Enhancing Microbial Pollutant Degradation by Integrating Eco-Evolutionary Principles with Environmental Biotechnology

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Environmental accumulation of anthropogenic pollutants is a pressing global issue. The biodegradation of these pollutants by microbes is an emerging field but is hampered by inefficient degradation rates and a limited knowledge of potential enzymes and pathways. Here, we advocate the view that significant progress can be achieved by harnessing artificial community selection for a desired biological process, an approach that makes use of eco-evolutionary principles. The selected communities can either be directly used in bioremediation applications or further be analyzed and modified, for instance through a combination of systems biology, synthetic biology, and genetic engineering. This knowledge can then inform machine learning and enhance the discovery of novel biodegradation pathways.

Do We Have Sufficient Solutions for Pollutant Management?

Pollution, in the atmosphere, soil, or water, is a serious challenge of the 21st century. Deleterious impacts on aquatic ecosystems are triggered by different sources of anthropogenic pollution including sewage, nutrients and terrigenous materials, crude oil, heavy metals, and plastics [1]. Importantly, oceans comprise the largest biome on the planet and operate as a sink for many pollutants, such as plastics. It is estimated that 80% of the plastic pollution in the ocean comes from land-based sources and reaches the ocean via rivers and wastewater treatment facilities [2]. In 2010, it was estimated that 5–13 million tons of plastic entered the ocean [3], where they accumulate in various habitats, such as marine sediments, and via ingestion at different trophic levels in the marine food web. Many of the pollutants are of global concern because they significantly affect human and ecosystem health around the world, for instance, contaminants of emerging concern (CECs) [4], persistent organic pollutants (POPs) [5], and endocrine disrupting chemicals (EDCs) [6]. Therefore, restoration and conservation of our ecosystems for future generations should be of utmost priority.

To date, different remediation techniques, such as physical, chemical, and biological, have been used for the removal of contaminants. Despite the fact that physical and chemical approaches have been practiced for decades, they still suffer from several drawbacks. These include high processing costs, increased requirements of reagents, and the undesirable generation of secondary pollutants [7]. By contrast, biological remediation (bioremediation, see Glossary) in the form of microbe-based treatments, is a cost-effective, eco-friendly, and socially acceptable way to remove pollutants such as heavy metals [8], pesticides [9], and hydrocarbons [10] from the environment. Nevertheless, while culturable bacteria were isolated from contaminated sites already 45 years ago [11], the approach of bioremediation has so far failed to provide convincible solutions in pollutant management. Classically, the majority of the studies performed in the field of bioremediation have aimed to isolate, culture, and characterize the organisms that are

Highlights

We advocate to shift research efforts in environmental biotechnology from searching for desired traits of monocultures to that of microbial communities. As these traits will be hard to identify with classical genome mining approaches, we recommend using artificial community selection as a tool to identify and to select for novel and/or enhanced functions.

Bioremediation and biodegradation with artificially selected microbial communities harbor great potential to become a fast, cost-effective, eco-friendly, and socially acceptable way to remove pollutants without prior knowledge of the involved species and degradation pathways needed.

The use of highly integrated multispecies microbial communities instead of monocultures in biodegradation processes will result in more stable and more productive cultures.

The novelty of our proposed approach lies in the combination of eco-evolutionary principles with applied biotechnology. This will stimulate new advancements in environmental biotechnology, and will likely result in the discovery of novel metabolic degradation pathways.

