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Review

Synthetic Biology: A New Era in Hydrocarbon Bioremediation

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Abstract: Crude oil is a viscous dark liquid resource composed by a mix of hydrocarbons which, after refining, is used for the elaboration of distinct products. A major concern is that many petroleum components are highly toxic due to their teratogenic, hemotoxic, and carcinogenic effects, becoming an environmental concern on a global scale, which must be solved through innovative, efficient, and sustainable techniques. One of the most widely used procedures to totally degrade contaminants are biological methods such as bioremediation. Synthetic biology is a scientific field based on biology and engineering principles, with the purpose of redesigning and restructuring microorganisms to optimize or create new biological systems with enhanced features. The use of this discipline offers improvement of bioremediation processes. This article will review some of the techniques that use synthetic biology as a platform to be used in the area of hydrocarbon bioremediation.

Keywords: synthetic biology; bioremediation; hydrocarbons; biosensors; consortium; genetically engineered microorganisms

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1. Introduction

Crude oil is a viscous dark liquid resource composed by a mix of hydrocarbons. Primarily it is composed of carbon and hydrogen, along with minor elements such as nitrogen, oxygen, sulfur, and metals. Petroleum components can be divided into two main groups: hydrocarbons and heterocompounds. Crude oil is separated by different distillation processes for the elaboration of distinct products. Petroleum in crude state has minimal applications. However, by refining, different useful products are obtained such as fuels, solvents, lubricants, plastics, oils, and asphaltic products, among others [1–3].

Due to the accelerated growth of industry and the high demand for oil, environmental problems and contamination from leaks or spills has become an important issue to be considered [4]. This can be caused by accidental leaks from reservoirs, refineries or transportation pipelines (not including petrochemicals products) [5], which lead to contamination of soil, groundwater, and oceans [2]. Environmental pollution causes direct damages on the ecological properties of many species, in addition to those that directly affect humans [5,6]. A major concern is that many petroleum components, such as benzene, toluene, ethylbenzene, xylenes (BTEX), and polycyclic aromatic hydrocarbons (PAH), are highly toxic due to their teratogenic, hemotoxic, and carcinogenic effects [2]. Considering this, along with the permanent damage to ecosystems, petroleum derivatives are one of the most persistent organic pollutants in the world. For this reason, oil pollution has become an environmental concern on a global scale, which must be solved through innovative, efficient and sustainable techniques [5].

Some of the most widely used procedures to totally degrade contaminants are biological methods such as bioremediation [5,7]. Here, the pollutants are used as carbon sources, which allows for elimination without altering the environment [2,7]. Microorganisms involved in bioremediation processes use multiple metabolic pathways where enzymes are the key actors for degradation [8]. Discovery of new techniques that consider the different forms of genetic and metabolic expression in order to choose the right microorganism and obtain better results is a new objective in the area [8,9]. The design, construction, and fine-tuning of a wholly-engineered organism for the monitoring and degradation of pollutants is a strategy provided by synthetic biology, creating a new era in bioremediation and making it the best option in terms of contamination removal [6,9].

1.1. Petroleum Composition: Crude Oil

Within the two groups of the petroleum components there are four fractions: saturated (aliphatic hydrocarbons), aromatic (cyclic hydrocarbons), resins, and asphaltenes. The main components of petroleum are hydrocarbons: hydrogen and carbon compounds with different molecular structures. The range of compounds extends from methane (natural gas), liquids that are refined into gasoline, to solid waxes [1–3].

Saturated or aliphatic hydrocarbons represent the higher percentage of oil compounds. Some of the common alkanes are methane, ethane, propane, and butane, and among the common cycloalkanes are naphthenes [1,3]. On the other hand, aromatic compounds are divided into (a) monocyclic aromatics, such as BTEX; (b) asphaltic compounds; and (c) polycyclic aromatic hydrocarbons [2,3,10]. Asphaltic compounds include resins and asphaltenes [1,3]. Aromatic derivatives are the most toxic components of crude oil, associated with chronic and carcinogenic effects [2,3,10].

1.2. Traditional Hydrocarbon Bioremediation Techniques

In order to choose the appropriate oil clean-up or remediation technique, time, cost and efficiency must be considered, as well as the type of hydrocarbon and geographic location [7]. Traditional physicochemical methods are effective to remove oil. However, they produce different immunotoxic and carcinogenic compounds that remain dangerous [7]. This problem, along with the increasing cost and limitation of physicochemical methods, led to the development of technologies based on the degradative capabilities of plants and microorganisms, an option that offers great benefits [2].

Bioremediation techniques can be classified according to the site of application, namely either ex situ and in situ (Table 1) [11,12]. Ex situ techniques involve the transportation of contaminants to another site for treatment. Performing treatment away from the contamination site could be costly and can disrupt natural sites. On the contrary, in situ techniques perform the treatment at the site of contamination to eliminate the toxic compounds. Therefore, this technique is less expensive as it does not require excavation nor transport, although certain environmental factors such as soil porosity, temperature, nutrient availability, humidity and pH must be evaluated [11,12].

Table 1. Examples of hydrocarbon bioremediation methods.

Bioremediation Technique	Treatment	% of Degradation	Reference
Biostimulation: compounds such as nutrients, oxygen, biopolymers, biosurfactants or fertilizers are added, in order to enhance microbial activity [13–15].	Mineral fertilizer with dolomite flour	Total petroleum hydrocarbon (TPH) decreased by 47% in 15 months.	[13]
Bioaugmentation: addition of autochthonous, exogenous or genetically engineered microorganisms with catabolic activity to the contamination site [14–16].	Microbial inoculum with *Alcanivorax* as the dominant genus.	TPH was reduced by 41% in 63 days.	[16]
	Acinetobacter sp. SCYY-5	TPH was removed by 69.17% in 10 days.	[17]

Table 1. *Cont.*

Bioremediation Technique	Treatment	% of Degradation	Reference
Biopile: contaminated soil is piled followed by the addition of nutrients and oxygen, which enhance degradation. A water system can be added or organic materials that acts as bulking agents [14,18,19].	Biopile system with crude oil, nutrients and Amnite P-300 (consortium of 10 strains mainly belonging to the *Pseudomonas* genus)	TPH were reduced by 77% in 156 days.	[20]
Phytoremediation: process based on plants and their associated microorganisms to degrade, remove or immobilize toxic compounds from the environment [14,15].	*Melilotus officinalis*	TPH by 42.2% and PAH by 49.9% in 6 months.	[21]

Microorganisms used in the bioremediation process can be isolated from soil, water, or air. In many occasions, these are isolated directly from the contaminated sites, as it has been found that they already have naturally developed the capacity to use or degrade these pollutants as an adaptation in response to the selective pressure that the contaminants in the environment exerted. Many microorganisms use hydrocarbons as an alternative carbon source, since they are compounds that naturally can provide energy [2,22]. Considering this, bioremediation techniques can be used to eliminate them [23].

2. Synthetic Biology and Bioremediation

Although bioremediation techniques offer great advantages, in many cases they are becoming traditional methods since new tools are emerging. Thanks to multi-omics analysis and advances in genetic engineering, it is possible to obtain the information necessary to choose the best microorganism for the remediation process [9].

