate the extraction of arsenic (As) from polluted soil through volatilization. Additionally, *Escherichia coli* equipped with the *ArsR* gene can enhance the bioaccumulation of arsenic in contaminated soil [50]. The GE *E. coli* SE5000 strain [51], classified as a “persistent pollutant,” may absorb Ni from aqueous solutions. Therefore, genetically designed bacteria can help with heavy metal bioremediation. For effective bioremediation, the use of genetically designed organisms that can battle environmental toxins is essential, and recombinant DNA technology provides a viable technique for doing so [52]. HMs, chlorinated hydrocarbons, pesticides, petroleum hydrocarbons, and explosives may all be removed from polluted places using DNA technology, as described by [53]. Mutagenesis techniques like DNA shuffling may be used to create biocatalysts and novel enzymes with improved degradation rates for chlorinated ethane and polyaromatic hydrocarbons [54]. It has been shown that horizontal gene transfer, a crucial part of bacterial evolution, plays a substantial role in this field [54]. The discovery of numerous degradative routes for the partial or total breakdown of dangerous pollutants is a major contribution of recombinant DNA technology for bioremediation of contaminated environments. Degradation of xenobiotic pollutants is a common use of this technique. Microbial communities containing 16S rRNA sequences, such as *Geobacter* species, can oxidize organic pollutants by reducing Fe³⁺, making them important for the degradation of trichloroethene (TCE) [55]. Phylogenetic oligonucleotides utilizing 16S rRNA genes are a key approach for

reducing sulfates in contaminated areas and can determine the phylogenetic relationships of bacteria that can be exploited for bioremediation purposes [55].

Table 2 provides an overview of various applications and strategies in the utilization of genetically modified microorganisms for bioremediation purposes. Each application represents a specific focus area where GE techniques are employed to enhance the biodegradation capabilities of microorganisms for environmental remediation.

Table 2. Applications and strategies for genetically modified microorganisms in bioremediation.

| Application | Description |
|---|---|
| Biodegradation of specific contaminants | Genetically modifying microorganisms to enhance their ability to degrade specific pollutants or contaminants [56] |
| Detoxification | Modifying microorganisms to metabolize and detoxify harmful chemicals or toxins present in the environment [57] |
| Bioaugmentation | Introducing genetically modified microorganisms into contaminated sites to enhance biodegradation capabilities [58] |
| Biostimulation | Modifying microorganisms to stimulate their natural biodegradation processes in the presence of pollutants [59] |
| Metal remediation | Genetically engineering microorganisms to remove or immobilize heavy metals from contaminated environments [60] |
| Nutrient removal | Modifying microorganisms to effectively remove excess nutrients, such as nitrogen and phosphorus, from ecosystems [61] |
| Oil spill cleanup | Utilizing genetically modified microorganisms to break down and degrade hydrocarbons present in oil spills [62] |
| Enhanced degradation pathways | Engineering microorganisms to express additional enzymes or pathways involved in the degradation of specific compounds [63] |

2.2. Genetic engineering tools for bioremediation and pollutant degradation

Bioaugmentation has emerged as a method to enhance the degradation of pollutants and waste in contaminated sites by introducing genetically engineered microbial strains [64]. Natural and wild microbial strains often exhibit lower potency and slower degradation rates, making bioaugmentation a valuable approach for efficient and rapid remediation [65]. Genetic modifications can involve various strategies such as replacement, hybridization, and induced mutation to alter the genetic composition of domesticated plants, animals, and microorganisms to achieve desired outcomes. GE specifically refers to the deliberate modification of specific

gene sequences in plants, animals, or microorganisms to produce desired effects. Genetically modified organisms (GMOs) encompass a wide range of plants, animals, or microorganisms, whereas genetically engineered microorganisms refer specifically to bacteria or fungi, including yeasts, that have been modified in vitro using molecular biology techniques. GMOs possess traits from multiple organisms due to the insertion of genes into a single bacterium [66]. Genetically engineered microorganisms can be effectively employed in bioremediation, which is a process that utilizes microorganisms or their enzymes to promote the degradation and elimination of contaminants from the environment. Bacteria, in particular, possess significant capabilities for degrading environmental pollutants [67]. Bacterial strains capable of degrading various pollutants, including nitroaromatics, chloroaromatics, polycyclic aromatics, biphenyls, polychlorinated biphenyls (PCBs), and components of oil, have been developed for use in bioremediation of polluted sites [67]. These include bacterial strains capable of degrading nitroaromatics, chloroaromatics, polycyclic aromatics, biphenyls, PCBs, and components of oil [68, 69]. Such engineered bacterial strains play a vital role in bioremediation efforts aimed at cleaning up polluted sites. Key enzymes play a pivotal role in catalyzing the degradation of pollutants. These enzymes are often tailored to target specific chemical structures within contaminants. For instance, dehalogenases are essential enzymes for the dechlorination of chlorinated compounds. These enzymes catalyze the removal of chlorine atoms from pollutants, rendering them less harmful to the environment.

Substrate Specificity: Genetically engineered microorganisms exhibit substrate specificity, meaning they are designed to degrade particular pollutants [70]. For example, some engineered strains excel at degrading chlorinated solvents like trichloroethylene (TCE), while others are optimized for the degradation of hydrocarbons like benzene or toluene. This substrate specificity is achieved through precise genetic modifications that fine-tune the microbial metabolism.

Reaction intermediates: During degradation, pollutants are transformed into various intermediates before being completely mineralized [71]. These intermediates may have different toxicity profiles and can influence the overall bioremediation process. Understanding the formation and fate of these intermediates is critical for assessing the effectiveness of genetically engineered microbes in pollutant removal. Many degradation reactions require cofactors or coenzymes for enzymatic activity. Genetically engineered microbes may be equipped with genes that code for these cofactors/coenzymes to ensure the efficient functioning of the degradation pathways. However, the toxicity of certain organic contaminants to existing microbial communities, along with the challenges posed by pollutant mixtures, remains a major hurdle in achieving successful microbial biodegradation. These challenges have paved the way for the development of

artificially engineered bacterial strains that possess efficient catabolic pathways and significant bioremediation capabilities [72]. Biostimulation and bioaugmentation are employed in various bioremediation techniques, including composting, electrobioremediation, microbial-assisted phytoremediation, and others [73]. With advancements in scientific research, genetic engineering is gaining popularity as a means to enhance the natural abilities of microorganisms for remediation purposes. A comprehensive understanding of biotechnology, ecology, field engineering techniques, and biochemical processes is crucial for the successful implementation of in situ bioremediation using GMOs [74].

3. Genetic engineering for enhanced biodegradation

3.1. Deep learning applications in biodegradation research

In recent years, deep learning techniques have found diverse applications in various scientific domains, including precision oncology [75]. The unique characteristics of gene expression data in biodegradation research pose challenges for traditional deep learning models. Therefore, the development of interpretable deep learning architectures tailored for transcriptomics studies is essential. To address these challenges, we introduce a novel interpretable deep learning model Transformer for Gene Expression Modeling (T-GEM) designed for modeling gene expression data. Scientists explore its application in predicting cancer-related phenotypes, such as cancer type and immune cell classification, and analyze its learning mechanisms. Furthermore, we investigate the potential of T-GEM to extract regulatory networks and identify network hub genes as potential markers for phenotypes of interest.

Biodegradation, the natural breakdown of organic compounds by microorganisms, plays a crucial role in environmental sustainability and waste management. GE has emerged as a powerful tool for enhancing the biodegradation capabilities of microorganisms, offering new possibilities for tackling environmental pollution and remediation challenges [76].

The initial understanding of genetic recombination and its significance in industrial applications, including biodegradation, was limited due to the low occurrence rate of natural genetic exchange processes in microorganisms. However, recent advancements in GE techniques have revolutionized our ability to manipulate microbial genomes, allowing for the deliberate introduction or modification of genes that enhance biodegradative capabilities [77].

