Microbial community design: methods, applications, and opportunities

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Microbial communities can perform a variety of behaviors that are useful in both therapeutic and industrial settings. Engineered communities that differ in composition from naturally occurring communities offer a unique opportunity for improving upon existing community functions and expanding the range of microbial community applications. This has prompted recent advances in various community design approaches including artificial selection procedures, reduction from existing communities, combinatorial evaluation of potential microbial combinations, and model-based *in silico* community optimization. Computational methods in particular offer a likely avenue toward improved synthetic community development going forward. This review introduces each class of design approach and surveys their recent applications and notable innovations, closing with a discussion of existing design challenges and potential opportunities for advancement.

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Introduction

Microbial communities have long been recognized for the important impact they have on human health, agriculture, and industry. In the context of health, specifically, recent studies have highlighted the key role that the human microbiome – the ensemble of microorganisms that live in and on the human body – plays in human physiology, immunity, development, and nutrition. For example, microbially produced butyrate contributes to colonic health via its function as an important energy source [1] and anti-inflammatory agent [2]. Proper immune system development also relies on the gut microbiome, which modulates aspects such as lymphoid structure development and T cell differentiation [3,4]. Beyond human health, microbial activity is important to a range of industrial applications including microbially mediated denitrification in wastewater treatment [5,6] and biofuel production [7,8]. These microbial influences also extend to agriculture, where bacteria support plant access to nitrogen [9,10] and phosphorus [11].

These myriad important functions suggest that microbial communities can serve as a prime target for medical, agricultural, and industrial advancement and have sparked recent interest in developing microbiome-based biotechnologies and therapeutics [12**,13]. Specifically, microbiome engineering – the manipulation of naturally occurring microbial communities or the construction of synthetic communities to produce a specific function – is a promising tool for improving and innovating upon various clinical and industrial applications. Such attempts to engineer a given community can be done by various approaches. One simple microbiome engineering technique is the modulation of environmental conditions to effect changes in community function. This approach is frequently applied to optimize bioreactor communities, which are used for the production and degradation of various compounds. Indeed, previous studies have shown that bioreactor operators can improve community function by changing substrate composition [14–16], aeration [17], pH [14,18], and temperature [19]. Another simple approach is to modify a community by adding beneficial species or removing undesirable ones. Antibiotics are perhaps the most prevalent example of this approach in therapeutics, acting as a tool for removing pathogenic species. Probiotics, in contrast, are an example of an additive tool, aiming to improve gut community function by introducing beneficial species [13,20,21].

Importantly, however, for microbiome engineering to realize its full potential, more sophisticated techniques must be employed to provide researchers with greater control when engineering microbial communities. This is especially important for the construction of synthetic communities that consist of a human-developed mixture of species. In the context of microbiome-based therapy, such synthetic communities could enable more precise modulation of the microbiome and bypass the negative

side effects of antibiotics usage [22,23] or potential inconsistency of probiotic engraftment [24,25]. Synthetic communities can also be useful for industrial applications, where the novel coupling of microbial metabolisms can lead to the improved production or degradation of economically important compounds [26]. More generally, synthetic communities provide a more flexible and powerful engineering approach, allowing researchers to engineer the community as a whole, rather than being limited to the perturbation of an existing community. However, one key challenge in developing such synthetic communities is the identification of novel species compositions that optimize, or at least improve upon, desired functions. This task, which can be thought of as 'rational design' for community engineering, is far from trivial and various techniques have been developed and applied to address this need.

In this review, we survey a variety of approaches for designing synthetic compositions with targeted functions, highlighting recent methodological innovations and applications. Importantly, we focus on methods for *designing synthetic compositions* (i.e. new mixtures of existing microbial species), rather than the use of genetically engineered microbes in synthetic compositions, which has been reviewed elsewhere [27,28,29•,30]. We begin with approaches that rely on selective pressures and adaptation of community composition to reach synthetic compositions with optimized functions. Next, we cover methods that use microbial isolates from existing communities to formulate reduced, well-defined compositions that recapitulate the desired functions of the source communities. We then describe techniques that evaluate possible synthetic compositions in a combinatorial manner to identify desirable compositions. Finally, we describe the recently expanding range of computational tools that identify candidate compositions predicted to optimize a desired function. We conclude by discussing the challenges and potential opportunities that are still present in the field of synthetic community composition design. Since therapeutic community design studies are currently limited, we illustrate the application of certain design approaches using examples from industrial settings.