For example, in order to identify catabolic genes, novel pathways or proteins involved in the biodegradation process, genomic, proteomics and metabolomic tools can be used. Transcriptomics also gives important information, such as how the cell responds after the exposure to toxic compounds and how it affects its metabolic state. On the other hand, in silico tools can give insight into the biochemical reactions that take place in the degradation of contaminants [24]

Synthetic biology is a scientific field based on biology and engineering principles, with the purpose of redesigning and restructuring organisms to optimize or create new biological systems with enhanced features [25–27]. This field uses molecular tools, systems biology and the reprogramming of the genetic framework, thus constructing synthetic pathways to obtain microorganisms with alternative functions [26,27]. The use of this discipline brings an improvement of bioremediation processes (Table 2).

Table 2. Examples of engineered microorganism in hydrocarbon bioremediation.

Genetically Engineered Microorganisms	Results	Reference
almA gene was inserted into two plasmids and transformed into *Escherichia coli.*	Total crude oil biodegradation was up to 32% and 50% in 72 h.	[28]
Enzyme consortium of three mutant alkane hydroxylases (*alkMa*, *alkMb*, and *almA*) belonging to *Acinetobacter venetianus* strain RAG-1.	Degradation of light crude oil by 88.65% [15.23% more than the wild type (WT)], viscous crude oil was degraded by 90.05% (21.65% more than WT), and high waxy crude oil by 60.52% (13.06% more than WT), in 10 days.	[29]
An improved dehalogenase gene (*dhaA31*) was cloned behind the constitutive *dhlA* promoter into *Pseudomonas putida* MC4.	1,2,3-Trichloropropane was degraded by 97% in 48 days. The enzyme showed 36-foldhigher activity and 26-foldhigher catalytic efficiency than the WT enzyme.	[30]
A recombinant strain was constructed from the integration of catechol 2,3-dioxygenase in *Acinetobacter* sp. BS3.	The biodegradation rate of the oil concentrations was 80% in 28 days.	[31]

Synthetic biology aims to design and construct an organism with a specific set of characteristics. Using computational models and engineering techniques, genetic circuits and metabolic pathways can be assembled and fine-tuned. To construct these microorganisms, modifications are encoded in vectors that are delivered into suitable hosts, known as chassis. This term in synthetic biology refers to an organism that acts as a carrier for the genetic components and allows them to function [32–34]. To build the appropriate chassis, there are two known approaches: (a) top-down, which generates synthetic organisms by manipulating existing genes or metabolic pathways; and (b) bottom-up, where de novo organisms are created from molecular building blocks [6,35–37].

In any case, a series of general steps can be followed to create an engineered organism for the degradation of pollutants: (1) selection and design of the microorganism, this includes the appropriate choice of the host and preliminary engineering; (2) metabolic or genetic optimization, improvements can be made at different levels to obtain better results in degradation; and (3) tolerance engineering of the chassis, with the aim of regulating or creating a response system to extreme conditions or stress, in order to increase biodegradation (Figure 1) [6].

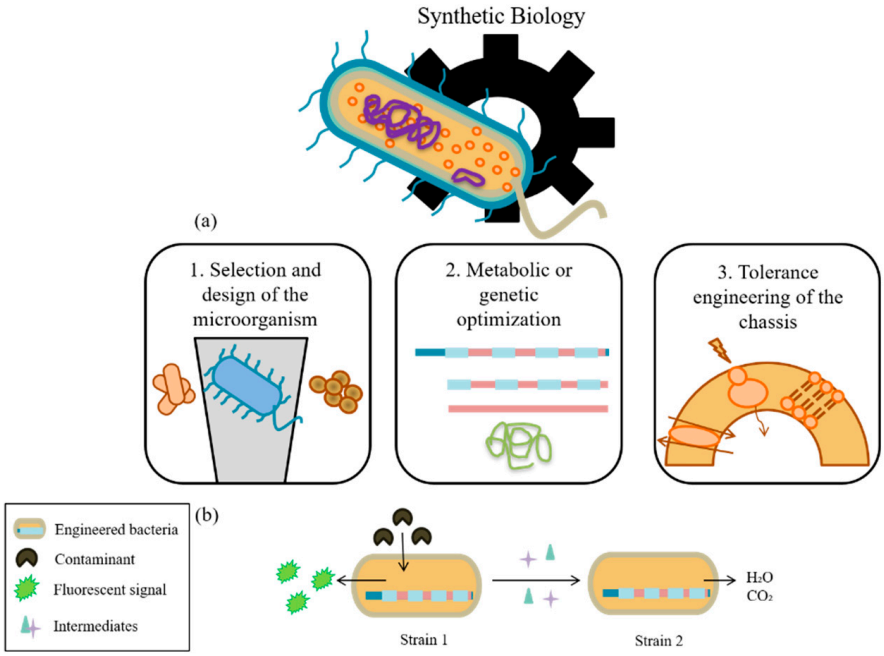

Figure 1. General workflow of the creation of an engineered microorganism and its possible uses in bioremediation. (**a**) Steps to create an engineered microorganism; (**b**) representation of a microorganism designed with synthetic biology for hydrocarbon bioremediation: the first strain functions as a biosensor by producing a bioluminescent signal. At the same time, it carries out the first steps of contaminant degradation, releasing intermediary metabolites. The second strain, having been modified, is now able to assimilate these products to finish the degradation process, reaching the mineralization of the pollutants.

Creation of genetic engineered microorganisms, design of biosensors and the use of consortia, are some of the strategies based on synthetic biology that offer the creation of innovative tools for increasing the efficiency of degradation. Here, this article will review some of the techniques that use synthetic biology as a platform to be used in the area of hydrocarbon bioremediation.

3. Genetically Engineered Microorganisms

Many microorganisms have the metabolic potential to use toxic compounds as a carbon source, and can even degrade some of these contaminants [38]. Thanks to advancements in

genetic and metabolic engineering, these properties can be enhanced to create new, efficient, and safe techniques that could overcome physicochemical ones [38–40].

One example is modifying or inserting the necessary metabolic machinery that will allow microorganisms to degrade specific contaminants [41,42]. Recombinant DNA technology allows the insertion of a gene of interest through a vector, so the microorganism will be capable of expressing the desired gene and gaining a new function [43]. There are different strategies to construct genetically engineered microorganism (GEMs) in bioremediation, although, there are two main techniques: (a) incorporation of the necessary degradation machinery, creating new metabolic pathways; and (b) genetic and metabolic optimization, to enhance affinity, specificity, and efficiency of the enzymes involved in degradation processes, as well as to improve substrate utilization, and increase bioavailability and genetic stability [40,42,44,45].

To create new metabolic pathways, it is necessary to identify degradative enzymes, their respective genes, and strains with catabolic capacity. Then, the chosen genetic sequence is inserted into a vector to subsequently transform the host. Specific experiments need to be performed to confirm the sequence integration into the microorganisms, as well as the efficiency of the biodegradation gene [40,45].

