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Toward merging bottom–up and top–down model-based designing of synthetic microbial communities

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The increasing interest of microbial communities as promising biocatalyst is leading an intense effort into the development of computational frameworks assisting the analysis and rational engineering of such complex ecosystems. Here, we critically review the recent computational and model-guided advances in the system-level engineering of microbiome, including both the rational bottom-up and the evolutionary top-down approaches. Furthermore, we highlight modeling and computational methods supporting both engineering paradigms. Finally, we discuss the advantages of combining both strategies into a hybrid top-down/bottom-up (middle-out) strategy to engineer synthetic microbial communities with improved performance and scope.

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Introduction

Microbial communities are defined as the set of co-occurring, and potentially interacting, microbes present in a defined habitat in space and time. Traditionally, they have been considered to play a critical role in ecosystem functioning, such as organic matter decomposition, biogeochemical cycling of nutrients, and xenobiotic degradation [1]. However, as our understanding of these microbial ecosystems broadens, their importance has exceeded previous considerations to the point that microbial communities have been proven to be key actors in human and animal nutrition, health, and agriculture [2]. Furthermore, increasing concerns over climate change and the deteriorating health of our planet are driving new applications for microbial ecosystems. For instance, they have started to be suggested as promising biocatalysts toward the replacement of petrochemicals with bio-based chemicals and materials [3,4].

The advantages of microbial-community-based biocatalysts over monocultures have been extensively reviewed and include division of labor (DOL), spatial organization, and robustness to perturbations [5,6]. However, it is important to note that microbial communities bring an additional level of biological complexity into play. Understanding and engineering such complex biocatalysts and using them to develop biotechnological applications requires system-level approaches often in the context of model-based frameworks.

In this review, we briefly discuss recent advances in system-level microbial-community engineering with a focus on biotechnological applications. We address i) rational, bottom-up and ii) evolutionary, top-down approaches, and describe how modeling and computational methods are increasingly supporting both engineering paradigms. Finally, we discuss the advantages and convenience of combining both strategies into a hybrid top-down/bottom-up (middle-out) strategy to deliver improved performance.

Top-down versus bottom-up engineering of microbial communities

Biology's nonlinearity and the functional complexity that is inherent to microbial communities render empirical attempts to decipher the specific roles of individual components in the provision of community-derived phenotypes largely unapproachable. Systematic strategies, including mathematical approaches, are thus needed to support a holistic understanding of microbial communities while tackling key microbial ecology issues and potential biotechnological applications (Table 1). These modeling approaches have proved powerful in assisting microbiome engineering, reducing costs, and enabling even new transformations [7]. Multiple community-level modeling approaches have been developed to gain insights into complex synthetic microbial consortia (SMC) following both bottom-up and top-down approaches [8–10].

Table 1

Selected software and key features.