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With the advancement of such recombinant DNA technologies, the field of bioremediation has been rejuvenated as it allowed for the creation of microbes and whole microbial communities hosting novel genes and enzymes with increased efficiencies (Box 1). Nevertheless, it is problematic to rely on genetically engineered organisms. We do not understand their effects on the Earth’s ecosystems, specifically if they interact with indigenous microbial communities during the bioremediation process and thus pose danger to the environment. This results in either strict containment protocols or administrative restrictions for applications in the field. Further, despite recent advances in metagenomics approaches (e.g., genomics, transcriptomics, proteomics, and metabolomics) that have generated data improving our understanding of the cellular processes, genetic control, and signaling networks in microbial communities [21], we still lack detailed knowledge of potential degradation pathways and enzymes. A new approach for linking genes to microbial community function has been experimentally showcased by Quistad et al. [22]. Here, selfish genetic elements were regularly added to replicate microbial communities under a particular environmental selection regime (i.e., under nitrogen-limited conditions). Over the course of the 48-week-long selection experiment, responsible for the remediation process [12]. While using such culture-based techniques has resulted in the identification of a number of microbes carrying out the biodegradation of specific environmental contaminants (Malla et al. [13] for examples), it suffers from important drawbacks. One is that more than 99% of the microorganisms that exist in the environment cannot be cultivated (easily) under laboratory conditions. This, known as the ‘great plate count anomaly’ [14], has made the recovery of specific isolates that are responsible for, or participate in, a given biodegradation process challenging. The biodegradation process for the so-called recalcitrant pollutants, such as microplastics and POPs, is particularly problematic as it is slow due to the lack of efficient microbial metabolic traits [15]. This can be exemplified by research on the degradation of the non-native polymer polyethylene terephthalate (PET), the sixth most produced plastic in Europe. Even though PET degradation is one of the best understood plastic degradation mechanisms to date, only a mere handful of verified active enzymes that degrade PET have been discovered in bacterial and fungal strains [16–18]. The application of these enzymes is hampered by their comparatively low conversion rates that do not suffice for industrial application. A current research focus therefore is the search for novel enzymes, as well as the improvement of already existing ones, for example, through rational protein engineering [19], or bioinformatically-aided mutagenesis [20].

Another promising approach directed at gene transfer to whole microbial communities is the new directed towards optimizing biocatalysts for different tasks, like increasing enantioselectivity, robustness in respect to solvents, or temperature. Besides mere enzyme optimization, enzyme engineering can be used to transform biocatalytic sites to perform novel functions and create artificial multifunctional enzymes [68]. Rational design can further be applied to design enzyme cascades, capable of combining different enzymatic activity to create ‘biological factories’ [69]. A comprehensive overview of current approaches of genetic engineering for bioremediation purposes is available [7,70].
the movement and enrichment of ecologically important genes, which were mainly involved in the nitrogen metabolism, could be detected within the communities by comparative metagenomics.

In this opinion article, we advocate to implement new approaches into bioremediation, specifically to integrate eco-evolutionary principles with environmental biotechnology. We believe that implementing artificial community selection will significantly speed up the process of discovering relevant enzymes and degradation pathways, as no prior knowledge is needed. The proposed solution relies on harnessing the unexplored diversity of microbial communities that reside within our ecosystems.

**Interactions within Microbial Communities: New Functions and Increased Stability**

Microorganisms usually do not exist as single, genetically identical strains, but live together with other microorganisms as taxonomically and metabolically diverse communities [23]. Such multi-species microbial communities are characterized by a spatial structure, which provides the physical vicinity for interactions between its members, for instance the exchange of metabolites [24]. These diverse communities offer several advantages relevant to environmental biotechnology. We know from macroecological surveys across ecosystems that there are positive relationships between biodiversity and ecosystem functioning. Here, high-diversity mixtures have been found to be more productive as compared with the equivalent monocultures [25]. Despite the fact that microbial communities were not included in this study, the same positive relationship can be expected between both parameters (i.e., diversity and productivity), within microbial communities. One of the reasons as to why diverse communities (at the genotype or species level) are probably more productive, includes their ability to utilize the different resources more efficiently when compared with monocultures, where initially all cells metabolize the same substrate. The increased resource utilization refers to both nutrients that are readily available in the external environment, as well as metabolites that are released by the fellow members of the community [26]. Comparative analyses of microbial genomes suggest that more than 98% of all sequenced microorganisms are auxotrophs and, thus, they rely on external nutrients and/or byproducts from other cells [27]. Further, in comparison to monocultures, diverse microbial communities are more robust against environmental and ecological disturbances, such as exposure to antibiotics, changes in oxygen or pH levels, invasion by non-native strains that were not part of the community before, or encountering protozoan predators and parasites [28]. Within these diverse communities, certain microorganisms are present in incredibly low abundances. It has however been recognized that, in particular, the rare bacterial biosphere fulfills essential functions in the degradation of pollutants and that they enhance the functionality of the more abundant microbes [29]. These observations strongly suggest that the increase in functionality is due to positive interactions between different members of diverse microbial communities [30]. One of the key processes underlying these interactions is metabolic cross-feeding [31]. As these positive interactions are key for microbial growth and survival, the different microbial entities must have coevolved as part of their respective community.