Community enrichment toward synthetic compositions

Naturally occurring microbial communities can carry out an amazing variety of functions, many of which could be harnessed toward clinical, industrial, and environmental applications. For example, the human gut microbiome can perform multiple metabolic processes that are crucial for the host, including harvesting energy from the diet [31], synthesizing important vitamins [32], and resisting pathogen colonization via competitive exclusion [33]. Indeed the transplantation of fecal microbiota from healthy donors has proven effective at treating several

gastrointestinal disorders [13,34–36]. Similarly, different soil communities can degrade various pollutants, such as diesel fuel [37] and polycyclic aromatic hydrocarbons [38], or prevent non-biodegradable pollutants, such as uranium [39], from contaminating water supplies by catalyzing their conversion to insoluble forms. However, the efficiency at which naturally occurring communities perform these functions may not be sufficient for industrial settings, necessitating an optimization procedure that can build upon such communities and ultimately produce synthetic communities with enhanced capabilities. A common approach to achieve this goal is through *enrichment* — a community design methodology that aims to reach a community composition with optimal desired capabilities by subjecting an existing community to environmental conditions that favor species that can perform the target function. To date, enrichment has primarily been used for biotechnological application, including microbial fuel cells (MFCs), biopolymer production, and biohydrogen production, which will be described below.

MFCs have become a prime target of microbial community engineering due to both the promise of efficient microbially mediated electricity generation and the wide range of substrates that they can utilize. This biotechnology was originally inspired by marine sediment communities that reduce various elements for energy in a manner that can be exploited to generate electricity [40–43]. Recent MFC applications can now utilize a wide range of substrates (depending on the specific microbial community employed) including glucose [44,45], acetate [44], lactate [46], cellulose [47,48], and ammonium [49]. Indeed, MFCs can even consume various industrial waste products, enabling the coupling of electricity generation with waste degradation [50–53]. Though naturally occurring communities can already achieve these tasks, MFCs seeded with such communities often undergo compositional changes and exhibit gradual improvement in efficiency over time as the community adapts to operating in the MFC environment. Such changes were shown to include enrichment for species potentially related to current generation [54] and degradation of the supplied substrate [55], and the observed changes in community composition during extended operation of an MFC were demonstrated to be linked to concurrent increases in MFC efficiency [56].

Notably, while MFC communities experience inherent and appropriate selective pressures, other biotechnologies may require the application of artificial selection procedures to optimize community function. For example, to increase the yield and efficiency of microbial communities grown and harvested for biopolymers used in biodegradable plastics, researchers have applied an artificial feast-famine cycle [57,58]. This cycle selects for communities that store energy (in the form of

biopolymers) more efficiently during the feast phase so that energy is available during the famine phase. This procedure can be further enhanced by introducing phosphate limitation, which can ensure that biopolymer production is advantageous while also reducing the growth of subcommunities that do not contribute to production [59]. A recent work has also demonstrated that photosynthetic communities can be enriched for biopolymer production without a famine step when oxygen is limited [60•].

Artificial selection procedures have also improved microbial community hydrogen production, though in this context, artificial section is often applied as a pretreatment rather than as part of post-enrichment operating conditions. Such pretreatments include heat shock, acidic or basic incubation, freeze drying, and chloroform treatment [61]. Each of these pretreatments aims to enrich for hydrogen-producing species in the original community while excluding hydrogen consumers. Interestingly, though, the efficacy of different pretreatments is inconsistent across different studies, likely due to difference in the set of species present in the initial community [62]. Consequently, the discovery of new promising hydrogen-producing communities necessitates the re-evaluation of these enrichment procedures to identify the best pretreatment [63].