Paradigm	Name	Input	Output	Interface	Language	Ref.
Bottom-up	Memote	GEM	Quality report	Webserver/ standalone	Python	[21]
	Cobramod	GEM	GEM+report	Standalone	Python	[22]
	Bigg	GEM	Model repository	Webserver	–	[23]
	AGORA	GEM	Model repository	Webserver	–	[24]
	Kbase	GEM	Model repository	Webserver	–	[25]
	Microbiome Toolbox 2.0	GEM	pan-GEM and pairwise growth rates	Standalone	Matlab	[27]
	μBialSim	GEM	Metabolite profile and growth rates	Standalone	Matlab	[28]
	COMETS 2.0	GEM	Metabolite profile and growth rates	Standalone	Matlab/Python/Java	[30]
	Surfin FBA	GEM	Metabolite profile and growth rates	Standalone	Python	[31]
	Community Gap-Filling	GEM	Reaction fluxes of the best community model	Standalone	Matlab	[32]
	DOLMN	GEM	Single-specie multiple GEMs	Standalone	Matlab	[34]
	ASTHERISC	GEM	Max–min driving forces of the proposed multistrain community	Standalone	Matlab/Python	[35]
	FLYCOP	GEM	Optimized configuration consortia	Standalone	Python/Shell/R	[36••]
	AutoCD	Genetic parts and their distributions	Topology and candidate models for synthetic microbial communities	Standalone	Python/C++/R	[38••]
	IndiMeSH	GEM	Metabolite profile and growth rates	Standalone	Matlab	[40]
	ACBM	GEM	Metabolite profile and growth rates	Standalone	Matlab/Java	[41]
Top-down	PICRUSt2	16s rDNA profile	EC, pathway, and 16S copy-number metagenome abundances	Standalone	Python/R	[46]
	Tax4Fun2	16s rDNA profile	EC, pathway, and 16S copy-number metagenome abundances	Standalone	R	[47]
	iVIkodak	16s rDNA profile	EC, pathway, and 16S copy-number metagenome abundances	Webserver	–	[48]
	Animalcules	16s rDNA profile metatranscriptome profile	Biomarkers and differential pathway results	Standalone	R	[49]
	METABOLIC	Metagenome sequencing	Metabolic profile	Standalone	Perl/R	[50]
	MICOM	GEMs, metagenomic, and metabolomics data	Community GEM and growth rates	Standalone	Python	[51••]
	CarveMe	Annotated Genomes	Community GEM	Standalone	Python	[52]
	MetaGEM	Metagenome sequencing	Community GEM	Standalone	Python/Shell/R	[53••]
	M2M	Annotated metagenomes	Minimal community GEM	Standalone	Python/R	[54]
	MiMiC	Annotated metagenomes	Minimal community GEM	Standalone	R/Shell/Perl	[55•]

Bottom-up involves piecing together systems to give rise to more complex systems, thus making the original systems subsystems of the emergent system. This approach requires measurements of the physicochemical and kinetic properties of the community components whose complexity is commensurate with the biological intricacy gradient as we progress from DNA parts toward microbial ecosystems. The bottom-up paradigm (and its successful application) requires a full understanding of the basic mechanisms of life in order to create biological systems from independent biomolecular, that is, less complex, components. Owing to this dependence of previous knowledge, bottom-up engineering is underpinned by rational guidance and it demands seasoned practitioners with the necessary background for smart engineering decision-making. Computational tools addressing the design and optimization of low-complexity-level components, including parts, pathways, or organisms [11,12], are not within the scope of this review. In contrast, tools focusing on rational design of microbial communities have just started to emerge and are carefully reviewed here.

Top-down engineering aims at reducing complexity using evolutionary engineering approaches. Contrary to bottom-up formalisms, prior knowledge of the community's functioning is not required to implement top-down attempts to engineer biological systems and identify molecular-interaction networks on the basis of correlated molecular behavior derived from (meta)genome-wide 'omics' studies. Despite being an apparently less rational approach, the support provided by evolutionary engineering is making it possible to unlock novel solutions in the shape of new functional modules encoded in the genotype space [13,14]. Computational methods assisting a rational top-down approach are already under development and on course to setting novel paradigms, and opening up new avenues, leading to successful engineering of superior synthetic microbial communities.

Bottom-up system engineering of synthetic microbial consortia

Out of the two main paradigms of synthetic biology engineering, bottom-up is the benchmark. Recent years have seen an outburst of rational efforts to design SMC with the ultimate aim of addressing increasingly complex endeavors. In the absence of computational support, researchers have often exploited OMIC-identified/synthetic interspecific relationships and functional synergisms to engineer SMC stability and functionality, respectively (Figure 1). For instance, an engineered synthetic mutualism comprising a chitin-metabolizing and lysine auxotrophic *E. coli* strain and a lysine overproducer *C. glutamicum* strain resulted in the successful production of this amino acid from chitin in a single-pot bioprocess [15].