GE techniques enable scientists to introduce specific genes into microorganisms to enhance their ability to degrade target pollutants [78]. This can involve the transfer of genes from other microorganisms or the modification of existing genes within the host organism. By identifying and incorporating genes encoding enzymes with desired degradation activities, microbial strains can be engineered to efficiently

break down a wide range of organic compounds, including recalcitrant pollutants that are difficult to degrade naturally [78].

One example of GE for enhanced biodegradation is the introduction of genes encoding enzymes involved in the degradation of aromatic compounds, such as benzene, toluene, ethylbenzene, and xylene (BTEX) compounds. These hydrocarbons are prevalent contaminants in petroleum-based industries and can persist in the environment, posing significant risks to ecosystems and human health [79]. Through genetic engineering, microorganisms can be modified to produce enzymes capable of efficiently degrading BTEX compounds, accelerating their breakdown and reducing their environmental impact.

Another approach in GE for enhanced biodegradation involves the optimization of metabolic pathways within microbial cells. By manipulating the expression of genes involved in specific metabolic pathways, researchers can enhance the production of enzymes or metabolites that contribute to biodegradation [80]. For instance, GE can be used to upregulate the expression of gene encoding enzymes responsible for the degradation of complex polymers, such as cellulose or lignin, present in plant biomass. This enables microorganisms to efficiently utilize these abundant and renewable resources as carbon and energy sources, opening new avenues for sustainable biodegradation processes.

Furthermore, GE allows for the creation of synthetic microbial consortia, where multiple genetically engineered microorganisms work together to synergistically degrade complex pollutants. Each member of the consortium can be engineered to possess specific degradation capabilities, enabling the consortium as a whole to efficiently degrade a broader range of pollutants or degrade them at a faster rate [81]. Synthetic microbial consortia offer enhanced versatility and adaptability in biodegradation applications, mimicking natural microbial communities while being engineered to optimize performance.

To facilitate GE for enhanced biodegradation, a variety of molecular tools and techniques are employed. These include gene cloning, DNA sequencing, gene synthesis, and gene expression regulation methods [81]. Advances in genome editing technologies, such as CRISPR-Cas systems, have significantly facilitated precise genetic modifications in microorganisms, enabling the targeted engineering of specific genes or gene clusters associated with biodegradation pathways [82].

Despite the Immense potential of GE for enhanced biodegradation, it is important to consider potential risks and ethical implications associated with the release of genetically modified organisms into the environment. Strains must be carefully evaluated for their safety, including the potential for unintended ecological consequences or gene transfer to other organisms. Strict regulatory frameworks are in place to ensure the responsible use of genetically engineered microorganisms and minimize any potential adverse impacts [83].

The degradation of chlorinated hydrocarbons, including PCBs and chlorinated solvents, has been facilitated through DNA technology. Engineered bacteria have been designed to express dehalogenase enzymes, enabling the breakdown of these persistent pollutants into less harmful compounds [84]. Bioremediation, as a practice, offers a robust foundation for making precise and steadfast comparisons with other remediation methodologies. Among these methods, in situ enhanced reductive dechlorination, which encompasses bioaugmentation and biostimulation, maintains its prominent position for treating chlorinated pollutants directly at contaminated sites. However, it is imperative to acknowledge and address the forthcoming challenges that will shape the successful application of in situ bioremediation in the future.

To ensure its effectiveness, the field must harness sophisticated molecular biological technologies for ascertaining and analyzing the in-situ presence of the requisite dechlorinators. Among these vital microorganisms, *Dehalococcoides* stand out as key players capable of inducing reductive dechlorination at contaminated sites. Nevertheless, due to the inherent complexity of isolating pure *Dehalococcoides* strains, the cultivation of these organisms as pure cultures remains a challenging endeavor. Optimizing the isolation procedures becomes paramount [84].

In the pursuit of advancing bioremediation, one crucial avenue is the identification of genes within *Dehalococcoides* that remain undiscovered. This discovery will empower researchers to design future bioremediation procedures with a deeper understanding and enhanced efficacy.

Moreover, to surmount the inhibitory effects of chloroethenes and PCBs on the reducers, a promising solution lies in the integration of microbial reductive dechlorination with physicochemical treatment [85]. This innovative combination holds the potential to unlock new possibilities and overcome longstanding challenges in the field.

Phytoremediation [86], the use of plants to remediate contaminated soils, has been revolutionized by recombinant DNA technology. Genetically modified plants, like poplar trees, have been designed to express metal-binding proteins and transporters, allowing them to efficiently accumulate and detoxify heavy metals from polluted soils. Such engineered plants have exhibited higher tolerance to toxic metals and improved remediation performance [86].

RNA-based technologies, including ribozymes and small interfering RNAs (siRNAs) [87], have been harnessed to regulate gene expression in microorganisms. These approaches enable precise control over the expression of enzymes involved in pollutant degradation pathways. By utilizing RNA technology, researchers have optimized the metabolic pathways of engineered microbes, leading to enhanced bioremediation efficiency [86].

Genetically engineered microbial consortia have proven to be highly effective for bioremediation scenarios involving complex pollutants. These consortia consist of multiple microorganisms, each designed to contribute specific degradation capabilities. This approach mirrors the complexity of natural microbial communities and offers improved pollutant degradation, especially for recalcitrant compounds [78].

Genetically modified bacteria have been pivotal in tackling chlorinated solvents like trichloroethylene (TCE) and perchloroethylene (PCE), commonly found in groundwater contamination. Engineered microbes can express dehalogenase enzymes, enabling them to detoxify and degrade these hazardous compounds [51].

Genetic modification of bacteria and plants has demonstrated great promise in addressing heavy metal contamination. Engineered organisms can express metal-binding peptides or transporters, facilitating the removal of metals such as cadmium, lead, and mercury from contaminated environments [88]. These examples underscore the versatility and success of genetic engineering, recombinant DNA technology, and RNA-based strategies in bioremediation efforts. As we explore the evolving landscape of bioremediation, these innovative approaches continue to shape a more sustainable and environmentally conscious future.

4. Enhancing the efficiency of producing recombinant proteins in microorganisms

It is possible to think of a living cell as a very sophisticated chemical reactor. More than a thousand proteins in a typical cell take part in numerous processes [17]. Cells contain a multitude of proteins that participate in various reactions, and changes in DNA can significantly affect the metabolic functions of organisms [89]. GE techniques can be utilized to enhance protein production by manipulating the cell's genetic makeup. This can involve increasing the production of a limiting enzyme or engineering the cell to synthesize a modified protein with improved catalytic efficiency [90]. By implementing these strategies, researchers have successfully developed genetically engineered bacteria capable of producing significant amounts of specific proteins, such as ethanol [90]. Another approach in optimizing protein production is metabolic engineering, which involves introducing enzymes that catalyze reactions not typically found in the cell. This alteration redirects cellular metabolism and establishes new biochemical pathways, allowing the synthesis of desired compounds using existing cellular resources. The manufacture of antibiotics, vitamins, and other compounds via fermentation, as well as the creation of bacteria with increased biodegradation capabilities for waste purification, are just a few examples of the possible uses of metabolic engineering. Organic solvents and acids are examples of bulk chemicals that may benefit from this research, and it can also

help in the development of better strains and manufacturing procedures for these compounds. Although metabolic engineering has many potential applications, its present focus is on the manufacture of high-value proteins (such as recombinant proteins). Isolating the gene encoding the target protein from a donor cell is the first step in the creation of recombinant proteins. Gene cloning is the technique of using a suitable host organism to insert the gene encoding the protein of interest. The efficiency with which the host organism can synthesise the protein is prioritised above that of the donor organism [91]. The basic focus of industrial production is to maximise output of the target protein. In many cases, this requires the host cell to accumulate proteins at quantities higher than 10% of the cell's dry weight [92]. On the other hand, if a protein is produced in excess, it may throw off the balance of the cell's metabolism and even cause cell death. Therefore, optimisation is necessary to keep the culture in this aberrant condition. Optimal bioreactors for protein production [93] need to take into account the specific properties and physiology of genetically modified cells. The present key problem in biochemical engineering is the scaling up of industrial techniques employing recombinant organisms. To combat this issue, scientists are constantly investigating new approaches to cellular physiology, fermentation, reactor design, mathematical modeling, instrumentation, and product recovery [94].