Community reduction from existing compositions

Some microbial community applications may impose specific restrictions on the species that can be present in the synthetic community. For example, microbiome therapeutics must meet various regulatory guidelines [64••], and be devoid of pathogenic species so as to avoid inadvertently infecting the recipient [12••]. However, it may not be possible to fully satisfy such restrictions using enrichment approaches due to the relatively broad and unspecified nature of environmental selection. For example, applying environmental conditions that inhibit the growth of pathogens may simultaneously negatively impact the growth of desirable species. This challenge can be addressed by a complementary design approach, referred to in this review as community *reduction*, wherein individual members of some initial community are isolated and characterized to rationally determine whether they should be used in the synthetic community. While some community members may be lost during the isolation step [65], this approach provides better control over community composition and enables a more principled selection of desirable species and the explicit exclusion of undesirable ones.

This design paradigm has been used, for example, to reconstruct synthetic communities for treating *Clostridium difficile* infection (CDI). CDI is a gastrointestinal infection where *C. difficile*, a spore-forming, antibiotic-resistant enteric pathogen, dominates the gut microbiome, causing inflammation and diarrhea [66]. Previous clinical studies have demonstrated that CDI can be effectively treated with fecal microbiota transplantation (FMT) from a healthy donor [35]. This makes CDI a prime target for synthetic FMTs that could potentially recapitulate the same beneficial effects using a simplified, well-defined community composition. Indeed, an early study used a mixture of ten previously identified and isolated intestinal species to formulate a reduced synthetic CDI treatment composition [67]. In this study, all five patients treated with the synthetic composition exhibited marked improvement, similar to that observed in a patient treated with a donor stool sample. Surprisingly, one of these five patients was previously treated unsuccessfully with a donor stool transplant, suggesting that reduced synthetic FMT compositions may not be strictly inferior in efficacy to traditional FMTs. In a more recent study, a synthetic FMT composition was designed by isolating as many individual species as possible from a single donor's stool [68••]. These isolates were screened for pathogens, and the remaining non-pathogenic isolates were mixed to form a synthetic community. Both patients treated with this synthetic community composition responded well to treatment, and longitudinal sampling revealed notable engraftment of the species in the synthetic composition, though this declined over time.

The community reduction approach has also shown success when used for non-CDI therapeutic applications. For example, rather than targeting *C. difficile*, Caballero *et al.* investigated the role that gut species play in resisting Vancomycin-resistant *Enterococcus* (VRE) colonization in mice [69]. As part of this larger study, the authors isolated ampicillin-resistant strains from mouse stool and examined which strains could confer VRE resistance. From these experiments, they identified a four-strain synthetic composition that both resisted VRE colonization and ameliorated pre-established VRE colonization. In another example, Atarashi *et al.* set out to design a synthetic composition that would induce T_{reg} cells in the mouse colon [70]. The authors isolated species from a human donor's chloroform-treated stool and found that a synthetic community composed of 17 isolates induced T_{reg} cells in germ-free mice to a similar extent as the original chloroform-treated stool. The same group, as part of a larger study of intestinal Th17 cell induction, also used community reduction to successfully identify a synthetic composition of 20 human gut strains with notable Th17 cell induction in mice [71]. These examples illustrate the potential of community reduction to recapitulate various important gut microbiome functions, making it a promising tool for future microbiome therapeutics. One important caveat to note, however, is that community reduction inherently cannot design synthetic compositions with novel functions, thus restricting its wider applicability.

Combinatorial evaluation of potential compositions

One of the unique benefits of synthetic communities is that they can include combinations of species that never co-occur in naturally occurring communities, potentially facilitating a wider range of metabolic capacities. Such synthetic communities may therefore be able to perform certain functions better than existing communities (or communities obtained via enrichment or reduction) or even perform entirely new functions. This is of particular interest for industrial applications such as the production of biofuel and other biological compounds [26]. Indeed, exploration of novel metabolic coupling in synthetic communities has already proved successful, demonstrating potential applications for the production of various resources including hydrogen [72], acetic acid [73–75], and lactic acid [76,77], as well as the degradation of undesirable substances including polycyclic aromatic hydrocarbons [78] and cellulose [79,80]. To go beyond a simple trial and error exploration approach for identifying such beneficial combinations, researchers can employ a more comprehensive process, referred to in this review as *combinatorial evaluation*, the systematic enumeration, construction, and evaluation of possible combinations of a set of species to identify the best-performing composition. The set of species used could, for example, consist of candidate species that are believed to contribute to the desired function.