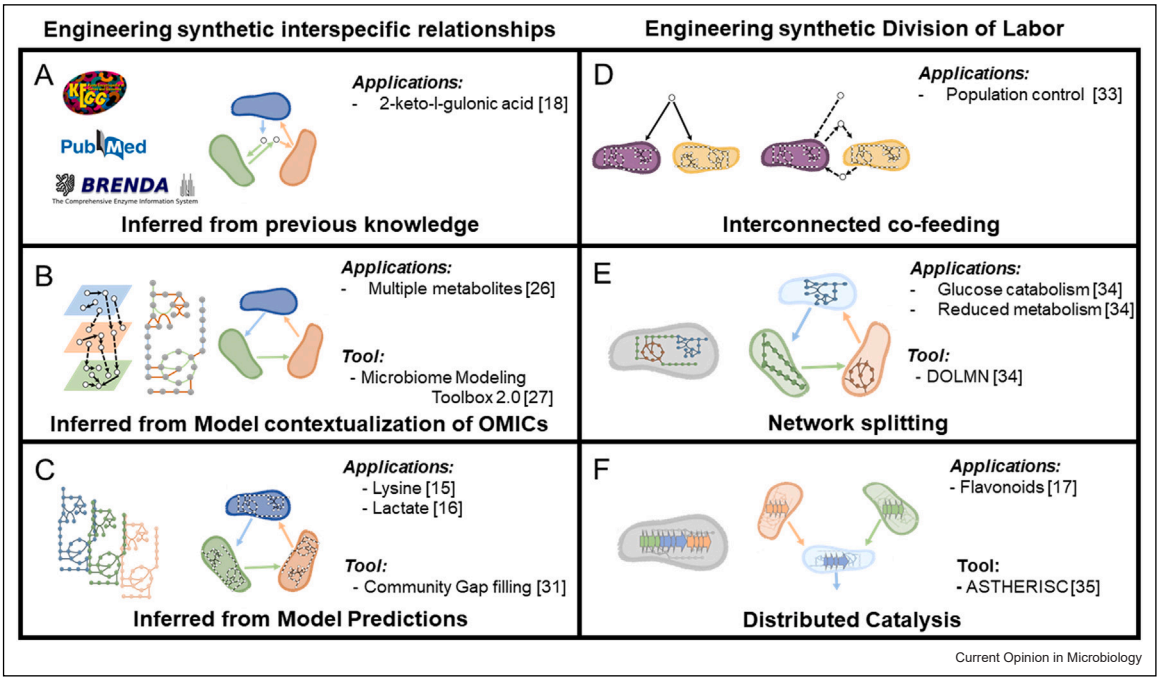
Engineering-distributed catalysis is another powerful strategy to optimize biotechnology applications dealing with complex bioprocesses and has been recently reviewed [6]. In a very elegant work, lignocellulose degradation was funneled to lactate as an intermediate building block in a primary microbial-degradation module. Subsequently, a library of secondary producer strains was engineered to produce a large variety of short-chain fatty acids using the lactate previously released [16]. Distributed catalysis is also very effective when optimizing nonlinear biosynthetic pathways because it minimizes metabolic burdens while maximizing carbon allocation to specific precursors. This approach has been successfully applied to the production of natural products such as flavonoids [17].

Prior knowledge of the performance of individual components is critical when applying bottom-up approaches. However, systematic assessment of consortium components is often overlooked, thus returning reduced titers and yield. In a recent work, a multikingdom (*S. cerevisiae*/*K. vulgare*) synthetic consortium overproducing 2-keto-L-gulonic acid, which was constructed *de novo* based on transcriptomics analyses, delivered a 1.49-fold yield increase compared with a *K. vulgare* monoculture [18].

Despite the growing interest in engineering SMC, current efforts are still heavily reliant on trial-and-error, which to a large extent limits the potential and scope of SMC-based biocatalysts. Hence, multiple computational methods have recently been launched to support microbial-community design and analysis (Table 1). Among these, COnstraint-Based Reconstruction and Analysis (COBRA), a mechanistic systems biology method supported by genome-scale metabolic models (GEMs) and powered by Flux-Balance Analysis (FBA), is becoming very popular and has been used successfully in over 100 studies [7,19]. The COBRA methodology has provided valuable insights into microbial ecosystem physiology, function, and evolvability. Within the COBRA framework, setting up quality GEMs is a critical step, and it is typically addressed following a bottom-up development process that is both labor-intensive and time-consuming. Multiple tools to automate the process have been developed in recent years, including microbial-community-modeling functionalities. A recent critical appraisal and overview of the capabilities of such tools is already available [20], so here, we address exclusively recent modeling developments and their applications.