The efficiency of rational metabolic engineering depends on the availability of genetic tools for the target microorganism. For non-model microorganisms, random mutagenesis remains a practical method to improve strain phenotypes. Conversely, model microorganisms benefit from rational metabolic engineering combined with novel technologies to generate recombinant strains with desired phenotypes [95].

5. Novel platform strains and functional tools

In recent years, there has been a growing emphasis on the development of novel platform strains and functional tools in biotechnology. These innovative approaches have the potential to revolutionize bioprocesses by enabling higher yields, improved metabolic efficiency, enhanced genetic stability, and greater control over cellular functions [96].

Genome reduction and streamlining is an innovative approach in genetic engineering that involves the deliberate removal of non-essential genes and regions from the microbial genome. By minimizing the genome, researchers can create novel platform strains with improved performance, metabolic efficiency, and genetic stability [97]. This technique has gained significant attention in recent years due to its potential to optimize microbial hosts for specific applications, including amino acid and nucleic acid production.

One of the primary objectives of genome reduction is to alleviate the metabolic burden on the organism. Microbial genomes often contain a large number of genes

that are not directly involved in the desired metabolic pathway or production of target compounds. These non-essential genes consume cellular resources and energy, diverting them away from the desired product synthesis. By selectively removing these genes through genetic manipulation, the metabolic load on the microbial cell is reduced, enabling it to allocate more resources towards the production of desired compounds [98].

In addition to improving metabolic efficiency, genome reduction also enhances genetic stability. Large genomes are more prone to genetic rearrangements, such as deletions, duplications, or insertions, which can negatively impact the stability and consistency of the desired phenotypic traits. By streamlining the genome, scientists can reduce the occurrence of genetic rearrangements and enhance the stability of the engineered strains. This is particularly important for long-term industrial applications where genetic stability is crucial for consistent and reliable production [99].

Recent advances in programmable synthetic biology tools based on biosensors, CRISPR–Cas, and RNA devices have greatly enhanced the ability to engineer microbial cell factories for energy biotechnology applications [100–102]. This review highlights these advancements and their potential impact on the field, showcasing how synthetic biology is driving innovation in the sustainable production of value-added chemicals from diverse biomass sources.

Genome reduction can be achieved through various techniques, including traditional genetic manipulation methods such as gene knockout or deletion, as well as more advanced tools like CRISPR–Cas9-based genome editing [103]. The choice of technique depends on the specific requirements of the target organism and the desired genetic modifications. Through careful planning and optimization, researchers can design and engineer platform strains with minimized genomes that are tailored for efficient production of amino acids and nucleic acids.

One of the key advantages of genome reduction and streamlining is the increased predictability and controllability of the microbial hosts. By removing non-essential genes, the metabolic pathways within the cells become more focused, allowing for better understanding and manipulation of the cellular processes [96]. This enables researchers to precisely control and optimize the production of desired compounds by fine-tuning the expression of key genes and pathways. It also facilitates the development of mathematical models and predictive tools that aid in the optimization of bioprocesses.

Furthermore, genome reduction and streamlining provide opportunities for modular engineering and genetic circuit design. By constructing a minimalistic genome, researchers can introduce synthetic genetic circuits and pathways without interference from redundant endogenous genes [104]. This modular approach allows

for easier manipulation and integration of new functionalities, enabling the development of more versatile and adaptable platform strains.

Overall, genome reduction and streamlining are powerful strategies in GE that offer immense potential for enhancing the production of amino acids and nucleic acids. These approaches optimize microbial hosts by reducing the metabolic burden, improving genetic stability, and providing a foundation for precise control over cellular processes. With further advancements in genome editing techniques and systems biology, genome reduction and streamlining will continue to play a significant role in the development of sustainable bioprocesses and the realization of environmentally friendly and economically viable solutions for the production of valuable compounds, ranging from amino acids and nucleic acids to biofuels and pharmaceuticals.

Table 3 provides an overview of the techniques used in genome reduction and streamlining, along with a brief description of each technique. These aspects include various strategies and tools that are employed to enhance the performance, stability, and control of microbial strains for specific applications, such as amino acid production.

Table 3. Aspects of novel platform strains and functional tools in biotechnology.

| Aspect | Description |
|-------------------------------|--|
| Reduced metabolic burden | Engineering platform strains with minimized non-essential pathways to improve metabolic efficiency [105] |
| Improved metabolic efficiency | Enhancing the efficiency of metabolic pathways through genetic modifications and optimization [106] |
| Enhanced genetic stability | Designing strains with improved genetic stability to maintain desired traits over extended periods [107] |
| Prophage-free strains | Developing strains free from prophages, which can reduce the risk of genetic instability [108] |
| Amino acid sensors | Utilizing transcriptional regulators or riboswitches to sense and regulate amino acid production [109] |
| CRISPR interference (CRISPRi) | Implementing CRISPR-based tools for precise gene regulation and interference [110] |
| Small regulatory RNAs | Employing small RNA molecules to modulate gene expression and metabolic pathways [111] |
| DNA scaffold systems | Designing synthetic DNA scaffold systems to organize and control the expression of multiple genes [112] |
| Optogenetic control | Utilizing light-inducible gene expression systems for precise control of gene expression [113] |

Metabolic engineering and pathway optimization is a powerful field within GE that focuses on the modification of cellular metabolic pathways to enhance the production of desired compounds, such as amino acids and nucleic acids [114]. By rewiring or introducing new enzymatic reactions into the metabolic network, researchers can redirect metabolic fluxes towards the synthesis of target molecules. This approach offers tremendous potential for improving bioprocesses and achieving higher yields of desired products.

One of the key strategies in metabolic engineering is pathway optimization. This involves optimizing the enzymatic reactions, gene expression, and regulation within a metabolic pathway to maximize the production of the desired compound [114]. Pathway optimization can be achieved through various techniques, including the overexpression of key enzymes, deletion or downregulation of competing pathways, and the introduction of novel enzymes or pathways [115].

An important aspect of pathway optimization is the fine-tuning of enzyme expression levels. By controlling the expression of key enzymes involved in the pathway, researchers can ensure that the flux through the desired pathway is maximized. This can be achieved through the use of strong promoters, regulatory elements, and genetic circuits that modulate gene expression. Additionally, optimizing the availability of cofactors and precursors required for the pathway can further enhance the efficiency of the metabolic network.

Furthermore, the rational design of metabolic pathways can lead to improved efficiency and yield. This involves identifying rate-limiting steps in the pathway and introducing modifications to overcome these limitations. For example, introducing enzymes with higher catalytic activity or substrate specificity can increase the flux through the pathway. Similarly, removing bottlenecks and optimizing the stoichiometry of reactions can improve the overall efficiency of the metabolic network [116].

Advancements in computational tools, such as metabolic modeling and simulation, have greatly facilitated pathway optimization. By using mathematical models to simulate and predict cellular metabolism, researchers can identify potential metabolic engineering strategies and guide experimental design. These models take into account various factors, including enzyme kinetics, gene expression levels, and nutrient availability, allowing for a more comprehensive and systematic approach to pathway optimization [117]. These tools enable scholars to design and engineer microbial strains for the efficient production of biofuels, chemicals, and other valuable compounds. Some commonly used computational tools include constraint-based modeling techniques like Flux Balance Analysis (FBA) and Constraint-Based Reconstruction and Analysis (COBRA). These tools allow scientists to predict cellular phenotypes and optimize metabolic pathways, making them indispensable for strain optimization in microbial engineering [118].