A very important part in creating new metabolic pathways is selecting a plasmid. Plasmids can carry degradative genes that encodes enzymes needed for the degradation of different contaminants, such as aliphatic, aromatic and polycyclic aromatic hydrocarbons [46,47]. Bacterial plasmids can be transferred through horizontal gene transfer (HGT) which may occur by different mechanisms: transformation, transduction, and bacterial conjugation. Transformation is the most used method to insert genes of interest into a microorganism, by using different vectors [47–49]. Some of the most commonly used plasmids in microbial engineering for hydrocarbon bioremediation are PHE, TOL, NAH, and OCT [46].

The PHE plasmid contains genetic information necessary for phenol metabolism to take place, the TOL plasmid encodes *xyl* genes that are necessary for the degradation of toluene and xylene. Naphthalene catabolic genes are part of the NAH plasmid and OCT plasmid is responsible for the octane degradation by *alk* genes [46,47,50].

Wang et al. [51] constructed an *E. coli* strain capable of converting phenol to a carbon source thanks to the integration of synthetic modules. First, phenol degrading genes were selected, isolated and modified. Then, two metabolic modules were constructed using two phenol hydroxylase genes and seven catechol-degrading genes, both of which were integrated into a vector to transform *E. coli* cells. Engineered strains degraded phenol rapidly in crude-contaminated wastewater: 5 mM in 7 h [51]. The study demonstrates the successful construction of a novel metabolic pathway capable of degrading phenol in *E. coli.* This is a great advantage considering that the bacteria do not naturally degrade phenol, making this synthetically modified strain one of the first to utilize phenol as a carbon source [51].

In contrast, to improve the genetic and metabolic performance of enzymes involved in the degradation process, fine-tuning can be performed at different stages of gene expression, such as transcription, translation and post-translation. Genetic optimization is carried out with the purpose of obtaining the best genetic platform to maximize metabolic efficiency. Some of these methods consist in the search of homologous genes or the optimization of codons, in order to match the host or to find the most appropriate degradation genes for the chosen microorganisms. It is also possible to regulate the number of copies of the plasmid and mRNA expression, or to modify the translation rate by modifying the ribosome binding site (RBS). All of these modifications could have different effects, such as minimizing bottlenecks, avoiding overexpression of an enzyme, or increasing the affinity or binding of the ribosomes to the transcripts [40,52]. Gene expression can be regulated by many factors, so fine-tuning allows to achieve a proper balance in pathways to maximize metabolic efficiency.

Research conducted by Jain et al. [53] focused on a in silico analysis of a protein involved in hydrocarbon degradation, alkane monooxygenase (ladA). An orthologous gene for this enzyme was found in *Burkholderia thailandensis* MSMB121. Homology modeling was performed in its structure and several amino-acids of the ladA protein were substituted. This achieved an improvement in the binding energy to chains of different alkanes which increases accessibility to the substrate, creating the basis for further validation of hydrocarbon degradation pathways in *B. thailandensis* [53]. This study is a great example of how genetic optimization is a tool to achieve metabolic improvements for degradation of various hydrocarbons.

Another approach of metabolic optimization is when metabolic pathways can be improved, following the theory that there is proximity between enzymes of the same pathway, allowing products to be immediately used, which generates a substrate tunneling effect. For this purpose, a synthetic protein scaffold can be designed, containing binding domains along with enzymes involved in the pathway. Therefore, scaffold aligns enzymes very closely, mimicking the substrate tunneling effect [40,52].

One example of a synthetic scaffold was made by Dueber et al. [54] with the aim of increasing the effective concentration of each component of a metabolic pathway. To achieve this, a scaffold of synthetic proteins that spatially recruited enzymes was constructed. The scaffold contained PDZ-SH3-GBD domains, which together with their binding peptides, was attached to each heterologous enzyme involved in a synthetic pathway, which mimicked the substrate tunneling effect. The use of this synthetic scaffold increased the efficacy of the pathway by 77-fold [40,54]

Most hydrocarbons are difficult to eliminate, and although there are microorganisms that have degradative capacities, it is not enough to achieve efficient biodegradation. For this reason, and thanks to genetic engineering advances, it has been possible to construct GEMs, which through genetic refinement or construction of new pathways offer several advantages, such as: metabolic optimization, integration of catabolic modules, increased enzyme expression and biodegradation rate, among others. Synthetic biology has given efficient results in the degradation of pollutants, allowing to create a new alternative in bioremediation areas.

4. Biosensors

Due to the harmful effects that oil contamination has on the environment, animal life and human health, it is very important to evaluate the risks this kind of pollutant may have [55,56]. In order to reduce the impact and contain the contamination as early as possible, the development of a detection unit for these compounds is necessary [56–58]. However, this represents a great challenge due to high costs, time spent and complex procedures. Establishing new techniques with high effectiveness and sensitivity, and rapid detection is necessary [55,58].

A biosensor is an integral and analytical device, which through biochemical reactions, can detect a signal to provide quantitative and precise information. Biosensors are composed of three main elements: a biological recognition element, a transducer, and a system that processes the signal (detector) [4,59,60].

The biological recognition elements (receptor) can be enzymes, antibodies, antigens, nucleic acids and even whole cells. The detected biochemical signals could be those derived from metabolic processes, gene expression, cellular toxicity, or enzyme activity. Finally, the transducer can be classified according to its physicochemical nature to detect the electrochemical, optical, calorimetric, or thermal signals [59,60].

When the biological sample comes in contact with the receptor, the transducer will convert it into a quantifiable electrical signal [4,56,59,60]. This means that the biological recognition element selectively identifies the analyte by generating a specific signal. The type of signal generated depends on the kind of transducer used. Then, the signal is quantified by the detector [56].

There are biological recognition elements that have the ability to detect multiple analytes which are useful when monitoring multiple toxic compounds. One of the recognition elements that have the ability to monitor multiple samples simultaneously, selectively and with fast response, are antibodies [56].

In the oil industry it is of great importance to perform environmental monitoring to ensure the safety of the processes and reduce possible contaminations [61]. Biosensors can be used to monitor oil spills along the process of bioremediation [62]. In this way, they help detecting toxic compounds on time, so potential risks can be eliminated [57].

There are physicochemical methods that evaluate oil contamination [61]. Although they are effective, they are also highly expensive, time-consuming and require solvents for extraction and a large sample volume [55,61]. Molecular tools offer an alternative that will overcome these limitations [61].

Biosensors can be constructed by isolating biological components or using whole microorganisms. These sensors have great advantages. For example, they are fast, easy-to-use and cost-effective tools that require less sample volume, no need for solvent extraction, are robust and have good compatibility for real-time application [56,61,62]. They can also be cultivated on a large scale, and even be engineered to resist harsh conditions such as extreme pH and temperatures, and environmental contamination [4,59].

Synthetic biology offers the possibility of constructing whole cell biosensors (WCB). WCBs have been used to monitor environmental pollutants such as heavy metals, pesticides, pharmaceutical residues, chemicals, and organic pollutants such as oil derivatives [10]. The use of microorganisms to monitor different pollutants is a result of the development of genetic engineering techniques which have made it possible to modify or design the necessary elements for the detection and processing of signals [63]. This new alternative has diverse bioreceptors that can be used along with different genetic mechanisms to overcome traditional sensors and be used in situ [61].