When the number of species to consider is small, combinatorial evaluation can be performed in its ideal form, constructing and assessing *all* possible combinations of the species of interest. For example, to optimize the biodegradation of dyes in textile wastewater, researchers isolated three species from a textile wastewater plant and evaluated the degradation capabilities of all combinations of these three species [81]. In fact, because of the relatively small number of species considered, they were also able to evaluate additional compositions that varied in the relative abundances of each species.

Importantly, however, as the number of candidate species grows, the number of potential compositions grows exponentially, quickly rendering the evaluation of all possible combinations impossible. This setting calls for techniques that can drastically reduce the number of evaluated compositions. One such technique is fractional factorial design (FFD). In general terms, FFD aims to estimate the effects of, and potentially the interactions between, particular components of a system on a specified output [82]. These effect and interaction measurements then provide a basis for mathematically identifying an optimal parameterization of these components. In the context of microbial community design, FFD reduces the required number of evaluated compositions by carefully selecting a subset of potential community compositions that can isolate specific species effects or interactions of interest. One important caveat is that FFD achieves this reduction in evaluated compositions

by assuming negligible effects of higher-order interactions. However, if later evidence suggests that one or more higher-order interactions have important contributions, a technique called foldover design can be applied to efficiently determine those specific interaction effects based on the findings of the original FFD experiment [82].

Microbial community function optimization via FFD has historically focused on factors external to the community. For example, various studies have used FFD to examine the impact of environmental factors such as substrate composition [15,37,83–85], pH [83,84,86], temperature [83], and heavy metal presence [86] on specific community functions. More recent efforts, however, have used FFD, or FFD-like techniques, to investigate the potential of individual species effect estimation. A recent study, for example, has used random gut community subsets to estimate individual microbial contributions to host phenotypes [87•]. Though these compositions were randomly selected (rather than specifically constructed to most efficiently separate individual contributions), the results of this study suggest that FFD could be applied in a similar manner for synthetic composition optimization by treating species as the components of interest. Indeed, one group has already applied FFD to develop wastewater treatment communities [88,89]. In this pair of studies, the authors used FFD to estimate the contributions of both individual species and interspecies interactions to total organic carbon (TOC) degradation [88] and substrate utilization rate [89]. They then employed this information to develop synthetic compositions with improved biodegradation capacities. Interestingly, both studies found that the optimized three-strain and four-strain communities performed better than a baseline mixture of all six strains evaluated, demonstrating the utility of FFD in synthetic community design.

Another technique for efficiently evaluating potential compositions is the definition of microbial consortia that will be treated as single units when enumerating possible species combinations (i.e. each combination will either include or exclude all species in a given consortium). This technique is particularly useful when a microbial consortium has previously demonstrated a desirable emergent function. For example, one group observed that a consortium of marine species, named the NPMC, could efficiently fix CO_2 [90]. They later treated this consortium as a single candidate community member when using a combinatorial evaluation approach to develop a synthetic community for CO_2 fixation [91•]. Importantly, in addition to reducing the pool of available species to six candidate community members (one of which was the NPMC), this approach also allowed the researchers to include species that could not be isolated from the NPMC in the final community. In a separate study, the same group designed a synthetic community for lignocelluloytic enzyme activity by considering a synthetic consortium previously designed for cellulolytic

activity [80] alongside several fungal strains [92]. These studies highlight the benefits of this approach, allowing researchers to evaluate designed communities with higher complexity without drastically increasing the number of evaluated compositions.