Genome-Scale Metabolic Models (GSMMs) are another crucial resource. They represent the entire metabolism of an organism and provide a comprehensive view of its metabolic network. Databases like the Kyoto Encyclopedia of Genes and Genomes (KEGG) and BiGG have become essential for simulating and optimizing metabolic pathways [119].

Dynamic modeling tools, such as Ordinary Differential Equation (ODE) solvers, enable researchers to study time-dependent changes in metabolic networks. This is particularly important for understanding how metabolic pathways function under varying conditions [120].

Metabolic Flux Analysis (MFA) is a valuable technique for quantifying intracellular metabolic fluxes in a cell. Tools like ^{13}C -Metabolic Flux Analysis (^{13}C -MFA) are used to gain insights into carbon flux distributions and optimize pathways accordingly [121].

A variety of optimization algorithms, including Genetic Algorithms (GAs), Particle Swarm Optimization (PSO), and simulated annealing, are employed to find optimal solutions for pathway design and optimization. These algorithms help researchers identify the most efficient configurations for metabolic networks [122].

Additionally, there are software tools designed specifically for pathway design and analysis, such as OptFlux and CellNetAnalyzer. These platforms streamline the process of engineering metabolic pathways and evaluating their performance [123].

For simulating and optimizing entire bioprocesses, including metabolic pathways, process simulation software like COMSOL and Aspen Plus is utilized. These tools consider various factors and parameters in a bioprocess to maximize efficiency.

Integrated platforms like Systems Biology Markup Language (SBML) and Systems Biology Workbench (SBW) provide comprehensive solutions for modeling and simulating complex biological systems. They are invaluable for understanding the intricate interactions within metabolic networks [118].

To visualize metabolic networks and identify key nodes for pathway optimization, researchers use tools like Cytoscape. Visualization platforms make it easier to comprehend complex metabolic relationships. The integration of machine learning and artificial intelligence techniques has introduced data-driven approaches to metabolic modeling and optimization. These methods harness vast datasets to enhance our understanding of metabolic networks and improve pathway design [124].

Another innovative approach in metabolic engineering is the use of synthetic biology tools, such as genetic circuits and biosensors, to control and regulate metabolic pathways. Genetic circuits can be designed to respond to specific signals or inputs, enabling dynamic control over pathway flux. Few examples of previous

work that illustrate the application of synthetic biology tools in metabolic engineering are as follows:

Tetracycline-inducible genetic circuits have been widely used to control gene expression in microbial hosts. Researchers have engineered microorganisms with tetracycline-responsive promoters, allowing for precise control over the production of specific enzymes involved in metabolic pathways. This technology has been employed in various bioprocesses, including the production of biofuels and pharmaceuticals [125].

Quorum sensing is a natural communication mechanism used by bacteria to coordinate gene expression based on cell density. Synthetic biology has harnessed this concept to design genetic circuits that respond to specific signals or metabolites. For example, engineered bacteria have been developed to produce biofuels in response to the accumulation of a particular substrate, enabling dynamic control over metabolic pathways [126].

Optogenetics involves the use of light-sensitive proteins to control gene expression. Researchers have incorporated optogenetic systems into microbial hosts, allowing for precise temporal control of metabolic pathways [127]. This technology has been applied to the production of bioactive compounds and the optimization of enzyme expression in response to light cues.

Synthetic biology has enabled the creation of biosensors that can detect specific metabolites or compounds within a microbial environment. These biosensors can trigger regulatory responses, such as turning on or off metabolic pathways, in the presence of target molecules. For instance, biosensors have been developed to detect environmental pollutants and initiate bioremediation processes in contaminated sites [128].

Riboswitches are regulatory elements found in the untranslated regions of messenger RNAs (mRNAs) that can sense small molecules and control gene expression. Synthetic biology has utilized riboswitches to engineer microbial strains that respond to specific metabolites. This approach has been used to optimize metabolic pathways for the production of valuable chemicals and biofuels [129].

Overall, metabolic engineering offers powerful strategies for enhancing the production of desired compounds. By rewiring and optimizing cellular metabolism, researchers can achieve higher yields, improved efficiency, and better control over bioprocesses. The integration of computational tools and synthetic biology approaches further enhances the capabilities of metabolic engineering, paving way for more sustainable and efficient bioproduction systems [117].

Table 4 provides an overview of the techniques and approaches used in metabolic engineering and pathway optimization, along with a brief description of each technique.

Table 4. Techniques for metabolic engineering and pathway optimization.

| Technique | Description |
|---------------------------------------|---|
| Overexpression of key enzymes | Increasing the expression levels of enzymes involved in the desired pathway to enhance pathway flux [130] |
| Downregulation of competing pathways | Inhibiting or reducing the expression of enzymes involved in competing pathways to redirect metabolic flux towards the desired pathway [131] |
| Introduction of novel enzymes | Introducing new enzymes or pathways into the metabolic network to enable the synthesis of desired compounds [132] |
| Fine-tuning of enzyme expression | Controlling the expression levels of key enzymes through the use of strong promoters, regulatory elements, and genetic circuits [133] |
| Rational design of metabolic pathways | Identifying and addressing rate-limiting steps in the pathway through enzyme engineering, pathway optimization, and stoichiometry adjustments [134] |
| Computational modeling and simulation | Using mathematical models to simulate and predict cellular metabolism for pathway optimization and guiding experimental design [135] |
| Synthetic biology tools | Utilizing genetic circuits and biosensors for dynamic control, regulation, and monitoring of metabolic pathways [102] |

6. Molecular cloning techniques in genetic engineering

Natural competence is an innate ability that certain bacteria possess allowing them to take in foreign DNA [136]. However, not all bacteria have this inherent capability. Increasing the permeability of a bacterium's cell membrane is the standard laboratory method for inducing competence. Chemical treatments that eliminate membrane charges are one option, as is exposing the bacteria to an electric field, which will cause the membrane to permeate at very small openings. Bacteria that are chemically or electrically competent are created using these approaches, respectively [137].

Following transformation, the bacterial cells are plated onto an antibiotic-containing growth medium to prevent the spread of untransformed host cells. For plasmid vectors like pUC19 that include the lacZ gene, blue-white screening is used. Colonies that are blue suggest that the beta-galactosidase enzyme is active because the lacZ gene has not been disrupted by an insertion of foreign DNA into the polylinker site. The linearized plasmid religating to itself is the typical cause of these colonies [138]. The absence of beta-galactosidase activity in white colonies is evidence of foreign DNA inserting into the polylinker site and interrupting the lacZ gene. As a result, the white colonies that emerge from the blue-white screening process include plasmids with insertions and may be studied

