Engineering Microbial Consortia towards Bioremediation

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Engineering microbial consortia is an effective way for the removal of heavy metals and organic pollutants. In the study, we discussed the molecular and ecological mechanisms of engineering microbial consortia with a particular focus on metabolic cross-feeding within species and the transfer of metabolites. Besides, the advantages and limitations of top-down and bottom-up approaches of engineering microbial consortia were discussed, together with their applications in bioremediation.

Keywords: engineering microbial consortia ; bioremediation ; cross-feeding ; top-down engineering ; bottom-up engineering

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1. Introduction

Microbes are ubiquitous organisms, found in air, soil, water, as well as animals, and plants [1]. They play vital roles in driving global biogeochemical cycles and have an immense impact on the survival, health, and development of mankind. A number of microbes have been isolated in laboratories that possess the ability to degrade organic pollutants and reduce or transform heavy metals [2]. However, the transforming efficiency of pollutants by a single species always declines when applied to in-site complex polluted sites [3]. Complex pollutants impose stress conditions on a single species and hinder their metabolism. In contrast, microbial consortia tend to show resistance and multifunctionality as varied species work together to efficiently utilize all forms of substrates [4][5].

Engineering microbial consortia may be an effective way to optimize the interaction within microorganisms and their environment and to ensure long-term stability. In the microbial communities, microbes compete for limited nutrients and consume metabolic products secreted by other species to gain fitness advantage [6]. It has been successfully applied in bioremediation of polluted sites, but also failed in some cases [7]. The metabolic interactions have a huge impact on the application of microbial consortia. The substances secreted by specific species can support or suppress the growth of other species, alter interactions between them and even influence the function of the whole community. Synthetic microbial consortia are defined as one that is created artificially by co-culturing of select (two or many) species under a (at least initially) well-defined media [8]. Unlike natural microbial consortia, it is feasible to reconfigure metabolic pathways and program social interactions of synthetic microbial consortia to obtain the desired function.

In this entry, we focused on the molecular mechanisms of microbial consortia, particularly metabolic cross-feeding between species (**Figure 1**). We reviewed two main ways of engineering microbial consortia: top-down engineering and bottom-up engineering. Besides, we addressed important principles for engineering microbial consortia for the bioremediation of pollutants.

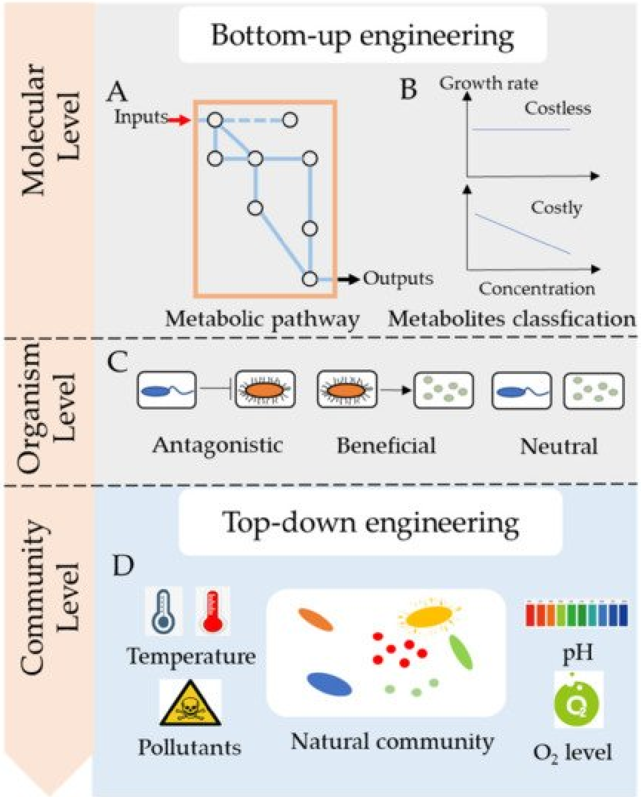

Figure 1. Approaches for engineering microbial consortia. There are two main approaches for engineering microbial consortia: bottom-up engineering and top-down engineering. Bottom-up engineering involves reconfiguring the metabolic pathway (**A**) and reprogramming social interactions (choose the species without reprogramming their metabolism). (**C**) The interactions between microbial consortia include antagonistic, neutral, or beneficial interactions (**B**) Metabolites are mainly divided into costless and costly depending on whether the secretion of metabolites causes a loss to fitness in monoculture (represented by growth rate). (**D**) Top-down engineering involves compelling a natural microbial community to perform desired functions by modifying environmental variables, such as temperature, pH, salt content, redox conditions, and carbon source.