When building WCBs it is important to consider the interaction between promoters and reporter genes. Promoters can be inducible by external factors or constitutively expressed, and reporter proteins must produce quantifiable signals with high sensitivity [63].

Commonly used reporter genes are: (1) *lacZ* gene from *Escherichia coli*, this gene encodes β-galactosidase. The enzyme degrades specific substrates producing colored compounds which are measured by colorimetry; (2) *GFP* gene from *Aequorea victoria*, encodes a green fluorescent protein (GFP). This protein absorbs light and emits it in a different wavelength, which can be easily measured; (3) *lux* operon, works via a quorum-sensing mechanism. The most used enzyme in WCB is luciferase (Luc), and it uses two types of configuration: *LuxCDABE*, from *Vibrio fischeri* (where the same cell synthesizes the necessary substrate for the luciferase) and *LuxAB*, from *Vibrio harveyi* (where the substrate needs to be added) [55,63,64].

The first genetically modified microorganism to be used as a WCB for the monitoring of the bioremediation process of contaminated soil was *Pseudomonas fluorescens* HK44 strain, which contained the pUTK21 plasmid. This plasmid holds the *nahG* gene (which controls the degradation of salicylate) fused with the *LuxCDABE* gene cluster. Another example of a WCB genetic construct is the fusion between the *Luc* gene and the *Pu* promoter, controlled by XylR activator protein to monitor toluene in the environment. This activator protein binds to toluene or its derivatives and activates Pu promoter; thus, creating a bioluminescent sensor [64].

Furthermore, the study of Patel et al. [65] developed two biosensing strains to detect hydrocarbons. Two vectors were designed with a promoter-operator fusion with fluorescent protein genes: *tbuT-gfp* (capable of detecting BTEX compounds) and *phnR-cfp* (capable of detecting naphthalene, phenanthrene, and related PAH compounds). Designed vectors were then transformed into *E. coli* DH5α. Both recombinant strains were capable of detecting mono and polyaromatic hydrocarbons, creating a method to measure contaminant levels [65].

To create an adaptable WCB capable of being tuned for a particular monoaromatic contaminant, Roy et al. [66] combined synthetic biology with a complementary design to construct a genetically rewired and selective biosensor, ideal to detect pollutants in contaminated water sources [66]. This study based its design on the phenol catabolism pathway, using the MopR protein, triggering the transcription of the gene cluster when binding to phenol. In this case, the difference was that to achieve sensitive detection, the catabolism gene cluster was replaced with a reporter gene (*Luc*); creating the *MopRLuc* biosensor, capable of detecting low and high concentrations of phenol in water. Given the efficiency of the constructed biosensor, Roy et al. [66] decided to use it as a model to create an array of WCBs for other pollutants. They discovered that changes in sensor profile were due to mutations in the variable region, so they could generate biosensors to detect xylenol, ethylbenzene and xylene. Not only this study was able to obtain successful results, but also was able to create a biosensor template that can be engineered for the detection of different aromatic pollutants [66].

There are still some areas in biosensor development that need to be studied to overcome limitations, such as sample interferences, cell stability, bioavailability of hydrocarbons and even legal considerations on the release of engineered microorganisms into nature [61,62]. However, synthetic biology brought new possibilities in the creation of biosensors to help achieve early detection of toxic compounds [57].

When it comes to choosing an appropriate bioremediation technique, it is important to know the conditions of the contamination site as well as the type of pollutant involved. Lack of information can cause setbacks in the elimination process or even affect the establishment of necessary control measures. Therefore, correct detection of contaminants is of great importance. Using microorganisms offers great advantages, given the fact that many of them are able to withstand high concentrations of contaminants, their adaptation is possible and makes them perfect candidates for monitoring. Considering many hydrocarbons are highly toxic, synthetic biology creates the possibility of engineering microorganisms with the necessary machinery to detect different pollutants. Studies carried out in this area provide data to position biosensors as new effective systems for detecting hydrocarbons in the environment.

5. Construction of Synthetic Consortia

A microbial consortium is a set of two or more microbial species that work synergistically to create a balanced community where mutual benefit exists [67,68]. The life cycle of a microbial community depends directly on the relationships existing among them, creating cooperative or competitive dynamics [67,69], as well as neutral, positive or negative effects [70]. These relationships are classified in: (a) symbiotic, where organisms obtain mutual benefit (e.g., mutualisms); and (b) antagonistic, where one species can be harmed and the other benefited (e.g., parasitism) [71].

A consortium tends to be more effective than a single microorganism due to the synergistic relationships resulting from complementary activities and metabolic capacity of each species [72]. Since metabolites of one microbe can be used by another one, microbial communities can use these mutual interactions to completely degrade contaminants [29]. On that account, when designing a consortium it is necessary to establish parameters that guarantee coexistence and stable interactions between all of the species [24,73].

Each environment has autochthonous microorganisms with different degradation capacities. However, the degradation of hydrocarbons by these individual microorganisms can be low. Consortia are capable of achieving complete mineralization of contaminants thanks to a sequential degradation due to the synergistic and metabolic activities they possess as a group [74]. Therefore, an alternative in hydrocarbon bioremediation is the addition and combination of allochthonous microorganisms for the creation of consortia, which is more effective and sustainable than traditional methods [75,76].

Molecular tools, along with systems biology, enable the analysis of genetic information and cell to cell interactions [8]. Omics tools can provide information on genes, proteins

and metabolic pathways that will help to understand microbial behavior to enhance the biodegradation processes. For example, they can be used to evaluate the structure and dynamics of microbial consortia in different environmental scenarios [24]. Thanks to this, synthetic biology offers the possibility of creating engineered microbial communities to make strong cellular functions and improve its microbial capabilities and cooperation [8]. Assembling synthetic consortia will improve efficacy in bioremediation. These modifications can be made by manipulating environmental conditions, communication networks, syntrophic interactions or the genetic framework and new genetic modules [8,24,34].

Synthetic biology tools can be used in microbial consortia to facilitate the interaction among microorganisms. Some of these tools are: (a) syntrophic interactions, to create a metabolic network where metabolites produced by one organism can be used by another one; (b) exogenous molecules, adding external inputs to control cell communities and gene expression; and (c) intercellular signaling, to control communication between cells and gene expression (e.g., quorum sensing) [34,77].

One example of a synthetic community is the study of a consortium consisting of two bacterial strains that were modified for phenanthrene degradation, by Jia et al. [78]. The used strains were: (a) *E. coli* HY, with two terminal dioxygenase modules and an electron transfer chain; and (b) *Pseudomonas aeruginosa* PH2, with a catechol 1,2-dioxygenase module. A gas chromatography-mass spectrometry (GC-MS) was performed to identify metabolites. The initial oxidation steps were made by *E. coli* HY1 (phenanthrene into 9,10-dihydroxy phenanthrene or 1,2-dihydroxy phenanthrene), and then ring cleavage was performed by *P. aeruginosa* PH2 to produce catechol. Further conversion between intermediates was through the tricarboxylic acid cycle (TCA). The modified consortium was able to degrade 71% of phenanthrene in nine days, while the wild type (*P. aeruginosa* PH1) only degraded 45% [78]. This confirmed the improved removal capacity of a constructed consortium compared to unmodified strains, confirming once again a new alternative for bioremediation of PAHs.