Computational model-based design of synthetic compositions

The design paradigms described above rely on various approaches for characterizing and assessing candidate community compositions, with techniques such as FFD and consortium inclusion allowing researchers to reduce the set of compositions ultimately evaluated. Importantly, however, such approaches may still entail evaluating many compositions that *a priori* might be expected to perform the desired function poorly based on existing knowledge of microbial ecology, genomics, and metabolism. Indeed, databases such as NCBI [93] and IMG [94] provide access to an ever increasing number of sequenced microbial genomes, which when coupled with various gene annotation databases, such as KEGG [95] and MetaCyc [96], can be used to infer the functional capacities of individual microbial species. Design methodologies that could harness such information to pinpoint community compositions that are likely to successfully and efficiently perform the desired function might dramatically reduce the time and labor needed for experimental evaluation. As a simple example, a collection of previously sequenced and genomically annotated microbial species could be searched to identify species whose genomes confer the capacity to perform a certain function, even when these species have never been experimentally tested for that function in the lab. In this review, however, we will focus on more sophisticated computational methods for designing synthetic community compositions, primarily highlighting methods that aim to *model* community-level metabolism and to identify synthetic compositions that are predicted to perform the desired function well. We refer to this approach as *computational model-based* design.

Microbial community metabolism can be modeled with varying levels of complexity and using a variety of modeling frameworks [97,98]. A relatively simple form of metabolic modeling, often referred to as network-based or topology-based modeling, represents each species as a directed network where nodes denote metabolites and edges connect substrates to products, reflecting the set of metabolic reactions the modeled species can catalyze. With this framework, community metabolism can be modeled as a collection of such networks, where outputs from one network can be used as input for another. A recently introduced design algorithm, termed CoMiDA [99•], has utilized this modeling framework to identify a minimal set of microbial species that collectively provide the enzymatic capacity required to synthesize a set of desired products from a predefined set of available substrates. To achieve this, the CoMiDA algorithm integrates a graph-theoretic

representation of network flow with the set cover problem to consider all possible metabolic paths from substrates to products and to detect the minimal set of species that can catalyze these reactions. The obtained solution can provide a starting point for further synthetic community experimentation and development. Another design algorithm, termed MultiPus [100], utilizes a similar framework but aims to minimize the number of reactions and inter-microbial transfers, rather than the number of species.

While network-based models are easy to construct and analyze, they generally only account for the potential metabolic capacity of each species, rather than for the way each species will behave in a given environment. Accordingly, communities designed by CoMiDA or Multi-Pus are indeed guaranteed to have the metabolic *potential* to carry out the desired function, but may not actually perform this function in reality. Instead, the accurate estimation of microbiome behavior requires a detailed model of microbial metabolism, one that can predict the specific activity of each species, the flux through each reaction, the uptake and secretion rate of environmentally available metabolites, and the growth rate of each species in a given environment. One such modeling framework utilizes constraint-based models and flux balance analysis (FBA) [101,102••]. Such models can predict the steady state metabolic activity of a given species by identifying a set of metabolic fluxes that maximize microbial growth while adhering to a set of thermodynamic constraints [103,104]. Recent years have witnessed an explosion of studies that aim to extend constraints-based modeling from single species models to community models that can predict community-level metabolism, species interactions, and community dynamics [105–108]. Building on these effort, a recent design method, termed FLYCOP [109••], has utilized a previously introduced community modeling framework to evaluate synthetic composition function *in silico*. Importantly, the underlying modeling framework accounts for community dynamics and spatial community organization, as well as metabolism-mediated species-interactions via changes to the shared environment [107]. Using this framework, FLY-COP explores potential synthetic compositions using a stochastic search procedure and identifies an optimized composition. Interestingly, FLYCOP is not restricted to optimizing metabolic activity, and can also consider a community's growth over time. This allowed the authors to identify an initial synthetic composition of four cross-feeding strains that optimized community stability. This ability to optimize community stability could be extremely important for therapeutics, where treatment may require the community to function for a prolonged period.