further to learn more about the foreign DNA they contain. The integrated DNA may be verified by sequencing the DNA fragment that was inserted. Molecular cloning may also be accomplished by the use of bacterial conjugation [138]. Fertility plasmids, or F plasmids, are shared between bacteria by conjugation. Conjugation allows the transmission of recombinant DNA from one set of bacteria to another when recombinant F plasmid-containing bacteria are combined with suitable, plasmid-free bacteria. The F pilus is a surface feature encoded by F plasmids that allows for interaction between cells with and without the plasmid [139]. When two cells are in close proximity to one another, a cytoplasmic bridge develops between them, allowing the cell harbouring the F plasmid to duplicate its plasmid and transmit a copy of the recombinant F plasmid to the recipient cell. After acquiring the recombinant F plasmid, the recipient cell is able to create its own F pilus, which aids in the spread of the plasmid to other cells. Recombinant F plasmids may be efficiently transferred to recipient cells by conjugation, which allows for the efficient introduction of recombinant DNA molecules into host cells. It is also possible to use bacteriophage-mediated transduction to introduce recombinant DNA into host bacteria. Laboratory work has shown that phagemids (plasmids containing phage sequences) may be modified to transport specific DNA pieces and then packaged into bacteriophages [140]. These phages may then infect bacterial cells and deliver the recombinant phagemids within. The recombinant DNA may either become part of the host bacterial genome (lysogeny) or reside as a plasmid in the cytoplasm [141], but this depends on the kind of phage used. Genomic libraries, which are the result of using molecular cloning procedures to create distinct bacterial clones, are collections of recombinant DNA plasmids that contain the whole or almost complete copy of an organism's genome [142]. Although all the cells in an organism have the same genomic DNA, the expression of distinct sets of genes in various tissues results in a wide range of mRNA profiles. Despite the fact that the insulin gene is present in the genomic DNA of every human cell, it is exclusively expressed in the pancreatic cells [143]. Since messenger RNA (mRNA) cannot be cloned directly, the retroviral enzyme reverse transcriptase is utilised to produce complementary DNA (cDNA) from mRNA in the lab. Researchers may build a cDNA library by reverse-transcribing the mRNA from an entire cell into cDNA molecules. These cDNA molecules then act as templates for DNA polymerase to make double-stranded DNA copies. Excluding introns, control sequences, and non-coding DNA [144], this collection only includes DNA from the genes that are actually used by the cell. Due to its exclusive emphasis on translated sequences, a cDNA library is unable to provide light on the genome's overall organisation. Eukaryotic cells present greater difficulties as hosts for recombinant DNA molecules than bacterial cells. Because of their inability to take up foreign DNA and keep plasmids, eukaryotic hosts make transfection much more challenging and need more intrusive procedures to successfully incorporate foreign DNA [145].

When producing large quantities of designed proteins, proteolytic breakdown becomes a major bottleneck. Even though *E. coli* is less susceptible to proteolysis than other microbial hosts like *Bacillus*, certain foreign proteins in *E. coli* are notoriously unstable [146]. Preproinsulin, an important pharmacological protein and insulin precursor, has been demonstrated to have a cytoplasmic half-life of less than two minutes in *E. coli* [147]. The stability of a protein may be affected by its amino acid sequence in a way that is not completely understood, and the impact might vary depending on the protein. Some recombinant proteins are quickly degraded by the cell because they are deemed “abnormal” in comparison to the cell’s original proteins. One possible indication for protein breakdown is in its initial amino acid sequence [148]. At least eight enzymes in *E. coli* are used to degrade proteins by targeting internal peptide links. Reduced protein breakdown is seen in La-deficient cells, suggesting a pivotal function for protease La in the clearance of aberrant proteins [149]. However, it has been shown that an increase in La levels, which speeds up product degradation [150], occurs when foreign proteins are produced in excess. Rapid breakdown of *E. coli*-produced recombinant interferon A was seen in glucose-starved or oxygen-deprived cells [151]. This discovery is noteworthy because recombinant protein synthesis often makes use of large-scale, high-density fermentations, which may lead to glucose and oxygen restrictions. Growing cells at less-than-ideal temperatures, like 30 °C [152], may be one way to reduce protein breakdown. However, further study is needed to learn how high cell density fermentation conditions affect product stability. Protein breakdown has been inhibited in two additional ways. Two further measures have been taken to slow protein breakdown. One strategy for stabilising an unstable protein includes connecting the gene generating the protein to another gene expressing a native *E. coli* protein [153]. When two genes are fused together in this way, a hybrid polypeptide is produced that has one gene’s amino acid sequence at the beginning and the other gene’s sequence at the end. This supplemental region seems to improve the hybrid protein’s stability and safeguard it against destruction. The resulting hybrid protein may be mass-produced and purified in the usual way. The hybrid’s additional sequence is often selected to make it more manageable to isolate. Following purification, enzymatic or chemical techniques may be used to break off the segment matching to the target polypeptide. Numerous tiny proteins [154] have been successfully produced using this method. However, proper cleavage of the product from the hybrid protein may be difficult to achieve, which may restrict its usage in certain circumstances. Alternatively, if the product aggregates into structures inside the cell called inclusion bodies, it is protected from destruction. Proteolytic enzymes do not seem to be able to get into these aggregates [155]. The process of protein excretion into the medium is less simple in yeasts than in bacteria because yeasts are more complicated organisms. Intracellular vesicles that are brought to the cell surface and discharge their contents are responsible for the

excretion of proteins. Foreign proteins have been reported to be excreted by *Saccharomyces cerevisiae*, despite the fact that this yeast does not ordinarily release many proteins into its medium [156]. Excretion of tiny recombinant proteins from yeast has been achieved thus far. Whether big proteins can be expelled as effectively or become stuck in the yeast cell wall's intricate structure remains to be seen. However, yeasts' ability to excrete foreign proteins is a major benefit. Proteins that are expelled go through post-translational changes such as disulfide bond isomerization and glycosylation, which bacteria just aren't capable of doing. Therefore, the end output can look quite similar to the original. Genetically altered cells have been used to manufacture a wide variety of proteins, with many more in the research and development or industrialization stages. The most often used microbial host for industrial production is *Escherichia coli* [157]. *Saccharomyces cerevisiae* and *Bacillus* were formerly utilised only in small amounts, but since previous technological hurdles are being surpassed, they are gaining wider acceptance [158]. *Bacillus subtilis* permits protein excretion with relative ease, but a key hurdle has been the quick breakdown of the extracellular product. Efforts are still being made to address product instability [159], including decreasing protease levels and changing growing conditions. There is now less reason to mistrust *Saccharomyces cerevisiae*'s capacity to synthesise large quantities of foreign proteins. New research also shows that *Saccharomyces* can properly change foreign proteins via post-translational modifications.

7. Essential elements for assessing the risks associated with bioactive molecules

Identify the specific nucleic acids involved, including RNA, DNA, and novel nucleotide polymers, as well as mixtures of nucleotides. Determine their role and potential applications, such as mutagenesis or gene silencing [160] and mixtures of nucleotides [161]. Regulatory evaluation: Assess whether the nucleic acids fall under existing GE regulations or require separate assessment. Note that nucleic acids may not always be regulated if they are not DNA, and their assumed lack of inherent chemical toxicity when used outside genetic engineering contexts [161]. Evaluate the purpose of the nucleic acids, such as controlling the viability or pathogenicity of pests or disease-causing organisms in pesticides. Understand the specific effects and potential risks associated with their application [161]. Consider the vehicles or carriers used to deliver the nucleic acids. Typically, both RNA and DNA are utilized, especially for topical applications. Assess their compatibility, effectiveness, and any safety concerns related to their use [161]. Determine whether the nucleic acids are intended for transient expression or long-term retention within the target organism. Evaluate the consequences and potential risks associated with their presence, activity, and persistence [162]. Understand how the nucleic acids interact with