Engineered consortia offer great advantages due to their efficient and improved levels of degradation in comparison to individual strains. This means that synthetic communities are a useful and a valid platform for bioremediation of hydrocarbons, even with efficient results. Analysis of each microbial community, separately and as a whole, is needed when designing and constructing one. It is important to remember synergistic relationships are the key to the consortium's success since metabolic capabilities and characteristics of each species can be integrated, enhancing biodegradation. Developing a microbial consortium with specific parameters to bioremediate hydrocarbon-contaminated areas is one of the most promising benefits that synthetic biology offers.

6. Risk Assessment of Synthetic Biology

Considering the bioremediation process will take place at the site of contamination, GEMs will be leaving the laboratory and entering a natural environment, which may entail risks or difficulties since it stops being a controlled environment [8,42]. The environmental risks relate to gene contamination, toxicity and competition with native species. The problems of gene contamination are related to horizontal gene transfer which leads to the delivery of the recombinant genes [79], modifying autochthonous microorganisms and altering their natural genetics [42]. One of the major risks is posed by plasmids containing antibiotics as resistance genes, as they can lead to the formation of antibiotic-resistant superbugs in nature [80]. It is also important to consider the release of compounds toxic to the environment or related to human health due to the change in microbial metabolism [42,80]. In regards to the competition with native species, as noted by de Lorenzo [81], the risk of altering the microbial composition by introducing GEMs into natural ecosystems is not as high as commonly thought. Due to the homeostasis of biological ecosystems and resistance to colonization, engineered microorganism have difficulties at establishing in a new environment, meaning that it is unlikely that the modified microorganisms could displace the indigenous community [80,81].

To minimize potential risks, strategies include using non-antibiotic selection markers and avoiding gene transfer to indigenous organisms [82]. To resolve this problem, biocontainment techniques based on cellular circuits, inducible systems or auxotrophy have been developed [83]. The most promising technique for biocontainment of genes in a natural environment is through toxin-antitoxin systems. This protection system will secure the genetic material from horizontal gene transfer [84]. A toxin is encoded in a plasmid, while the antitoxin is encoded into the genome. Therefore, if a gene transfer occurs, the new host would die as it incorporated the plasmid with the toxin but does not possess the antitoxin [83]. Another strategy that can be used to protect the inserted genes is the use of conditional replication origins. The origin needs an initiator protein to replicate the plasmid, which is inserted into the chromosome of the host. In this way, the replication of the plasmid is blocked if it is transferred to another cell [84].

It must be taken into consideration that legal regulations play an important role when implementing these new alternatives, as well as the possible environmental risks it may involve. The good news is genetic engineering and synthetic biology offer to mitigate these biological risks through different biocontainment mechanisms. Therefore, based on the different bioinformatics studies and laboratory results obtained, it is important to carry out more in situ tests to analyze the behavior of GEMs in an uncontrolled environment, as well as their proper biomonitoring, in order to establish this alternative in bioremediation as one of the most effective and safe.

7. Conclusions

Petroleum-derived pollutants are highly toxic, creating serious and harmful consequences in any environment. Developing innovative, fast, safe, and cost-effective techniques for their elimination is of great importance. Bioremediation as a contaminant removal technique has been very successful, and although several microorganisms possess degradative capacities, optimizing these techniques is necessary due to the pollutant's persistence. Over the years, advances in different areas of science have led to improvements in various degradation techniques. Knowledge in systems biology, molecular tools, and multiomics are the basis of synthetic biology, which creates a new era in bioremediation. Analysis, design, construction and fine-tuning of genetically and metabolically optimized microorganisms maximize toxic compounds degradation.

Creating biosensors to detect and monitor contaminants, understanding microbial dynamics to construct synthetic consortia, as well as creating new metabolic pathways or enzymatic enhancement, are some of the possibilities offered by synthetic biology. It is still necessary to carry out more in situ experiments to support different results obtained in laboratories, as well as establishing the necessary safety parameters for an engineered microorganism to enter the environment. The most important thing is that now it is possible to create ideal techniques to degrade persistent and harmful pollutants such as hydrocarbons. Even though some areas need further research, synthetic biology puts science on the right track. With these new tools at hand, bioremediation positions itself as one of the best and most effective pollutant removal process available today.