An often-neglected aspect of microbial ecosystem modeling is the mandatory requirement to have not just high-quality, but also intelligible, syntax-compatible models that are able to support the emergence of interspecific relationships. In this sense, recent

Figure 1



Details of engineering synthetic interspecific relationships (left) and DOL modeling (right) in SMC design with bottom-up approach. The relationships between consortia members can be inferred from **(a)** previous knowledge stored in metabolic databases and literature legacy, **(b)** using model contextualization of OMICs, or **(c)** the use of GEMs. The main designing strategies addressing DOL include **(d)** cofeeding, **(e)** network splitting, and **(f)** distributed catalysis. Key tools and applications are indicated.

community-driven initiatives such as MEMOTE [21] and COBRAMOD [22] provide the optimal framework to assess the quality, version, and annotation control of new models being released. Microbial-community-level modeling is thus being facilitated by the development of repositories of metabolic models featuring compatible syntaxes, which supports their use as a source of prebuilt GEMs for a *la carte* SMC assembly. Such repositories include both broad-spectrum (BIGG [23]) and niche-specific microorganisms (AGORA [24]), as well as universal and non-organism-specific metabolic reconstructions available on Reactome (KBase [25]). Accordingly, methods are now being developed to support a rational approach to condition-specific SMC design. For instance, by integrating metatranscriptomics, metabolomics, and the COBRA approach to phenotyping, it was possible to reveal condition-dependent secretion and cross-feeding of metabolites in a synthetic phototrophic community [26•]. On the other hand, Microbiome Modeling Toolbox 2.0 features improved scalability and efficiency, thus supporting large-scale interrogation of hundreds or even thousands of microbial-community models using AGORA as a source of GEMs [27].

Alongside this significant surge of new GEMs and modeling methods, the demanding computational requirements of community-level modeling have recently begun to drive the development of novel, optimized FBA-based methods, with an emphasis on dynamic modeling. In this sense, μBialSim [28] is a dynamic Flux-Balance-Analysis-based (dFBA) [29] numerical simulator able to predict evolution in terms of microbiome composition and activity of microbiomes containing hundreds of species in batch or chemostat mode. In addition, an updated version of the popular dFBA-based method computation of microbial ecosystems in time and space (COMETS) is now available. This supports evolutionary analysis of microbial communities across time and space [30]. COMETS provides dynamic prediction of microbial-community composition, population size, and metabolite yield. Finally, an efficient dFBA method has been developed to support improved parameter fitting to time-longitudinal data, thus reducing computational burdens and significantly increasing the scope of dynamic modeling within microbial communities [31].

Many of the recent advances in GEM-based microbial-community analysis have focused on modeling and broadening our understanding of these complex assemblages at the system level. Remarkably, by exploiting well-known microbial-community features, elegant methods addressing *in silico* design of optimal SMC-based biocatalysts have started to be developed (Figure

1). A recent community-level gap-filling algorithm was developed to predict cooperative and competitive metabolic interactions between species [32]. This method directly addresses the prediction of metabolic interactions among microorganisms, which in turn, are a key driving force for the resulting SMC's function and structure.