cellular components, such as enzymes and guide molecules. Assess the potential for DNA damage, gene silencing, or interference with gene expression pathways [163]. Evaluate the potential environmental effects of the nucleic acids, including their spread, persistence, and impact on non-target organisms and ecosystems. Consider any long-term consequences in subsequent generations [164]. Assess the potential risks and unintended consequences associated with the use of nucleic acids, both in terms of human health and ecological balance. Evaluate their impact on genetic stability and any potential hazards [162]. The RNA molecules used in external treatments are often called inhibitory RNA, short/small inhibitory RNA (siRNA), piwi-interacting RNAs (piRNA), and micro-RNA (miRNA) and are involved in causing RNA interference (RNAi), post-transcriptional gene silencing (PTGS), or antisense gene silencing. All of these RNAs are dsRNAs, or double-stranded RNAs [165]. Silencing genes means effectively switching them “off” in certain cells, animals, or environments. In certain cases, the silencing of a gene may persist across several cell divisions (during the life cycle of the organism) and even multiple generations [166]. The common characteristic of RNA-based gene silencing methods is their reliance on dsRNA molecules, which play a crucial role in the biochemistry of these reactions. This dsRNA structure can form between two complementary single-stranded RNA molecules or through intramolecular pairing, resulting in a “stem-loop” structure. While initially described in animals, dsRNA-mediated gene silencing has also been observed in fungi, plants, and bacteria, although with different biochemical mechanisms [66, 67]. RNA-based technologies show promise in controlling infectious diseases, enhancing agricultural productivity, and reducing unwanted secondary metabolites in food. They are seen as an attractive alternative to GE by the biotechnology industry [167]. However, it should be noted that dsRNA molecules can interact with DNA and induce changes in sequences, similar to mutagens. These changes can be heritable, either through epigenetic modifications or ongoing replication. Therefore, the lack of consideration for dsRNA treatments as potentially generating genetically modified organisms alongside DNA-based approaches raises questions [168]. Gene/genome editing techniques have gained significant attention due to their simplicity and the potential for their products to be exempt from classification as genetically modified organisms. Few products have been commercialised so far, and even fewer have been subjected to risk assessment as genetically modified organisms. Depending on the jurisdiction and the product’s intended purpose, subsequent products may also be exempt from such review [168]. Whether they are planned or accidental, the direct harmful effects of bioactive chemicals on exposed species must be taken into account in any risk assessment. Although the impacts on exposed creatures may be compounded in the environment, especially via the reproduction of those organisms, this evaluation is different from that for harmful substances. When considering the potential dangers of bioactive molecules and the vehicles that transport them, it is important to

consider both their toxicity and their heritability. Overall, understanding the mechanisms and potential risks associated with RNA-based gene silencing methods, as well as considering the broader implications and possible heritability, is essential for conducting comprehensive risk assessments of bioactive molecules and their applications. Nucleic acids can be introduced into plants and insects by encapsulating them in tissue-penetrating agents. Researchers in Australia have developed a nanoparticle formulation called Bioclay, which exhibits properties such as binding and stabilizing dsRNA molecules for extended periods, strong adhesion to plant leaves, controlled release of dsRNA over several days, and induction of RNA interference (RNAi) when delivered. Bioclay-based formulations have potential applications as herbicides, antiviral agents, or antifungal agents. Similar delivery formulations have been explored for targeting insect pests, where the pests ingest RNA-loaded bioclay particles that have been adhered to plant leaves. The dsRNA is protected by the clay particles from nucleases and chemical reactions until it is exposed to the basic pH of the insect's digestive tract [71, 72]. Overproducing main metabolites is the goal of the genetic engineering efforts being put into creating new strains. In species with complicated regulatory systems, where numerous genetic alterations are required for deregulation, this strategy includes assembling desirable features using recombinant DNA methods in vitro. Plasmid maintenance during fermentation is a major obstacle to employing plasmid-based strains. Antibiotic pressure during fermentation is one approach; this selects organisms that are able to tolerate antibiotics thanks to a plasmid-borne resistance gene. Synergistic effects may be achieved by combining deregulation with plasmid amplification [73, 74]. In order to improve overproducing strains, new genetic methods must be developed. Accelerating strain improvement programmes [169] are projected to be ongoing genome sequencing efforts, the availability of sequences from model organisms, sophisticated DNA microarray and proteomic technologies, and mutagenesis and recombination procedures. Molecular breeding methods allow in vitro homologous recombination to simulate natural recombination. Recombining DNA fragments is only one side effect of DNA shuffling, which also creates low-frequency, controlled point mutations. Remarkable progress has been made in a very short amount of time using this technique of pooling and recombining fragments of homologous genes from several species or strains. Whole genome shuffling combines the benefits of multi-parental crossing made possible by DNA shuffling with the recombination of whole genomes [76, 77], making it a novel strategy for strain enhancement.

The most common approach for transferring modified plasmids into bacteria is called transformation. Bacteria take up free DNA from their surroundings throughout the transformation process. Lysed bacterial cells are a common source of this free DNA in the wild. Free DNA from recombinant plasmids is delivered to the area around a bacterial cell in the lab [170].

8. Alternative technologies for biodegradation and amino acid production

While GE approaches have shown great promise for biodegradation and amino acid production, alternative technologies have also been reported in the literature. These alternative approaches offer different advantages and may be suitable for specific applications or environmental conditions.

Chemical treatments involve the use of specific reagents or processes to degrade pollutants or extract amino acids from biomass. For biodegradation, chemical oxidation or reduction processes can be employed to break down pollutants into less harmful substances [171]. However, chemical treatments often require harsh conditions, involve the use of hazardous chemicals, and may generate toxic byproducts, limiting their applicability in environmentally sensitive areas.

Enzymatic processes utilize enzymes derived from microorganisms, plants, or animals to degrade pollutants or produce amino acids [172]. Enzymes can exhibit high specificity and catalytic activity, making them efficient biocatalysts. Enzymatic biodegradation processes offer advantages such as mild reaction conditions, reduced energy consumption, and the potential for selective degradation. However, the availability and stability of enzymes, as well as the cost of enzyme production are the limiting factors for large-scale applications [172]. Several noteworthy case studies demonstrate the effectiveness of alternative technologies for biodegradation and amino acid production. For instance, in the field of enzymatic processes, the use of ligninolytic enzymes derived from white rot fungi has been successful in breaking down lignin-containing compounds in soil and water environments [173]. This approach has been applied in the bioremediation of sites contaminated with recalcitrant pollutants like polycyclic aromatic hydrocarbons (PAHs) [118]. Evaluating the environmental impact of these technologies is crucial. Enzymatic biodegradation processes, such as those involving laccases or peroxidases, often operate under mild conditions, reducing energy consumption and minimizing the generation of harmful byproducts [174]. These enzymatic processes align well with environmentally sensitive areas. When considering the application of alternative technologies, it's important to navigate regulatory requirements. In many regions, the use of GMOs in bioremediation may require adherence to specific guidelines and permits. Enzymatic processes, on the other hand, may have fewer regulatory hurdles but could still require approval for large-scale applications.

Scaling up alternative technologies from laboratory to industrial settings may pose challenges. For example, physicochemical processes such as membrane separation techniques are effective in controlled environments but may require specialized equipment and significant energy inputs for large-scale applications [175]. Overcoming these challenges involve careful reactor design and process optimization.

Continuous advancements in alternative technologies hold great promise. Researchers are exploring novel enzymes and microbial consortia for more efficient and sustainable biodegradation and amino acid production [176]. The development of enzyme cocktails and robust microbial consortia could further enhance these approaches. Comparing these alternative technologies reveals their relative strengths and weaknesses. For instance, while microbial consortia offer increased metabolic diversity and resilience, they require ongoing monitoring and optimization to maintain stability. In contrast, enzymatic processes are highly specific and efficient but may have limitations in terms of enzyme availability and production costs.

Synergy between different technologies is a growing area of interest. Combining genetically engineered microorganisms with enzymatic processes has the potential to enhance the biodegradation of complex pollutant mixtures [177]. These integrated strategies leverage the strengths of each approach to achieve superior results.

Assessing the commercial viability of alternative technologies is essential. Enzymatic processes, due to their milder conditions and lower energy requirements, often offer cost-effective solutions for amino acid production [178]. This economic advantage can be a key factor in determining their suitability for large-scale production.

Microbial consortia consist of multiple microbial species working together to achieve biodegradation or amino acid production [176]. Each species in the consortium performs specific functions, such as hydrolysis, fermentation, or degradation of complex substrates. Microbial consortia offer advantages such as increased metabolic diversity, enhanced resilience to environmental fluctuations, and the ability to perform complex biotransformations. However, the maintenance and stability of microbial consortia can be challenging, and interactions between different species can be difficult to control and optimize [176].