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References

1. Speight, J.G.; El-Gendy, N.S. (Eds.) Chapter 1—Petroleum Composition and Properties. In *Introduction to Petroleum Biotechnology*; Gulf Professional Publishing: Boston, MA, USA, 2018; pp. 1–39.
2. Varjani, S.J. Microbial degradation of petroleum hydrocarbons. *Bioresour. Technol.* **2017**, *223*, 277–286. [CrossRef] [PubMed]
3. Selley, R.C.; Sonnenberg, S.A. (Eds.) Chapter 2—The Physical and Chemical Properties of Petroleum. In *Elements of Petroleum Geology*, 3rd ed.; Academic Press: Boston, MA, USA, 2015; pp. 13–39.
4. Bilal, M.; Iqbal, H.M.N. Microbial-derived biosensors for monitoring environmental contaminants: Recent advances and future outlook. *Process Saf. Environ. Prot.* **2019**, *124*, 8–17. [CrossRef]
5. Wu, M.; Wu, J.; Zhang, X.; Ye, X. Effect of bioaugmentation and biostimulation on hydrocarbon degradation and microbial community composition in petroleum-contaminated loessal soil. *Chemosphere* **2019**, *237*, 124456. [CrossRef] [PubMed]
6. Xiang, L.; Li, G.; Wen, L.; Su, C.; Liu, Y.; Tang, H.; Dai, J. Biodegradation of aromatic pollutants meets synthetic biology. *Synth. Syst. Biotechnol.* **2021**, *6*, 153–162. [CrossRef]
7. Baniasadi, M.; Mousavi, S.M. A Comprehensive Review on the Bioremediation of Oil Spills. In *Microbial Action on Hydrocarbons*; Kumar, V., Kumar, M., Prasad, R., Eds.; Springer: Singapore, 2018; pp. 223–254.
8. Jaiswal, S.; Shukla, P. Alternative Strategies for Microbial Remediation of Pollutants via Synthetic Biology. *Front. Microbiol.* **2020**, *11*, 808. [CrossRef] [PubMed]
9. Kumari, A.; Chaudhary, D.R. 21—Engineered microbes and evolving plastic bioremediation technology. In *Bioremediation of Pollutants*; Pandey, V.C., Singh, V., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 417–443.
10. Imam, A.; Suman, S.K.; Ghosh, D.; Kanaujia, P.K. Analytical approaches used in monitoring the bioremediation of hydrocarbons in petroleum-contaminated soil and sludge. *TrAC Trends Anal. Chem.* **2019**, *118*, 50–64. [CrossRef]
11. Pal, A.K.; Singh, J.; Soni, R.; Tripathi, P.; Kamle, M.; Tripathi, V.; Kumar, P. 10—The role of microorganism in bioremediation for sustainable environment management. In *Bioremediation of Pollutants*; Pandey, V.C., Singh, V., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 227–249.
12. Azubuike, C.C.; Chikere, C.B.; Okpokwasili, G.C. Bioremediation techniques-classification based on site of application: Principles, advantages, limitations and prospects. *World J. Microbiol. Biotechnol.* **2016**, *32*, 180. [CrossRef]
13. Myazin, V.A.; Korneykova, M.V.; Chaporgina, A.A.; Fokina, N.V.; Vasilyeva, G.K. The Effectiveness of Biostimulation, Bioaugmentation and Sorption-Biological Treatment of Soil Contaminated with Petroleum Products in the Russian Subarctic. *Microorganisms* **2021**, *9*, 1722. [CrossRef]
14. Ossai, I.C.; Ahmed, A.; Hassan, A.; Hamid, F.S. Remediation of soil and water contaminated with petroleum hydrocarbon: A review. *Environ. Technol. Innov.* **2020**, *17*, 100526. [CrossRef]
15. Sakshi; Singh, S.K.; Haritash, A.K. Polycyclic aromatic hydrocarbons: Soil pollution and remediation. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 6489–6512. [CrossRef]
16. Yaman, C. Performance and Kinetics of Bioaugmentation, Biostimulation, and Natural Attenuation Processes for Bioremediation of Crude Oil-Contaminated Soils. *Processes* **2020**, *8*, 883. [CrossRef]
17. Cai, Y.; Wang, R.; Rao, P.; Wu, B.; Yan, L.; Hu, L.; Park, S.; Ryu, M.; Zhou, X. Bioremediation of Petroleum Hydrocarbons Using *Acinetobacter* sp. SCYY-5 Isolated from Contaminated Oil Sludge: Strategy and Effectiveness Study. *Int. J. Environ. Res. Public Health* **2021**, *18*, 819. [CrossRef] [PubMed]
18. Hussain, A.; Rehman, F.; Rafeeq, H.; Waqas, M.; Asghar, A.; Afsheen, N.; Rahdar, A.; Bilal, M.; Iqbal, H.M.N. In-situ, Ex-situ, and nano-remediation strategies to treat polluted soil, water, and air—A review. *Chemosphere* **2022**, *289*, 133252. [CrossRef] [PubMed]
19. Jørgensen, K.S.; Puustinen, J.; Suortti, A.-M. Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. *Environ. Pollut.* **2000**, *107*, 245–254. [CrossRef]
20. Benyahia, F.; Embaby, A.S. Bioremediation of Crude Oil Contaminated Desert Soil: Effect of Biostimulation, Bioaugmentation and Bioavailability in Biopile Treatment Systems. *Int. J. Environ. Res. Public Health* **2016**, *13*, 219. [CrossRef] [PubMed]
21. Steliga, T.; Kluk, D. Assessment of the Suitability of *Melilotus officinalis* for Phytoremediation of Soil Contaminated with Petroleum Hydrocarbons (TPH and PAH), Zn, Pb and Cd Based on Toxicological Tests. *Toxics* **2021**, *9*, 148. [CrossRef] [PubMed]
22. Xu, X.; Liu, W.; Tian, S.; Wang, W.; Qi, Q.; Jiang, P.; Gao, X.; Li, F.; Li, H.; Yu, H. Petroleum Hydrocarbon-Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis. *Front. Microbiol.* **2018**, *9*, 2885. [CrossRef]
23. Jariyal, M.; Yadav, M.; Singh, N.K.; Yadav, S.; Sharma, I.; Dahiya, S.; Thanki, A. 8—Microbial remediation progress and future prospects. In *Bioremediation of Pollutants*; Pandey, V.C., Singh, V., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 187–214.
24. Sharma, B.; Shukla, P. Designing synthetic microbial communities for effectual bioremediation: A review. *Biocatal. Biotransform.* **2020**, *38*, 405–414. [CrossRef]
25. Rylott, E.L.; Bruce, N.C. How synthetic biology can help bioremediation. *Curr. Opin. Chem. Biol.* **2020**, *58*, 86–95. [CrossRef]
26. Xin, F.; Dong, W.; Dai, Z.; Jiang, Y.; Yan, W.; Lv, Z.; Fang, Y.; Jiang, M. Chapter 9—Biosynthetic Technology and Bioprocess Engineering. In *Current Developments in Biotechnology and Bioengineering*; Singh, S.P., Pandey, A., Du, G., Kumar, S., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 207–232.
27. Bhattacharjee, G.; Gohil, N.; Singh, V. 14—Synthetic biology approaches for bioremediation. In *Bioremediation of Pollutants*; Pandey, V.C., Singh, V., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 303–312.

