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Bioenergy, Biofuels and Biochemicals – Review

Top-down and bottom-up microbiome engineering approaches to enable biomanufacturing from waste biomass

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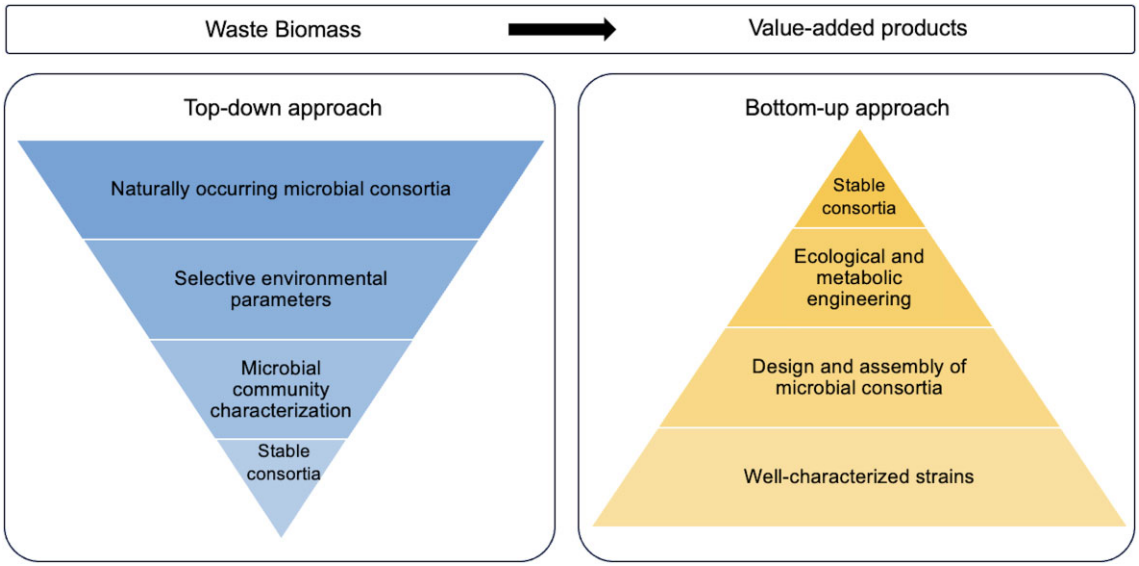
Abstract: Growing environmental concerns and the need to adopt a circular economy have highlighted the importance of waste valorization for resource recovery. Microbial consortia-enabled biotechnologies have made significant developments in the biomanufacturing of valuable resources from waste biomass that serve as suitable alternatives to petrochemical-derived products. These microbial consortia-based processes are designed following a top-down or bottom-up engineering approach. The top-down approach is a classical method that uses environmental variables to selectively steer an existing microbial consortium to achieve a target function. While high-throughput sequencing has enabled microbial community characterization, the major challenge is to disentangle complex microbial interactions and manipulate the structure and function accordingly. The bottom-up approach uses prior knowledge of the metabolic pathway and possible interactions among consortium partners to design and engineer synthetic microbial consortia. This strategy offers some control over the composition and function of the consortium for targeted bioprocesses, but challenges remain in optimal assembly methods and long-term stability. In this review, we present the recent advancements, challenges, and opportunities for further improvement using top-down and bottom-up approaches for microbiome engineering. As the bottom-up approach is relatively a new concept for waste valorization, this review explores the assembly and design of synthetic microbial consortia, ecological engineering principles to optimize microbial consortia, and metabolic engineering approaches for efficient conversion. Integration of top-down and bottom-up approaches along with developments in metabolic modeling to predict and optimize consortia function are also highlighted.

One-Sentence Summary: This review highlights the microbial consortia-driven waste valorization for biomanufacturing through top-down and bottom-up design approaches and describes strategies, tools, and unexplored opportunities to optimize the design and stability of such consortia.