Physicochemical processes combine physical and chemical methods to degrade pollutants or extract amino acids. Examples include ultraviolet (UV) irradiation, electrochemical processes, or membrane separation techniques. These processes can achieve efficient pollutant removal or amino acid recovery through physical mechanisms such as photolysis, oxidation/reduction, or molecular sieving. Physicochemical processes are generally energy-intensive and may require specialized equipment [179]. They may also have limitations in terms of selectivity and efficiency for complex pollutant mixtures or biomass sources [180].

Fermentation strategies involve the use of microbial cells to produce amino acids through metabolic pathways. These approaches often utilize non-genetically modified microorganisms and focus on optimizing fermentation conditions, media composition, and process parameters. Fermentation can offer cost-effective and scalable production processes, especially for amino acids that are naturally produced

by microorganisms. However, the production yields and product purity achievable through fermentation alone may be limited compared to GE approaches [181].

It is important to note that each of these alternative technologies has its own strengths and limitations, and their suitability depends on specific application requirements, target pollutants, or desired amino acids (Table 5). Table 5 presents a comparison of alternative technologies for biodegradation and amino acid production, highlighting their respective advantages and disadvantages. These technologies offer promising approaches to address the challenges associated with traditional methods. Furthermore, the choice of technology may also depend on factors such as cost-effectiveness, environmental impact, regulatory considerations, and scalability to industrial production.

Table 5. Comparison of alternative technologies for biodegradation and amino acid production: advantages and disadvantages.

| Technique | Advantages | Disadvantages | References |
|---------------------------|--|---|------------|
| Genetic engineering | Precise modification of microorganisms for targeted biodegradation and amino acid production | Potential risks associated with the release of genetically modified organisms | [182] |
| Chemical treatments | Can degrade pollutants and extract amino acids | Requires harsh conditions and hazardous chemicals May generate toxic byproducts | [183] |
| Enzymatic processes | Mild reaction conditions | Availability and stability of enzymes can be limiting Cost of enzyme production | [184] |
| Microbial consortia | Increased metabolic diversity | Maintenance and stability of consortia can be challenging Difficult to control and optimize interactions between different species | [185] |
| Physicochemical processes | Efficient pollutant removal and amino acid recovery | Energy-intensive and may require specialized equipment Limitations in selectivity and efficiency for complex pollutant mixtures or biomass sources | [186] |
| Fermentation strategies | Cost-effective and scalable production processes | Production yields and product purity may be limited compared to genetic engineering approaches | [187] |

9. Risk assessment and bioactive molecules

Risk assessment plays a crucial role in the evaluation and management of bioactive molecules, ensuring their safe and responsible use in various applications. Bioactive molecules encompass a wide range of compounds, including pharmaceuticals, pesticides, food additives, and industrial chemicals, which may have biological effects on living organisms [188].

The purpose of risk assessment is to identify and evaluate potential hazards associated with bioactive molecules and determine their potential impact on human health, ecosystems, and the environment. It involves a systematic analysis of various factors, such as exposure levels, toxicity, persistence, and potential ecological effects. The goal is to provide a scientific basis for decision-making and to develop appropriate risk management strategies.

The process of risk assessment typically involves several key steps. These include hazard identification, which involves identifying the potential adverse effects of the bioactive molecule on humans and the environment. This is followed by exposure assessment, where the levels and duration of exposure to the bioactive molecule are estimated for different population groups or environmental compartments. Toxicity assessment is then conducted to determine the potential harmful effects of the molecule at the given exposure levels. Finally, risk characterization combines the results of the previous steps to quantify the potential risks and inform risk management decisions.

In the context of bioactive molecules, risk assessment considers factors such as the mode of action, target receptors, dose-response relationships, and potential cumulative effects. It takes into account various routes of exposure, such as ingestion, inhalation, or dermal contact. The assessment also considers vulnerable populations, including children, pregnant women, and individuals with pre-existing health conditions.

To support risk assessment, a range of scientific tools and methodologies are employed. These include *in vitro* and *in vivo* testing, computational modeling, structure-activity relationships, and exposure modeling [189]. These tools provide valuable insights into the potential hazards and risks associated with bioactive molecules and help inform risk management decisions.

The outcomes of risk assessment guide the development of risk management strategies and regulatory frameworks. These may include setting exposure limits, establishing safety standards, implementing control measures, and monitoring programs. Risk communication also plays a vital role in ensuring that relevant stakeholders, including policymakers, industries, and the public, are informed about the potential risks and understand the measures in place to mitigate them [190].

Quantitative Structure-Activity Relationship (QSAR) models are computational tools used to predict the biological activity and potential hazards of bioactive molecules based on their chemical structure and properties [191]. These models play a crucial role in drug discovery, toxicology studies, and chemical safety assessments by providing insights into the activity and behavior of compounds before they are experimentally tested.

The foundation of QSAR models lies in the assumption that the biological activity of a molecule can be quantitatively related to its physicochemical properties and structural features. These properties include molecular weight, solubility, lipophilicity, electronic properties, and the presence of functional groups [191]. By analyzing the relationships between these properties and the observed biological activities of a dataset of known compounds, QSAR models can make predictions for new or untested molecules.

To develop a QSAR model, a training dataset is created, consisting of a diverse set of compounds with known biological activities. The dataset is divided into two subsets; a training set used for model development and a test set used for validation. Various mathematical and statistical techniques are then employed to correlate the structural and physicochemical descriptors of the compounds with their corresponding biological activities.

The most commonly used approach in QSAR modeling is multiple linear regression analysis, where a mathematical equation is derived to relate the molecular descriptors to the activity values. Other methods, such as machine learning algorithms (e.g., neural networks, support vector machines), and 3D-QSAR techniques (e.g., comparative molecular field analysis, comparative molecular similarity indices analysis), can also be utilized to improve the accuracy and robustness of QSAR models [192].

Once a QSAR model is established, it can be used to predict the activity of new compounds by inputting their structural information and physicochemical properties. These predictions provide valuable insights into the potential activity, potency, toxicity, or other relevant properties of the compounds [192]. QSAR models can also be used for virtual screening of large chemical databases, prioritizing compounds for further experimental testing, and identifying structural alerts that indicate potential hazards.

However, it's important to note that QSAR models have certain limitations. They rely on the availability of accurate and reliable training datasets, which can be challenging to obtain for rare or unexplored compounds. QSAR models are also limited by their inability to capture complex interactions and mechanisms that may influence biological activities. Therefore, experimental validation and further testing are essential to confirm the predictions made by QSAR models.

Despite these limitations, QSAR models have proven to be valuable tools in predicting the activity and potential hazards of bioactive molecules [193]. They offer a cost-effective and time-efficient approach to screen large chemical libraries, prioritize compounds for further evaluation, and aid decision-making processes in drug discovery, toxicology, and chemical safety assessments.

To overcome the limitations of QSAR models, several approaches and strategies can be employed. First and foremost, it is crucial to have high-quality and diverse training datasets. Building robust QSAR models requires comprehensive datasets that cover a wide range of chemical structures and biological activities. Efforts should be made to gather reliable and accurate experimental data for known compounds, including both active and inactive molecules. Collaborations between researchers, sharing of data, and the development of public databases can contribute to the availability of high-quality training datasets, improving the performance of QSAR models [194].

In addition to improving the datasets, the incorporation of additional molecular descriptors can enhance QSAR models. By including a broader range of descriptors that capture various aspects of chemical structure and properties, the models can capture more complex interactions and provide more accurate predictions [194]. These descriptors can be derived from quantum chemical calculations, molecular dynamics simulations, or other computational methods.

Another strategy is to employ more advanced machine learning algorithms. While multiple linear regression is a commonly used method in QSAR modeling, more advanced algorithms such as random forest, support vector machines, or neural networks can be employed to improve model performance. These algorithms can capture non-linear relationships and interactions between descriptors and biological activities, leading to more accurate predictions.