28. Meng, L.; Bao, M.; Sun, P. Construction of long-chain alkane degrading bacteria and its application in bioremediation of crude oil pollution. *Int. J. Biol. Macromol.* **2018**, *119*, 524–532. [CrossRef]
29. Liu, J.; Zhao, B.; Lan, Y.; Ma, T. Enhanced degradation of different crude oils by defined engineered consortia of *Acinetobacter venetianus* RAG-1 mutants based on their alkane metabolism. *Bioresour. Technol.* **2021**, *327*, 124787. [CrossRef] [PubMed]
30. Samin, G.; Pavlova, M.; Arif, M.I.; Postema, C.P.; Damborsky, J.; Janssen, D.B. A *Pseudomonas putida* strain genetically engineered for 1,2,3-trichloropropane bioremediation. *Appl. Environ. Microbiol.* **2014**, *80*, 5467–5476. [CrossRef] [PubMed]
31. Xie, Y.; Yu, F.; Wang, Q.; Gu, X.; Chen, W. Cloning of Catechol 2,3-Dioxygenase Gene and Construction of a Stable Genetically Engineered Strain for Degrading Crude Oil. *Indian J. Microbiol.* **2014**, *54*, 59–64. [CrossRef] [PubMed]
32. Kim, J.; Salvador, M.; Saunders, E.; González, J.; Avignone-Rossa, C.; Jiménez, J.I. Properties of alternative microbial hosts used in synthetic biology: Towards the design of a modular chassis. *Essays Biochem.* **2016**, *60*, 303–313. [PubMed]
33. Chi, H.; Wang, X.; Shao, Y.; Qin, Y.; Deng, Z.; Wang, L.; Chen, S. Engineering and modification of microbial chassis for systems and synthetic biology. *Synth. Syst. Biotechnol.* **2019**, *4*, 25–33. [CrossRef] [PubMed]
34. McCarty, N.S.; Ledesma-Amaro, R. Synthetic Biology Tools to Engineer Microbial Communities for Biotechnology. *Trends Biotechnol.* **2019**, *37*, 181–197. [CrossRef]
35. Göpfrich, K.; Platzman, I.; Spatz, J.P. Mastering Complexity: Towards Bottom-up Construction of Multifunctional Eukaryotic Synthetic Cells. *Trends Biotechnol.* **2018**, *36*, 938–951. [CrossRef]
36. Mutschler, H.; Robinson, T.; Tang, T.-D.; Wegner, S. Special Issue on Bottom-Up Synthetic Biology. *ChemBioChem* **2019**, *20*, 2533–2534. [CrossRef]
37. Roberts, M.A.J.; Cranenburgh, R.M.; Stevens, M.P.; Oyston, P.C.F. Synthetic biology: Biology by design. *Microbiology* **2013**, *159*, 1219–1220. [CrossRef]
38. Varjani, S.J.; Upasani, V.N. A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. *Int. Biodeterior. Biodegrad.* **2017**, *120*, 71–83. [CrossRef]
39. Hussain, I.; Aleti, G.; Naidu, R.; Puschenreiter, M.; Mahmood, Q.; Rahman, M.M.; Wang, F.; Shaheen, S.; Syed, J.H.; Reichenauer, T.G. Microbe and plant assisted-remediation of organic xenobiotics and its enhancement by genetically modified organisms and recombinant technology: A review. *Sci. Total Environ.* **2018**, *628–629*, 1582–1599. [CrossRef] [PubMed]
40. Tran, K.M.; Lee, H.; Thai, T.D.; Shen, J.; Eyun, S.; Na, D. Synthetically engineered microbial scavengers for enhanced bioremediation. *J. Hazard. Mater.* **2021**, *419*, 126516. [CrossRef] [PubMed]
41. Chaudhary, D.K.; Kim, J. New insights into bioremediation strategies for oil-contaminated soil in cold environments. *Int. Biodeterior. Biodegrad.* **2019**, *142*, 58–72. [CrossRef]
42. Kumar, A.; Singh, R.; Kumar, A.; Rai, R.; Singh, V.; Bhadouria, R. Genetically engineered bacteria for the degradation of dye and other organic compounds. In *Abatement of Environmental Pollutants*; Singh, P., Kumar, A., Borthakur, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 331–350.
43. Pant, G.; Garlapati, D.; Agrawal, U.; Prasuna, R.G.; Mathimani, T.; Pugazhendhi, A. Biological approaches practised using genetically engineered microbes for a sustainable environment: A review. *J. Hazard. Mater.* **2021**, *405*, 124631. [CrossRef] [PubMed]
44. Liu, L.; Bilal, M.; Duan, X.; Iqbal, H.M.N. Mitigation of environmental pollution by genetically engineered bacteria—Current challenges and future perspectives. *Sci. Total Environ.* **2019**, *667*, 444–454. [CrossRef]
45. Miri, S.; Naghdi, M.; Rouissi, T.; Kaur Brar, S.; Martel, R. Recent biotechnological advances in petroleum hydrocarbons degradation under cold climate conditions: A review. *Crit. Rev. Environ. Sci. Technol.* **2019**, *49*, 553–586. [CrossRef]
46. Boronin, A.M.; Kosheleva, I.A. The Role of Catabolic Plasmids in Biodegradation of Petroleum Hydrocarbons. In *Current Environmental Issues and Challenges*; Cao, G., Orrù, R., Eds.; Springer: Dordrecht, The Netherlands, 2014; pp. 159–168.
47. Bhatt, P.; Bhandari, G.; Bhatt, K.; Maithani, D.; Mishra, S.; Gangola, S.; Bhatt, R.; Huang, Y.; Chen, S. Plasmid-mediated catabolism for the removal of xenobiotics from the environment. *J. Hazard. Mater.* **2021**, *420*, 126618. [CrossRef]
48. Emamalipour, M.; Seidi, K.; Zununi Vahed, S.; Jahanban-Esfahlan, A.; Jaymand, M.; Majdi, H.; Amoozgar, Z.; Chitkushev, L.T.; Javaheri, T.; Jahanban-Esfahlan, R.; et al. Horizontal Gene Transfer: From Evolutionary Flexibility to Disease Progression. *Front. Cell Dev. Biol.* **2020**, *8*, 229. [CrossRef]
49. Yutin, N. Horizontal Gene Transfer. In *Brenner's Encyclopedia of Genetics*, 2nd ed.; Maloy, S., Hughes, K., Eds.; Academic Press: San Diego, CA, USA, 2013; pp. 530–532.
50. Abbasian, F.; Lockington, R.; Megharaj, M.; Naidu, R. A Review on the Genetics of Aliphatic and Aromatic Hydrocarbon Degradation. *Appl. Biochem. Biotechnol.* **2016**, *178*, 224–250. [CrossRef]
51. Wang, B.; Xu, J.; Gao, J.; Fu, X.; Han, H.; Li, Z.; Wang, L.; Tian, Y.; Peng, R.; Yao, Q. Construction of an *Escherichia coli* strain to degrade phenol completely with two modified metabolic modules. *J. Hazard. Mater.* **2019**, *373*, 29–38. [CrossRef]
52. Jones, J.A.; Koffas, M.A.G. Chapter Eight—Optimizing Metabolic Pathways for the Improved Production of Natural Products. *Meth. Enzymol.* **2016**, *575*, 179–193.
53. Jain, C.K.; Gupta, M.; Prasad, Y.; Wadhwa, G.; Sharma, S.K. Homology modeling and protein engineering of alkane monooxygenase in *Burkholderia thailandensis* MSMB121: In silico insights. *J. Mol. Model.* **2014**, *20*, 2340–2343. [CrossRef] [PubMed]
54. Dueber, J.E.; Wu, G.C.; Malmirchegini, G.R.; Moon, T.S.; Petzold, C.J.; Ullal, A.V.; Prather, K.L.J.; Keasling, J.D. Synthetic protein scaffolds provide modular control over metabolic flux. *Nat. Biotechnol.* **2009**, *27*, 753–759. [CrossRef]