DOL has been also profusely exploited when designing SMC. For instance, FBA and unstructured kinetic modeling have been used to investigate the robustness and behavior of synthetic consortia in terms of stability and population control. This hybrid approach provided an *in silico* interconnected carbon cross-feeding system based on strains of *Escherichia coli* and *Acinetobacter baylyi* ADP1, which was further experimentally validated [33]. Division of labor in Metabolic Networks (DOLMN) [34] uses a mixed-integer linear programming formulation to explore the space of feasible multistrain metabolic networks derived from splitting a single parent network. This method predicted metabolic pathway partitions difficult to assess manually, thus providing paths to design synthetic competitive advantages over individual organisms. When dealing with DOL along metabolic pathways, the thermodynamic feasibility of the predicted subnetworks becomes critical. Algorithmic Search of THERmodynamic advantages in Single-species Communities (ASTHERISC) [35] faces this challenge by designing multistrain communities of a single species, splitting a production pathway into smaller fragments and distributing them between the strains. This approach maximizes the thermodynamic driving force for product synthesis. ASTHERISC exploits the fact that compartmentalization of a product pathway into modules and their subsequent allocation to a set of specialist strains can circumvent thermodynamic bottlenecks arising in the context of the entire pathway. Beyond multiple microbial analysis and assembly, FLexible sYnthetic Consortium OPtimization (FLYCOP) robustly combines COMETS with a local search algorithm to design SMC with a desired function [36••]. FLYCOP uses the list of community members and growth-medium nutrients as its inputs and returns optimal interspecific relationships, growth-medium composition, and relative microbe abundances for specific community-level goals.

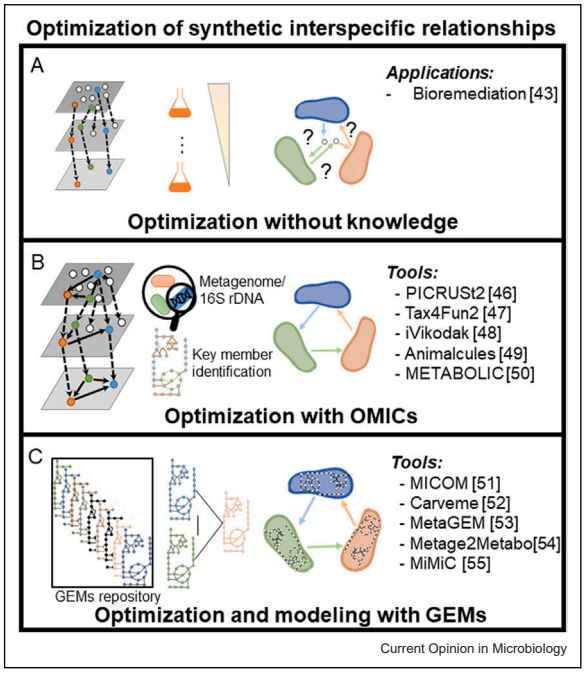
Promising Bayesian inference methods have been developed as alternatives to GEM-based approaches. They lack mechanistic understanding, but are easy to construct in return. Using this approach in the context of an ad hoc gLV modeling, a variety of synthetic human gut microbiomes producing butyrate have been recently designed and experimentally validated [37]. Automated synthetic microbial Community Designer (AutoCD) [38••] is a general method based on Approximate Bayesian computation combined with sequential Monte Carlo sampling (ABC SMC). AutoCD uses an ordinary differential equation (ODE) model to generate stable, steady-state communities capable of fulfilling specific goals. It also considers competition for nutrients and quorum sensing in order to deliver robust synthetic communities. An important disadvantage of AutoCD is that it is limited to SMC with a small number of components.

Among other modeling formalisms, agent-based modeling (ABM) has demonstrated that it is a powerful approach to dealing with the spatiotemporal composition of synthetic microbial consortia, including the dynamic modulation of average cell length of constituent strains [39]. Individual-based Metabolic network model for Soil Habitats (IndiMeSH) [40] is another ABM-based method specifically designed to address the dynamic responses to environmental changes using adaptive metabolic networks and spatial organization. Finally, analysis of the spatiotemporal dynamics of microbial communities has been addressed using an interesting FBA–ABM hybrid method called an integrated Agent and Constraint Based Modeling of microbial communities (ACBM) [41]. ACBM models cell population in three dimensions and predicts spatial and temporal dynamics and metabolic interactions.