Validation and external testing are crucial to assess the reliability and predictive power of QSAR models. Rigorous validation using independent test sets helps evaluate the model's performance and generalizability. External validation involves testing the model on compounds that were not used in the model development, providing an unbiased assessment of its performance and determining its applicability domain [195].

To enrich QSAR models, integration of additional data sources can be considered. This includes incorporating bioactivity data, structural databases, biological pathways, and omics data such as genomics and proteomics. The integration of diverse data types can provide a more comprehensive understanding of the relationships between chemical structures and biological activities, leading to more accurate predictions.

Hybrid approaches that combine QSAR models with other computational techniques, such as molecular docking, molecular dynamics simulations, or 3D-QSAR methods, can offer a more comprehensive analysis of compound activities. These hybrid approaches leverage the strengths of different methods and can provide a more holistic understanding of the interactions between molecules and their biological targets [196].

It is important to note that experimental validation is crucial for confirming the predictions made by QSAR models. While QSAR models are valuable predictive tools, experimental testing should be performed on selected compounds to verify their biological activities and compare them with the predicted values. This iterative feedback loop between modeling and experimentation helps refine and improve the QSAR models.

By implementing these strategies, the limitations of QSAR models can be mitigated, leading to more accurate predictions and reliable assessments of the activity and potential hazards of bioactive molecules. Collaboration among researchers, data sharing, and continuous improvement of modeling techniques contribute to the advancement of QSAR modeling and its applications in various fields of research and industry [196].

High-throughput screening (HTS) assays are powerful techniques used in drug discovery and chemical biology to rapidly test large compound libraries against biological targets or cellular systems. HTS assays enable the screening of thousands to millions of compounds in a relatively short period, providing valuable information about their activity, potency, and selectivity [197].

The process of conducting HTS assays begins with the selection of a target or a biological pathway of interest [198]. This can range from receptors, enzymes, ion channels, to cellular processes involved in disease pathways. The target is usually chosen based on its relevance to a specific disease or biological function.

Next, a diverse library of compounds, often consisting of synthetic or natural product-based molecules, is prepared. These compound libraries are commercially available or can be custom-designed based on specific chemical structures or functional groups of interest. The libraries are typically stored in microtiter plates, allowing for HTS in an automated fashion.

HTS assays employ various assay formats, including biochemical, cell-based, or phenotypic assays. In biochemical assays, the target protein or enzyme is isolated, purified, and then exposed to the compound library to assess its inhibitory or activating effect. These assays often utilize colorimetric, fluorometric, or luminescent readouts to measure the target activity [199].

Cell-based assays involve the use of intact living cells to assess compound activities. Cells can express the target protein or have a specific genetic background

that mimics a disease state. Upon exposure to the compound library, changes in cell viability, proliferation, or specific molecular pathways are measured using fluorescence-based probes, reporter genes, or high-content imaging systems.

Phenotypic assays focus on the observation of a specific cellular phenotype or behavior induced by the compounds [200]. These assays provide a holistic view of compound activity and can reveal potential therapeutic effects beyond the initial target. Phenotypic assays are particularly useful when the underlying mechanism of a disease is complex or not well understood.

HTS assays are performed using automated liquid handling systems, robotics, and plate readers, allowing for the simultaneous screening of multiple compounds in a high-throughput manner. The data generated from HTS assays are typically analyzed using computational tools and statistical methods to identify active compounds, determine their potency, and prioritize hits for further characterization [201].

One of the key advantages of HTS assays is their ability to accelerate the drug discovery process by rapidly identifying compounds with desired activity. This significantly reduces the time and cost involved in traditional one-by-one compound screening approaches. HTS assays also enable the exploration of chemical space and the identification of novel lead compounds with potential therapeutic applications [201].

However, HTS assays also have certain limitations. False positives and false negatives can occur due to assay variability, compound interference, or assay artifacts. Therefore, hit compounds identified from HTS assays require further validation and confirmation using secondary assays with orthogonal readouts. Additionally, the selection of appropriate assay controls and the optimization of assay conditions are critical to ensure assay robustness and reliability.

HTS assays themselves are not new or innovative techniques, as they have been used in drug discovery and chemical biology for several decades [202]. The concept of screening large compound libraries in a high-throughput manner to identify active compounds has been widely adopted in the pharmaceutical industry and academic research settings.

However, it is worth noting that there have been continuous advancements and innovations in HTS technologies, automation, and data analysis methods over the years. These advancements have significantly improved the speed, efficiency, and reliability of HTS assays, making them more accessible and cost-effective.

In recent years, there have been notable advancements in miniaturization and parallelization of HTS assays, allowing for even higher throughput and screening of larger compound libraries. Technologies such as microfluidics and lab-on-a-chip systems have enabled miniaturization of assays and reduced reagent consumption, while maintaining high data quality [199].

Moreover, the integration of robotics, liquid handling systems, and automation has streamlined the HTS workflow, enabling the screening of large compound libraries in a highly efficient and reproducible manner. This automation has also led to increased standardization of assays, reducing assay-to-assay variability and improving data quality.

In terms of data analysis, the development of computational tools and machine learning algorithms has facilitated the efficient processing and interpretation of large datasets generated from HTS assays. These tools aid in hit identification, hit-to-lead optimization, and the prediction of compound properties, enhancing the decision-making process in drug discovery [203].

Additionally, the application of HTS assays has expanded beyond traditional drug discovery efforts. HTS is now being used in chemical biology, functional genomics, and personalized medicine research. Assays have been developed to screen compound libraries against specific cellular pathways, protein-protein interactions, or disease-relevant models, allowing for a more comprehensive understanding of complex biological processes [204].

The continual advancements in automation, miniaturization, data analysis, and their expanded applications have made the overall approach more innovative and powerful. These advancements have accelerated the pace of drug discovery, improved the efficiency of compound screening, and expanded our ability to explore chemical space and understand biological systems.

10. Conclusion

The production of amino acids and nucleic acids from genetically engineered microbial cells holds immense potential for biodegradation and environmental sustainability. By leveraging genetic engineering techniques, microbial cells can be modified to efficiently degrade diverse pollutants, contributing to a cleaner and healthier environment.

Genetic recombination processes, including conjugation, transduction, transformation, and internal genetic rearrangements, play a crucial role in reshaping microbial genomes and enhancing their biodegradation capabilities. Through genetic manipulation, microbial strains can be engineered to possess specific traits, such as increased enzyme production or the ability to utilize alternative carbon sources, which are essential for efficient biodegradation processes.

The utilization of alternative carbon sources, such as lignocellulosics or agro-industrial residue, offers sustainable bioprocesses for amino acid production. Genetic engineering allows for the construction of amino acid-producing base strains, where gene expression is modulated at the transcriptional level using

transcription regulators and attenuators. This fine-tuning of gene expression maximizes amino acid production and provides a resource-efficient approach to meet the growing demand for amino acids.

Furthermore, the development of novel platform strains has revolutionized amino acid production by reducing metabolic burden, improving metabolic efficiency, enhancing genetic stability, and eliminating prophages. Functional tools, including amino acid sensors, CRISPR interference, small regulatory RNAs, and optogenetic control, have greatly accelerated strain development, enabling high-throughput screening and rapid validation of target genes. These tools have enhanced overall metabolic efficiency and allowed for light-inducible gene expression, offering precise control over the production of amino acids.

The production of amino acids and nucleic acids from genetically engineered microbial cells not only offers sustainable solutions for biodegradation but also presents opportunities for various industrial applications. The advancements in genetic engineering techniques and functional tools have paved the way for development of innovative and efficient bioprocesses. By harnessing the power of microbial cells, we can address environmental challenges, reduce waste, and promote a more sustainable future.

Conflict of interest

The authors declare no conflict of interest.

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