Another modeling approach that can be useful for community design efforts, especially when stability is an important consideration, aims to model community ecological dynamics rather than community metabolism. These models capture how the abundance of each community

Table 1

Summary of synthetic community design methods

Design approach	Description	Requirements	Benefits	Limitations	Examples of usage
Enrichment	Environmental conditions are modified to promote the growth of species that perform a desired function	• Initial community with capacity for the desired function • Selection procedure that favors growth of species that can perform the desired function	• Optimizes community function without identifying individual species involved	• Undefined or partially defined communities may be unsuited for therapeutics • Optimization time can be extensive depending on the application and initial community	Fradinho et al. [60•] Korkakaki et al. [59] Kumari et al. [63]
Community reduction	Species are isolated from an existing community, screened to keep desirable species and/or exclude undesirable species, and then reconstituted into a simplified community that recapitulates the source community's function	• Initial community with capacity for the desired function • Appropriate media for culturing microbial isolates • Screening procedures for isolated species	• Produces a defined synthetic composition • Can explicitly exclude pathogenic or otherwise undesirable species	• Cannot design communities for novel functions • May fail if important species cannot be cultured	Petrof et al. [68••] Atarashi et al. [71] Caballero et al. [69]
Combinatorial evaluation	All possible combinations of a set of candidate species are evaluated for their performance of a desired function and the best-performing composition is selected	• Microbial isolates suspected to contribute to the desired function • Screening procedures for candidate communities	• Produces a defined synthetic composition • Can explicitly exclude pathogenic or otherwise undesirable species • Can combine species from distinct sources	• Number of potential compositions to evaluate grows exponentially with the number of candidate species	Hu et al. [91•] Hu et al. [92]

Table 1 (*Continued*)

Design approach	Description	Requirements	Benefits	Limitations	Examples of usage
Computational model-based design	Mechanistic models of microbial functional capacities and activities and/or ecological models of community dynamics are used to evaluate potential community compositions *in silico*, identifying one or more optimized compositions for further experimental validation	• Mechanistic knowledge of individual microbial function *and/or* • Ecological models of community dynamics	• Produces a defined synthetic composition • Can explicitly exclude pathogenic or otherwise undesirable species • Can combine species from distinct sources • Reduces time and labor required for experimental evaluation	• Well-curated mechanistic information is unavailable for many species • Mechanistic models of non-metabolic functions are lacking	Eng and Borenstein [99•] Julien-Laferrière et al. [100] Garcia-Jiménez et al. [109••] Stein et al. [112••]

member impacts the abundances of others over time [110•,111]. Such models can be especially useful when interactions between species may not be mediated via metabolism or when detailed metabolic models are not available. In one example, a group optimized a synthetic composition for both a non-metabolic function and community stability through a combination of ecological modeling and experimental characterization of individual microbial activity [112••]. In this case, the authors aimed to develop a community for T_{reg} induction in the mouse colon that would persist over time. To achieve this, the authors first created a model of community induction effectiveness for a set of *Clostridia* strains using data on T_{reg} induction contributions. They then simulated the community's ecological dynamics using a previously published ecological model for those strains [110•], and used their induction model to estimate T_{reg} induction over time. This enabled them to predict each potential composition's inductive effect and stability simultaneously. Such integration of different modeling framework may be a promising avenue for future expansion and improvement of computational design capabilities.

Synthetic composition design challenges and opportunities

The previous sections have surveyed recent applications of, and advances in, synthetic composition design. Importantly, however, there are still many daunting challenges, as well as exciting opportunities, for future development in this field. Clearly, each design approach described above has its own strengths and weaknesses that make it more suitable for certain applications and less appropriate for others and that impose a specific set of challenges and opportunities (Table 1). Enrichment can often work without detailed knowledge of individual species, instead relying on understanding the desired biological process in order to create a selection procedure. However, each novel application may require a completely new selection procedure, which could be challenging to develop [113]. Additionally, the time required for optimization can be extensive, with some experiments showing continued improvement over the course of months [58,114]. The community reduction and combinatorial evaluation approaches similarly avoid the need for detailed mechanistic data while also incorporating greater control over the specific species used. Unfortunately, due to our inability to culture a large fraction of microbial species [65], community reduction can suffer from a failure to isolate a set of species sufficient to recapitulate the original community's function. Additionally, community reduction does not inherently optimize a synthetic composition's function, but rather only identifies a well-defined synthetic composition with the desired function. Combinatorial evaluation, in contrast, can suffer from tractability issues regarding the number of compositions to evaluate, as described above. Finally, computational model-based design can drastically decrease the time and labor needed to identify optimal, or near-optimal