While monocultures are often considered suitable for biotechnological purposes, this is not representative of the situation that exists in nature. Synergistic interactions within taxonomically and metabolically diverse microbial communities facilitate the emergence of complex biosynthetic functions that would be difficult or impossible to achieve in monocultures [28, 32]. An area, where the benefit of species interactions is already harnessed is the removal of nutrients from wastewater. Here, it has been demonstrated that synergistic interactions in natural and artificial microalgae–bacteria consortia lead to an enhanced overall uptake of nutrients [33].

Mathematical modeling has shown that monocultures suffer from the burden of complex metabolic pathways, resulting in a decrease of the system’s overall productivity [34]. As a result, strains
showing division of labor, where distinct parts of the population perform complementary steps of the pathway, outperform uniform populations [34]. This compartmentalization of reactions into different populations increases the modularity of the system [35]. Further advantages of multispecies microbial communities that are important to consider for downstream applications, are that these systems tend to be more resistant and resilient to disturbances presented by changing ecological and environmental conditions, which we predict would lead to the breakdown of a given bioprocess based on monocultures. All these findings emphasize that we should not think about microbes as individually occurring cells and/or isolated strains because they live as part of communities in their natural habitats [36]. Therefore, diverse microbial communities cannot be easily deconstructed into their individual components. Instead, microbial communities should be appreciated as differentiated groups of cells that are highly integrated, which is analogous to the cellular differentiation and integration that can be observed in multicellular organisms.

Engineering microbial consortia has the potential to advance the field of biotechnology from biosynthesis (e.g., bioproduction of medicines, biofuels, and biomaterials) to bioremediation. Synthetic microbial communities can perform highly complex tasks that are challenging to be realized with monocultures. Advances in systems biology allow us to design and control synthetic consortia and to program their behavior so that they perform a specific function (synthetic biology tools and design strategies have been reviewed in Tsoi et al. [35]). Despite recent advances in designing synthetic consortia, this strategy is dependent on detailed prior knowledge of the respective inter-species interactions that result in the degradation of the pollutant of interest, which in many cases is still lacking.

Artificial Selection for Increased Community Function

A complementary approach to designing synthetic microbial consortia, and the one we advocate here, is artificial community selection of microbes (Table 1 for a comparison of artificial selection with genetic engineering). The technique of artificial selection per se is not novel and is well accepted; humans have performed it for more than 10 000 years [37], since moving from societies of hunter-gatherers to being farmers and pastoralists. By breeding animals and plants, desired traits of individuals get selected until the desired phenotype (and underlying genotype) is achieved. This technique has also been successfully applied to single microbial strains that have, for example, been selected for an increase in atrazine degradation [38]. While important advances have been made in this area, we propose that our focus needs to shift from traits of monocultures
to traits of communities. Our reason for this stems from the realization that the majority of microbial functions are not the product of single cells but rather the result of interactions between members of highly diverse multispecies communities [39]. Thus, the microbial interaction as a trait of the community needs to become the trait of interest for artificial selection. Pioneering work in 2000 [40,41], demonstrated that selection of communal microbial traits is possible (which was at the time referred to as ‘artificial ecosystem selection’). Of particular relevance for the field of pollutant degradation through microbes, is a study where artificial community selection resulted in the improvement in biodegradation of the environmental pollutant 3-chloroaniline, in the majority of the experimental replicates under selection [41]. Despite the promising start, research in the area of artificial microbial community selection has shown little progress. This is likely due to the misconception regarding the importance of selection working at levels above the individual (i.e., groups, populations, and ecosystems) [42–44] and because of the complexity and scale of artificial community selection experiments. Nevertheless, artificial community selection has been resurrected, with several recent theoretical and experimental studies addressing this topic [45–49]. Here, different strategies and setups of selection experiments using artificial microbial communities have been tested and challenges have been identified [50–52]. Reported problems concern various steps of the entire experimental procedure, such as unstable communities, species extinctions, invasion of mutants that do not contribute to the trait of interest, selection on growth rate (rather than on the trait of interest), and the loss of variation between communities.

In the next section, we draw attention to common pitfalls and misconceptions that likely undermine desired outcomes of community selection. There are multiple requirements for selection to work at the level of communities: there must be variation between competing communities in the community trait, communities must be able to replicate, and the community trait must be heritable [53]. These requirements ‘dictate’ many factors of the design of the artificial selection regime. By comparing two setups (Figure 1), we highlight critical aspects that need to be taken into account when performing artificial community selection: the ‘Transient communities’ strategy (called ‘migrant pool’ in classical group selection experiments) is comparable with the original experiment performed by Swenson et al. [41]. This is contrasted with the ‘Persistent communities’ strategy (known as the ‘multispecies propagule pool’), which differs from the ‘Transient communities’ strategy in the way the communities are replicated (see review by Goodnight [54] for an overview and comparison of multiple group selection strategies).