Top-down systems engineering of synthetic microbial consortia

Top–down is a complementary engineering paradigm accounting for reductionist processes where previous knowledge of the system is not required. Complexity reduction is addressed under multiple evolutionary frameworks [13,14,42], yielding smaller microbial communities with the desired phenotype. Top-down approaches have been profusely used in bioremediation and to power catabolism of recalcitrant compounds by reducing the metabolic space of natural communities present in the habitats exposed to target compounds [43]. Given the nature of this approach, optimal functionality is often achieved with no consideration of the molecular mechanisms underpinning the desired phenotypes and, in principle, it does not require a strong computational assessment (Figure 2). However, a detailed characterization of the consortium's composition and structure is useful in order to unravel the dynamics of the final microbial community, including identification of key species and/or description of the minimal consortium required to mimic the functionality of the evolved SMC. Therefore, it is hardly surprising that increasingly sophisticated computational methods are beginning to be developed in an attempt to understand and rationally manage evolutionary processes that are inherent to the top-down approach (Table 1). In any case, rational top-down design of SMC is still in its infancy, so, it is mainly evolutionary engineering approaches that are currently being used.

Figure 2



Optimization of microbial consortia following a top-down approach. The incremental process of optimization has three main levels: **(a)** optimization following a top-down approach with no prior knowledge. **(b)** Inclusion of OMIC data (amplicons, metagenomics) to support identification of consortium components. This provides crucial knowledge and control of the metabolic interactions, metabolic potential, and consortium dynamics. Based on this knowledge, a minimal consortium with similar characteristics to the original community can be defined. **(c)** The last level of optimization is driven by GEMs. The individual components are modeled separately and integrated into a community model with increasing levels of metabolic accuracy and cross-feeding interactions. Key tools and applications are indicated.

OMIC-derived technologies, including amplicon sequencing, metagenomics, and metatranscriptomics, are now widely used to characterize complex microbial communities (Figure 2). 16S/18S rDNA amplicon sequencing has recently become very popular due to its low cost compared with other technologies. Despite its lower resolution, third-generation sequencing supports identification at species level and even strain level [44]. In addition, recent advances have removed certain known taxonomical classification biases when using 16S rDNA amplicon data, thus providing higher reliability and confidence [45]. Largely powered by these improvements, a variety of interesting reference-based tools have been developed to predict and analyze functional profiles of microbial communities. PICRUSt2 [46] integrates existing open-source tools to predict and analyze genomes of environmentally sampled 16S rRNA gene sequences. Tax4Fun2 [47] is another inference method that additionally considers habitat-specific genomic information to improve the accuracy and robustness of predicted functional profiles. iVikodak [48] is a comprehensive web platform supporting multiple functional, structural, and comparative analyses of natural communities. In addition, Shotgun meta sequencing is able to handle full-genome assemblies, thus supporting higher-resolution microbial ecosystem studies. Bespoke new methods to analyze microbial communities defined by such data have subsequently emerged — see the above-mentioned PICRUSt2, a robust tool that also uses metagenomics data. Animalcules [49] is another highly versatile tool integrating 16S rRNA sequencing, metagenomics, and metatranscriptomics profiling data. Animalcules also combines novel and existing analytics, visualization methods, and machine-learning models to infer microbiome structure and functionality. Advanced computational methods include METABOLIC [50], a software designed to analyze community-scale functional networks, which uses metagenomics data to i) return annotated, biochemical, and metabolic pathway analysis and ii) size microbes' contributions to individual biogeochemical transformations and cycles.