2. Microbial Cross-Feeding in Microbial Consortia

Microbial cross-feedings are common in natural environments [9] and microbes frequently exchange substances [10]. Many studies have reported that microbes significantly benefit from this exchange of metabolites [11]. In order to improve microbial consortia's performance in bioremediation, it is essential to delineate the mechanisms of engineered microbial consortia on the molecular level by identifying metabolites and mechanisms of transfer.

Microbes commonly secrete metabolites across the membrane into the environment for utilization by other species. Several metabolites such as carbon and nitrogen resources, hydrogen (H 2), amino acids, vitamin, or growth factors may be exchanged between microbes [7]. These metabolites could be classified by molecule type, connection to metabolism, and fitness cost. Recently, more researches tended to focus on costly and costless metabolites, as they have different influences on the stability of microbial consortia [6][12]. For example, Bidirectional of costly metabolites may promote the growth of each species [13]. In contrast, unidirectional exchange of costly metabolites harms the secretors, and may lead the microbial consortia to collapse [14]. Besides, the exchange of costless metabolites doesn't harm the secretors, instead benefits the receivers, thus, it causes no effect on the stability of microbial consortia. So, in this article, secreted metabolites are mainly classified into two categories, costly metabolites, and costless metabolites, depending on whether the secretion of metabolites causes a loss to the fitness of the organism.

Costly metabolites usually benefit the receivers but harm the producers as the increased secretion leads to reduced fitness. Creating a bidirectional exchange of costly metabolites could benefit the microbial consortia, which may help construct a stable community to improve bioremediation. In a study, an Escherichia coli mutant unable to synthesize methionine was co-cultured with Salmonella enterica ser. Typhimurium, which was able to receive metabolites from Escherichia coli [13]. After several generations, the Salmonella strain underwent mutations to excrete methionine, thereby aiding E. coli growth. That is to say, Salmonella gained fitness by receiving nutrients from enhanced E. coli growth, which helped Salmonella to overcome the fitness cost of high methionine excretion. Therefore, the bidirectional exchange of costly metabolites contributes to a reciprocal nutrient exchange that is beneficial for the whole community. However, there was little research on determining whether the metabolites of pollutants were costly or not. It causes risks of disrupting the functional microbial consortia when unknown species are added.

Microbes commonly secrete waste products with no fitness cost to the producer, that is, secretion does not alter the growth rate of the producer. It was proposed that costless metabolites can be a prominent driver of microbial interactions and influence the functions of microbial consortia [6]. The exchange of costless metabolites could offset competition for nutrients and yield stable specific partnerships, such as pollutants-degraders, which might help improve the bioremediation of pollutants. However, the cost of metabolite secretion and metabolic interactions of an organism may change in different environments [15] and are difficult to determine by experimental procedures. Using genome-scale models of metabolism, Pacheco et al. identified a large spectrum of costless metabolites [6].

3. How to Engineer Microbial Consortia towards Bioremediation

The engineering of microbial consortia is a complex task involving the assembly of different genera and species of microbes. There are two main approaches and several important principles of engineering microbial consortia towards bioremediation.

Bottom-up engineering is based on the metabolism and interactions between species. In the past, it was a challenge to predict and precisely manage microbial consortia; however, with the development of multi-omics and automation technology, many researchers have found ways to manipulate metabolic networks and microbial interactions [16][17][18].

Metabolism determines the nutrients that a species consumes and the metabolites that are excreted into the environment. There are several pathway models for the molecular mechanisms, such as glycolysis, the Kreb's cycle, and glutaminolysis. Constraint-based methods, such as flux balance analysis, were also used for bottom-up engineering. Based on metabolic fluxes and interacting networks, it is possible to predict the desired output. Generally, the genome sequences of the strains in microbial consortia are needed to reconstruct the metabolic pathways and quantitative models are used to investigate the dynamics of the consortia [18]. Designing microbial consortia with defined social interactions is also an important part of bottom-up engineering. Microbial social interactions, such as competition and cooperation, are common in microbial communities and are essential in specifying ecosystem dynamics [19]. A model based on social-interaction programming can successfully predict the behaviors and dynamics of a community comprising of up to four species [20].