Competing communities are seeded with heterogeneous inocula in both regimes. Importantly, each community occupies its own vial so that it is discrete from the other communities but it is nevertheless part of the metapopulation of, in our example, eight competing communities. Ideally, the setup of the metapopulation gets replicated, as signs of parallel evolution [55,56] between metapopulations might facilitate the identification of the mechanisms underlying the community trait. An alternative option for identifying the genes underlying the trait of interest is their enrichment within the community via selfish genetic elements [22].

The next step in the selection regime involves growing the communities until the peak activity in the trait of interest is measured [57] or a stable community composition is achieved. This ensures selecting for complex and stable interactions instead of selecting for the fastest growing community members [47,50,58]. After the incubation phase, the trait of interest and the biomass of the community are quantified, and the communities are ranked according to their trait values per biomass. We advocate accounting for biomass (instead of ranking solely according to trait value) as this will select against fast growth and aim for a higher trait value (i.e., productivity), per cell [50]. Next, the best-functioning communities get chosen, where, based on theoretical predictions, the ‘top-tier’ should be preferred over the ‘top-dog’ strategy. In contrast to the
Heterogeneous initial inocula

Culture incubation

Phenotyping
Trait & biomass quantification

Ranking & selection

Mix

Keep separate

Transient communities

Persistent communities

(See figure legend at the bottom of the next page.)
‘top-dog’ strategy, where the best-functioning community replaces as many communities as possible, followed by the second best and so on, the ‘top-tier’ strategy is one whereby the best communities, for example, the best 10%, get equal opportunities to replace the bottom ranked communities. Importantly, several communities above a certain threshold (and not only the best one) get transferred [47]. This not only ensures high productivity per cell [50] but also maintains a higher fraction of variable communities.

The next step contrasts the methods of community replication (reproduction) and is the step where both regimes differ. The approach that has been applied in most studies so far is a mixed approach (‘Transient communities’), where the chosen communities get pooled before inoculating the next community generation. We compare this with the approach we favor, where the chosen communities are kept as distinct lineages (‘Persistent communities’). The difference seems to be subtle, but it crucially affects the efficacy of the artificial community selection [49]. While the maintenance of inter-community variation is highlighted as one advantage of the ‘Transient community’ design, we propose that this design also selects for fast growth/high biomass, as the strains that dominate the communities will be rewarded (r-strategists i.e., fast-growing strains). If participating in the desired interaction/community function is costly for the cell, this step might also select for the fast-growing cells that do not contribute to the trait of interest (i.e., free riders). Further, the mixing of communities leads to reduced heritability and decrease in variation between communities. This would thus lead to a reduced efficacy of selection at the community level. All of these pitfalls are avoided in the ‘Persistent community’ regime, where variation between populations and heritability of population composition (and thus of interactions) are maintained. Competition within communities is reduced, so that fast describes growth at the expense of maintaining the interactive trait is not rewarded. Albeit with the challenges in the setup of artificial community selection studies [50,51], well controlled community selection experiments [22,46,49,52] in combination with theoretical studies [47,48] and new technologies, such as microfluidics [59], have already made headway and have demonstrated the efficiency of artificial community selection in selecting for functions that arise from community interactions.

Revising the Roadmap for Bioremediation
We propose that artificial community selection of microbes in itself or as a step before applying systems biology approaches, will significantly aid in characterizing pollutant biodegrading microbial consortia and enhance the discovery of novel biodegradation pathways as well as the tailoring of existing ones (Figure 2, Key Figure). The method is achievable and can proceed without detailed knowledge of the organisms and their interactions, and can result in the selection of successful combinations of organisms (and genes within organisms) that would be ‘difficult or impossible to discover otherwise’ as pointed out by Swenson et al. [41].