The above-mentioned methods define the potential metabolic space of a given microbial community. Interestingly, this metabolic space can be easily interrogated within the COBRA framework, thus paving the way for top-down engineering of complex natural communities (Figure 2). Computational frameworks have indeed been developed to fulfill this aim: MICOM [51••] uses metagenomics and metabolomic data to identify key functional partners in microbial communities, and predicts interspecific interactions using AGORA as a curated source of metabolic models. MICOM has been used successfully to improve our understanding of the metabolic features driving microbiome interactions in an anaerobic biogas-production system. This highlights this approach's potential for rational SMC design. Nevertheless, top-down design formalisms necessarily require large databases of high-quality GEMs. CarveMe [52] uses a top-down approach to build single-species and community models in a fast and scalable manner. CarveMe was used to build a collection of 74 human gut bacteria models and a database of 5587 bacterial models, thus contributing to make microbial-community model construction more feasible. MetaGEM [53••] is another top-down modeling method attempting to push the boundaries by creating automatic GEMs and community metabolic models. Finally, ingenious methods such as Metage2Metabo (M2M) [54] and MiMiC [55•] have emerged to tackle minimal SMC designs capable of delivering target objectives in full.

Middle-out approaches for enhancing the system design of synthetic microbial consortia

When applied individually, both bottom-up and top-down design paradigms have multiple strengths, but also important weaknesses. Overall, while bottom-up offers high levels of control for rational design, the metabolic space suitable for design purposes is limited and solutions are often rendered suboptimal. On the other hand, top-down displays a larger initial metabolic space. However, large portions of it remain unexplored due to knowledge gaps and thus become unavailable for design purposes. Therefore, the synergistic application of both formalisms, also known as the middle-out approach, will likely increase the chances of finding optimal solutions.

Intuitively, SMC constructed following either bottom-up or top-down approaches is liable to functional enrichment via migration of components (cells) from the opposite formalism. In an elegant work, an ammonium-assimilation microbiome was constructed following this approach [56••]: first, a nitrifying SMC was isolated and acclimatized to high-salinity synthetic wastewater using a top-down design from an activated sludge. In the subsequent bottom-up step, the 'domesticated' SMC was combined with the well-known ammonium-removing and granular-biofilm-producing *Psychrobacter aquimaris* strain. The resulting ammonium-assimilating microbiome achieved efficient nutrient-removal performance with over 80% of ammonium, total nitrogen, and total phosphorus removed. In a different setting, two top–down engineered lignocellulolytic SMC were enriched with *Lactobacillus plantarum* to significantly improve their ability to degrade structural carbohydrates and transform soluble carbohydrates into lactic acid [57].

On the other hand, nonrational evolutionary procedures such as Adaptative Laboratory Evolution (ALE) have the potential to be used to fine-tune and optimize the performance of an SMC that was originally bottom-up. This idea was applied to the optimization of a *L. plantarum*-based SMC displaying cellulolytic enzymes, while the further adaptive laboratory process served to significantly increase enzymatic activity within the SMC [58]. Similarly, the performance of a bottom-up *E. coli* modular coculture designed to produce pinene was greatly improved by using ALE [59] to increase its productivity and tolerance to pinene toxicity.

Such evolutionary engineering methods do not necessarily exclude the use of computational tools. In fact, GEMs are able to qualitatively assess a single mutant's fate in a given scenario in a ALE experiment. This approach was used to enhance secretion of multiple metabolites within the model and to improve metabolite secretion in the *L. plantarum–S. cerevisiae* consortium [60] above.

Overall, the potential of computational approaches supporting middle-out SMC design remains largely underutilized. Likewise, certain methods and tools supporting bottom-up and top-down SMC design still require integration into standardized computational workflows, despite their many potential applications in the field.

Outlook

The scope of synthetic biology continues to expand significantly and is no longer limited to assembling DNA, pathways, or cells. Cells can now be used as building blocks to assemble and build functional consortia with improved functionality and performance. However, this extra level of biological complexity and the existing knowledge gaps concerning the basic principles underlying the assembly of natural microbiomes largely hampers rational design of such complex ecosystems. While computational methods guiding bottom-up design are in good shape, the full biocatalytic potential of novel computational methods supporting top-down and middle-out SMC engineering is yet to be achieved in areas as diverse as industrial biotechnology, human and animal health, agriculture, food and beverage, ecosystem restoration, climate change, and even exoplanet terraforming.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- • of special interest
- •• of outstanding interest

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