compositions, but it requires detailed and thorough mechanistic and/or ecological data about the species being considered. Understanding these differences between approaches, as well as considering the available time, labor, and knowledge resources, will help future designers select the approach best suited for their specific application.

While each design technique offers unique benefits, the power and flexibility of computational model-based design, combined with the recent expansion of available genomic, metabolic, and other mechanistic data, render such computational design efforts an especially promising route toward rapid advancement in synthetic community design. For example, methods that can concurrently optimize multiple community functions could enable synthetic therapeutic communities to simultaneously treat diverse health concerns, such as metabolic deficiencies and pathogenic infections, while also ensuring community stability. There are, however, substantial obstacles that must be overcome for model-based design to reach its full potential. One key challenge is the inability of many currently modeling frameworks to directly incorporate knowledge of non-metabolic microbial interactions into models of community function, which recent evidence suggests can be important factors in shaping the human gut microbiome [115]. Ecological models that are based on observed community dynamics may only partially capture the outcomes of such interactions, and it is likely that the nuanced effects of key interaction mediators are ignored [116•]. The potentially important functional impact of higher-order interactions (i. e. interactions involving multiple species in the community) [117–119] poses another challenge for computational methods, specifically when such methods evaluate only a subset of possible compositions. This calls for more sophisticated methods that efficiently search the space of potential compositions while adequately accounting for higher-order interactions, which would be especially advantageous.

Perhaps the most promising avenue for advancement in synthetic design might entail the integration of multiple design approaches such that the weaknesses of one approach would be addressed by the incorporation of a complementary strategy. Indeed, there is already evidence that communities designed using one method can be further improved via orthogonal design techniques. The computationally designed synthetic T_{reg} induction community described above [112••] was developed from a T_{reg} induction community originally designed using community reduction [70]. In this case, community reduction identified a set of culturable species that could form a synthetic T_{reg} induction community and computational design optimized the composition to improve its function. Pairing enrichment with combinatorial evaluation or model-based design could also offer potentially fruitful composite approaches. Specifically, the enrichment procedure could begin with compositions identified and pre-optimized by other design techniques, rather than environmentally sampled or randomly constructed initial communities. Such a method integration could potentially reduce the time required to select for an optimal composition since the initial community is hopefully closer in composition to the final community that enrichment would achieve. Additionally, this could help augment combinatorial evaluation or computational design, which may result in suboptimal communities due to insufficient coverage of evaluated compositions or insufficient mechanistic and ecological knowledge respectively. Such innovative combinations of design approaches could enhance or enable the development of synthetic compositions that may have previously been challenging due to the various limitations of each individual approach.

Conclusions

In this review, we have surveyed various methods for designing synthetic microbial communities, highlighting their utility in formulating and optimizing communities for a wide variety of applications. These methods range from experimentally driven techniques, including enrichment, community reduction, and combinatorial evaluation, to computational approaches that employ mechanistic models of microbial function and ecological models of community dynamics. We have described various successful applications of these methods to both industrial and therapeutic synthetic community design, noting interesting and important observations made during the design process. Perhaps the most important of these observations is that optimized communities are often smaller and less complex than naturally occurring communities. Indeed, as in some cases mentioned above, simpler synthetic communities with fewer strains have achieved better performance than their more complex counterparts. This suggests that design methods will continue to play an important role in identifying the particular subcommunities that best achieve specific functions. Given this, we believe that continued advancement in all classes of design approaches will greatly expand the uses of, and improve the efficacy of, synthetic microbial communities.

Conflict of interest statement
Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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