![Figure 1. Comparison of Two Artificial Microbial Community Selection Regimes: Transient Communities versus Persistent Communities.](image-url)
Figure 2. Environmental sampling yields communities collected from natural habitats where pollutants accumulate, for example, from the marine environment, which form the inocula for the artificial selection regime. The evolved communities can either directly be used in biotechnological applications or analyzed in detail using systems biology. A subsequent bottom-up approach allows engineering of synthetic microbial communities, pathways, or enzymes (genetic engineering). Results from all above processes can inform machine learning, which will likely result in the identification of novel types of biodegradation enzymes and pathways.
Sampling and isolation of bacterial strains/communities from contaminated sites, such as landfills or sewage treatment plants, are well established procedures. As oceans function as a sink for many pollutants, for example microplastics, we recommend sampling several marine microbial habitats (Figure 2). Harnessing the underexplored marine microbial biodiversity has the potential to result in the discovery of novel biodegradation consortia and pathways. After sampling microbial communities and distributing them over a number of microcosms, they will be put through an artificial selection regime (Figure 1 for details). The evolved communities will not only be well adapted with respect to the selected trait but will likely remain stable after relaxation of the selection regime, an important prerequisite for use in biotechnological applications [41]. These communities may then be applied in the field or cultured in bioreactors without worrying that they might lose their properties, for example, through the loss of a community member or the invasion of fast-growing free riders. With regard to maintenance and storage for use in future work or at different treatment sites, we envision a strategy akin to the Microbiota Vault initiative, which aims at conserving the diverse microbiota important for human health [60]. After performing artificial community selection, chosen communities could be preserved in a biobank for bioremediation, and could provide a versatile resource/toolbox for addressing emerging environmental problems for researchers worldwide. For technical aspects (sample collection and preservation), one could adopt approaches from the feasibility study of the Microbiota Vault (https://www.microbiotavault.org/feasibility-study/) as this initiative focuses on preserving microbial communities, which is in contrast to culture collections where usually single microbial strains are preserved. We regard the step of freezing/preserving the communities as important as it will conserve the status quo and will avoid the potential pitfall of adaptation of the communities to alternate environmental conditions. We could, for example, imagine that a regular transfer regime, which is typically employed in traditional culture collections for the maintenance of some strains, may lead to undesired shifts in community composition.

If required/desired, the evolved communities can subsequently be analyzed in detail, making use of recent advances in systems biology. Specifically, we envision a process, where it is possible to link the biological processes at the molecular with the ecosystem level [61]. Particular setups of the artificial community selection experiment will likely facilitate the identification of novel pathways or functions based on traces of parallel evolution.

The community could also be taken apart and the pure microbial cultures could be used for constructing synthetic consortia, ‘designer’ microbes with altered metabolic pathways, or improved and novel enzymes. When doing so, not only the performance of the community should be considered but also its stability against abiotic and biotic disturbances. Artificial selection could also advance the systemic biology component and workflow [62]. In addition we see high potential for the subsequent use of machine learning in yielding discovery of novel enzymes and pathways. Whereas this approach is new in the field of biodegradation, it has been successfully applied in medical microbiology. A recent study that employed the application of deep learning neural networks led to the discovery of new types of antibiotics [63].

Concluding Remarks
Here, we have advocated integrating ecological and evolutionary principles with biotechnological applications. We have specifically highlighted the potential of artificial community selection for accelerating current procedures in bioremediation, and for complementing established techniques. This strategy is fast and straightforward and promising results can be achieved without the requirement for large-scale sequencing efforts and tedious bioinformatic analyses (but see Outstanding Questions). Having said this, we do not opt for neglecting omics approaches, they are extremely useful, powerful, and very much needed, but rather to reverse the current order
when searching for promising routes of pollutant management. Time itself is the key component in environmental pollutant management; the longer we wait, the more pollutants will accumulate to a critical and dangerous mass. The biggest pitfall of all, the lack of communication between ecologists, evolutionary biologists, and biotechnologists, can be avoided by fostering interdisciplinary interactions across these disparate fields.

Acknowledgments
This work was supported by the Federal Ministry of Education and Research (BMBF), Germany as part of the Funding Initiative ‘New Biotechnological Processes based on Marine Resources – BioProMare’, 2020-2023 (PLASTiSEa, Funding Reference Number: 031BO867A) (awarded to U.H. and E.B.). P.D. appreciates support from ProBone, a Marine Biotechnology ERA-NET (ERA-MBT) funded project under the European Commission’s Seventh Framework Programme (Grant Agreement Number: 604814) (awarded to U.H.). K.H. thanks the Hamburg Institute for Advanced Study (HIAS) and the Joachim Herz Foundation for support. We thank Susanne Landis @scienstration for the artwork.

Declaration of Interests
None are declared.

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