The core function of synthetic microbial consortia in the bioremediation of pollutants is the degradation of hazardous substances. Therefore, degraders are indispensable in a synthetic microbial consortium. However, there are still other important partner species with varied functions in the consortia that could contribute to the improvement of bioremediation (**Figure 2**). When dealing with low water-soluble organic pollutants, the surfactant-producing function of microbial consortia needed to be considered. When the nutrient of polluted sites was poor, adding nitrogen fixers and carbon producers was important to provided degraders with enough nutrients for bioremediation. For instance, a Bacillus strain, part of a pyrene-degrading consortium, is unable to degrade pyrene, but can enhance the bioavailability of pyrene by producing biosurfactant [21]. A polycyclic aromatic hydrocarbons (PAHs)-degrading microbial consortium consisting of Pseudomonas and Actinobacteria strains also show emulsifying activities in the presence of PAHs, which notably helps the solubilization of PAHs during biodegradation [22].

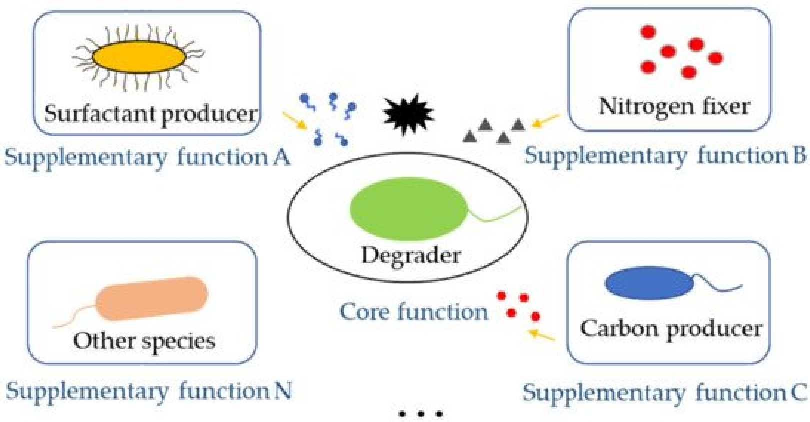

Figure 2. Multifunctionality in a microbial consortium. Degradation of pollutants is a core function of a synthetic microbial consortium. Supplementary functions, such as producing surfactant, nitrogen, or carbon resources, help the degraders to achieve the core function.

4. Engineering Microbial Consortia Promotes Bioremediation

In recent years, engineering microbial consortia have been used to study the degradation of organic pollutants or the removal of heavy metals. Certain synthetic microbial consortia have exhibited promising potential for bioremediation of polluted sites (**Table 1**).

Table 1. Cases of engineering microbial consortia towards bioremediation.

Pollutants	Microorganism	Bioremediation Efficiency	References
Pyrene	**(*Mycobacterium spp*. PO1 and PO2, *Novosphin-gobium pentaromativorans* PY1, *Ochrobactrum* sp. PW1, and *Bacillus* sp. FW1**	**Three-fold higher degradation rate for pyrene than the individual degrader.**	[21]
Atrazine	***Arthrobacter* sp. DNS10, *Bacillus subtilis* DNS4 and *Variovorax* sp. DNS12, *Arthrobacter* sp. DNS9**	**Removed 100% of atrazine at initial concentration of 100 mg/L, faster than single species.**	[23]
PAHs	***Rhodococcus* sp., *Acinetobacter* sp., and *Pseudomonas* sp.**	**100% degradation of Fl and Phe in sediment-free liquid medium after 4 weeks of growth.**	[24]
Cr(VI)	***Streptomyces* sp. A5, A11, M7, MC1**	**Removed 86% of Cr(VI) at initial concentration of 50 mg/kg in soil.**	[25]
Lindane		**Removed 46% of lindane at initial concentration of 25 mg/kg in soil.**	
Cd	***Bacillus* sp. strain H9, *Ralstonia eutropha* JMP134**	**Removed 42% of phenanthrene at initial concentration of 24 mg/L.**	[26]
2,4-D		**Removed 100% of 2,4-D at initial concentration of 500 mg/L.**	