55. Jiang, B.; Song, Y.; Liu, Z.; Huang, W.E.; Li, G.; Deng, S.; Xing, Y.; Zhang, D. Whole-cell bioreporters for evaluating petroleum hydrocarbon contamination. *Crit. Rev. Environ. Sci. Technol.* **2021**, *51*, 272–322. [CrossRef]
56. Behera, B.K.; Das, A.; Sarkar, D.J.; Weerathunge, P.; Parida, P.K.; Das, B.K.; Thavamani, P.; Ramanathan, R.; Bansal, V. Polycyclic Aromatic Hydrocarbons (PAHs) in inland aquatic ecosystems: Perils and remedies through biosensors and bioremediation. *Environ. Pollut.* **2018**, *241*, 212–233. [CrossRef] [PubMed]
57. Nandimandalam, H.; Gude, V.G. Indigenous biosensors for in situ hydrocarbon detection in aquatic environments. *Mar. Pollut. Bull.* **2019**, *149*, 110643. [CrossRef]
58. Paitan, Y.; Biran, I.; Shechter, N.; Biran, D.; Rishpon, J.; Ron, E.Z. Monitoring aromatic hydrocarbons by whole cell electrochemical biosensors. *Anal. Biochem.* **2004**, *335*, 175–183. [CrossRef]
59. Rajkumar, P.; Ramprasath, T.; Selvam, G.S. 12—A simple whole cell microbial biosensors to monitor soil pollution. In *New Pesticides and Soil Sensors*; Grumezescu, A.M., Ed.; Academic Press: Cambridge, MA, USA, 2017; pp. 437–481.
60. Nigam, V.K.; Pratyoosh, S. Enzyme Based Biosensors for Detection of Environmental Pollutants-A Review. *J. Microbiol. Biotechnol.* **2015**, *25*, 1773–1781. [CrossRef]
61. Voon, C.H.; Yusop, N.M.; Khor, S.M. The state-of-the-art in bioluminescent whole-cell biosensor technology for detecting various organic compounds in oil and grease content in wastewater: From the lab to the field. *Talanta* **2022**, *241*, 123271. [CrossRef]
62. Moratti, C.F.; Scott, C.; Coleman, N.V. Synthetic Biology Approaches to Hydrocarbon Biosensors: A Review. *Front. Bioeng. Biotechnol.* **2022**, *9*, 804234. [CrossRef]
63. Tecon, R.; Van der Meer, J.R. Bacterial Biosensors for Measuring Availability of Environmental Pollutants. *Sensors* **2008**, *8*, 4062–4080. [CrossRef]
64. Plotnikova, E.G.; Shumkova, E.S.; Shumkov, M.S. Whole-cell bacterial biosensors for the detection of aromatic hydrocarbons and their chlorinated derivatives. *Appl. Biochem. Microbiol.* **2016**, *52*, 347–357. [CrossRef]
65. Patel, R.; Zaveri, P.; Mukherjee, A.; Agarwal, P.K.; More, P.; Munshi, N.S. Development of fluorescent protein-based biosensing strains: A new tool for the detection of aromatic hydrocarbon pollutants in the environment. *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109450. [CrossRef] [PubMed]
66. Roy, R.; Ray, S.; Chowdhury, A.; Anand, R. Tunable Multiplexed Whole-Cell Biosensors as Environmental Diagnostics for ppb-Level Detection of Aromatic Pollutants. *ACS Sens.* **2021**, *6*, 1933–1939. [CrossRef] [PubMed]
67. Padmaperuma, G.; Butler, T.; Shuhaili, F.; Almalki, W.; Vaidyanathan, S. Microbial consortia: Concept and application in fruit crop management. In *Fruit Crops*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 353–366.
68. Khoo, K.S.; Chew, K.W.; Yew, G.Y.; Leong, W.H.; Chai, Y.H.; Show, P.L.; Chen, W. Recent advances in downstream processing of microalgae lipid recovery for biofuel production. *Bioresour. Technol.* **2020**, *304*, 122996. [CrossRef]
69. Kong, W.; Meldgin, D.R.; Collins, J.J.; Lu, T. Designing microbial consortia with defined social interactions. *Nat. Chem. Biol.* **2018**, *14*, 821–829. [CrossRef]
70. Zhang, Z.; Yan, C.; Zhang, H. Mutualism between antagonists: Its ecological and evolutionary implications. *Integr. Zool.* **2021**, *16*, 84–96. [CrossRef]
71. Drew, G.C.; Stevens, E.J.; King, K.C. Microbial evolution and transitions along the parasite–mutualist continuum. *Nat. Rev. Microbiol.* **2021**, *19*, 623–638. [CrossRef]
72. Bidja Abena, M.T.; Sodbaatar, N.; Li, T.; Damdinsuren, N.; Choidash, B.; Zhong, W. Crude Oil Biodegradation by Newly Isolated Bacterial Strains and Their Consortium Under Soil Microcosm Experiment. *Appl. Biochem. Biotechnol.* **2019**, *189*, 1223–1244. [CrossRef]
73. Qian, X.; Chen, L.; Sui, Y.; Chen, C.; Zhang, W.; Zhou, J.; Dong, W.; Jiang, M.; Xin, F.; Ochsenreither, K. Biotechnological potential and applications of microbial consortia. *Biotechnol. Adv.* **2020**, *40*, 107500. [CrossRef]
74. Srivastava, A.K.; Singh, R.K.; Singh, D. Chapter 20—Microbe-based bioreactor system for bioremediation of organic contaminants: Present and future perspective. In *Microbe Mediated Remediation of Environmental Contaminants*; Kumar, A., Singh, V.K., Singh, P., Mishra, V.K., Eds.; Woodhead Publishing: Sawston, UK, 2021; pp. 241–253.
75. Chen, W.; Kong, Y.; Li, J.; Sun, Y.; Min, J.; Hu, X. Enhanced biodegradation of crude oil by constructed bacterial consortium comprising salt-tolerant petroleum degraders and biosurfactant producers. *Int. Biodeterior. Biodegrad.* **2020**, *154*, 105047. [CrossRef]
76. Guo, G.; Tian, F.; Ding, K.; Wang, L.; Liu, T.; Yang, F. Effect of a bacterial consortium on the degradation of polycyclic aromatic hydrocarbons and bacterial community composition in Chinese soils. *Int. Biodeterior. Biodegrad.* **2017**, *123*, 56–62. [CrossRef]
77. Xu, C.; Yu, H. Insights into constructing a stable and efficient microbial consortium. *Chin. J. Chem. Eng.* **2021**, *30*, 112–120. [CrossRef]
78. Jia, X.; He, Y.; Jiang, D.; Liu, C.; Lu, W. Construction and analysis of an engineered *Escherichia coli-Pseudomonas aeruginosa* co-culture consortium for phenanthrene bioremoval. *Biochem. Eng. J.* **2019**, *148*, 214–223. [CrossRef]
79. Wu, C.; Li, F.; Yi, S.; Ge, F. Genetically engineered microbial remediation of soils co-contaminated by heavy metals and polycyclic aromatic hydrocarbons: Advances and ecological risk assessment. *J. Environ. Manag.* **2021**, *296*, 113185. [CrossRef]
80. Wang, F.; Zhang, W. Synthetic biology: Recent progress, biosafety and biosecurity concerns, and possible solutions. *J. Biosaf. Biosecurity* **2019**, *1*, 22–30. [CrossRef]
81. de Lorenzo, V. Environmental biosafety in the age of Synthetic Biology: Do we really need a radical new approach? *Bioessays* **2010**, *32*, 926–931. [CrossRef]

82. Cases, I.; de Lorenzo, V. Genetically modified organisms for the environment: Stories of success and failure and what we have learned from them. *Int Microbiol.* **2005**, *8*, 213–222.
83. Torres, L.; Krüger, A.; Csibra, E.; Gianni, E.; Pinheiro, V.B. Synthetic biology approaches to biological containment: Pre-emptively tackling potential risks. *Essays Biochem.* **2016**, *60*, 393–410.
84. Kim, D.; Lee, J.W. Genetic Biocontainment Systems for the Safe Use of Engineered Microorganisms. *Biotechnol. Bioprocess Eng.* **2020**, *25*, 974–984. [CrossRef]