4.1. Organic Pollutants

Organic pollutants such as high molecular weight polycyclic aromatic hydrocarbons (PAHs) are not efficiently degraded by a single strain [19][27]. However, a synthetic microbial consortium may perform better degradation under the approach of bottom-up engineering. A microbial consortium comprising of five culturable bacteria shows a three-fold higher degradation rate for pyrene than the single bacterial strain [21]. The biodegradation of pyrene was enhanced because of the cooperation between different species. Pyrene was initially degraded by *Mycobacterium*, while *Novosphingobium pentaromativorans* PY1, *Bacillus* sp. FW1, and *Ochrobactrum* sp. PW1 degraded the intermediates of pyrene. Besides, a biosurfactant was produced by *Bacillus* sp. FW1, which enhanced the dissolution of pyrene. The approach of top-down engineering also showed potential. In another study [24], mixed PAHs of fluorene (Fl), phenanthrene (Phe), and pyrene were effectively degraded by a synthetic microbial consortium consisting of three bacterial strains. The degradation rate of Fl and Phe was up to 100% after 4 weeks. Engineering microbial consortia exhibit a promising potential in the bioremediation of organic pollutants. However, the common approach is top-down engineering and microbial consortia are enriched from the in-situ environment. This is a time-consuming procedure and synthetic microbial consortia cannot be effectively applied in different sites. In the future, engineering microbial consortia based on bottom-up engineering requires more attention.

4.2. Heavy Metals

Engineering microbial consortia is an effective way for the removal of heavy metals. In a study [28], microbial consortia enhance the bioremediation of acid mine drainage, as biofilms formed by heterotrophic acidophiles decrease the dissolution rate of heavy metals. Biofilms play vital roles in the removal of heavy metals as they protect microbial consortia from diverse environmental stresses. Extracellular polymeric substances, such as polysaccharides, in biofilms, can easily bind to heavy metal ions [29]. However, the interactions of microbial consortia in biofilms are still not clear and need more research. Furthermore, microbial consortia are found to be more effective for Cr(VI) bioremediation, such as sulfate-reducing microbial consortia reduces the toxicity of Cr(VI) by reducing it to Cr(III) [30]. The interactions within microbial consortia may promote the growth of Cr(VI)-reducing bacteria, and then contribute to Cr(VI) bioremediation [31]. Though engineering microbial consortia succeed in some applications, the costs analysis needed to be considered in the future.

4.3. Complex Pollution

Recent empirical studies have shown that bacteria significantly benefit from trading metabolites with others, and cross-feeding within microbial consortia augment their ability to survive in complex polluted environments [27]. In a synthetic microbial consortium, *Escherichia coli* ATCC 33456 and *Pseudomonas putida* DMP-1 contribute to the simultaneous degradation of phenol and reduction of Cr(VI) [32]. Polti et al. also constructed a microbial consortium including *Streptomyces* sp. M7, MC1, A5, and *Amycolatopsis tucumanensis* AB0, and the synergistic removal efficiency of Cr(VI) and lindane was up to 69.5% and 54.7%, respectively [25]. Microbial consortia are robust and resist stress by complex contaminants. Recently, interest in synthetic microbial consortia that can perform complex functions, unlike a single community, is gaining momentum.

5. Conclusions

The advancement of biotechnology helps in engineering microbial consortia with desired functions associated with bioremediation. Top-down engineering compels a natural microbial community to perform the desired functions by modifying environmental variables, while bottom-up engineering reconfigures the metabolic pathway and reprograms social interactions among microbes. Both approaches could be used for engineering microbial consortia towards bioremediation. Top-down engineering is effective and costless when dealing with simple and easily degraded pollutants. While bottom-up engineering has advantages in degrading complex pollutants. Besides, in the progress of engineering, principles such as making division of labor in metabolic pathways, keeping multifunctionality, and eliminating stress need to be considered. In the future, more works need to be focused on metabolic interactions between species to make accurate control over the microbial consortia to obtain desired functions and stable effects. Although there are many limitations in the application of engineered microbial consortia, it is still a promising approach for bioremediation.

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