Keywords: Biomanufacturing, Biomass, Microbial community, Synthetic microbial consortia, Value-added products

Graphical abstract



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Introduction

A staggering 85% of the world's energy consumption is derived from nonrenewable fossil fuels (Cleveland & Morris, 2014; World Energy Use, 2022). This reliance has led to greenhouse gas (GHG) emissions and severely impacted natural ecosystem and biodiversity, necessitating sustainable alternatives to petrochemical-derived products. Biomanufacturing harnesses the power of microorganisms or enzymes to produce biofuels and bioproducts thus reducing our dependence on fossil fuels. Biomass-based products could replace up to 16% of crude oil consumption in the U.S., generating an additional $812 billion profit (Bioproducts to Enable Biofuels Workshop Summary Report, 2015). Biomass provided 5% of the total energy consumption in the U.S. in 2022, reducing the reliance on traditional energy sources (Biomass Explained, 2023). To achieve the ultimate goal of achieving a net-zero emission by 2050 and a carbon-neutral economy, biobased products will be further incentivized the U.S.

It is estimated that 2.01 billion tons of municipal solid waste is generated annually with more than 33% of total waste managed in an environmentally unsafe manner (Kaza et al., 2018). According to the U.S. Environmental Protection Agency, 66.2 million tons of food waste was generated from food retail and an additional 40.1 million tons from food processing and manufacturing, causing significant economic loss and environmental issues in 2019 (2019 Wasted Food Report, 2023). Traditional waste management methods, such as composting, landfill disposal, and incineration have limitations such as low process efficiency and negative environmental impacts, including space requirements, generation of odor, contaminated leachate, and toxic pollutants, and ash emissions (Phua et al., 2019; Andraskar et al., 2021; Parvin & Tareq, 2021). Inexpensive and readily available wet waste (Tayou et al., 2022), lignocellulosic waste (Li et al., 2020; Shahab et al., 2020a; Wongfaed et al., 2023), and C1 waste (CO_2, CO) (Diender et al., 2016; Jiang et al., 2021), are promising renewable feedstocks for the production of value-added products to enable biomanufacturing. Implementing innovative technologies for converting such untapped waste to useful products can address negative impacts due to waste accumulation and management problems, substitute fossil fuel-based products, and strengthen the bioeconomy.

Microbial communities greatly impact various aspects of life ranging from biogeochemical cycles, medicine, bioremediation, and public health, to biomanufacturing and resource recovery. Microbial consortia are suited to convert complex waste biomass due to their high enzyme diversity and the concerted and syntrophic activity of microorganisms belonging to different functional groups. Biotechnologies employing microbial consortia for sustainable waste valorization have emerged as promising alternatives to the petrochemical refinery processes to produce valuable biofuels, bioplastics, biochemicals, enzymes, and single-cell proteins (Venkateswar Reddy & Venkata Mohan, 2012; Zhou et al., 2017; Chi et al., 2018; Reddy et al., 2018; Valentino et al., 2018; Li et al., 2020; Pagliano et al., 2020; Tayou et al., 2022). Several of these biotechnologies have moved beyond lab and pilot scales and seen commercialization and expansion in recent years, contributing to the bioeconomy. For instance, anaerobic digestion (AD) has been widely adopted as a biological waste treatment and resource recovery technology to produce biogas which is further converted into electricity and heat. According to the World Biogas Association, an estimated 132 000 small, medium, and large-scale ADs are operational globally (World Biogas Association Global Report, 2019). The advancement in AD has been possible by understanding the role of microbial consortia and engineering it for efficient degradation of a wider range of feedstocks, tolerance to inhibitory compounds, and resilience to environmental perturbations (Werner et al., 2011; Blair et al., 2021).

Microbial consortia-based biotechnologies harness the metabolic capacity of microorganisms and their synergistic interactions by employing either top-down or bottom-up approaches for microbiome engineering. The top-down approach involves providing selective pressure by manipulating environmental or operating conditions to steer the structure and activity of the natural microbial consortia toward a desired function. On the contrary, the bottom-up approach starts by understanding individual microbial characteristics to rationally assemble native or engineered microorganisms into a new synthetic consortium. However, such microbial consortia-based processes still suffer from undesirable side reactions, low process efficiency, and inability to control and maintain stability for a long term, which is partly due to exposure to external perturbations and unpredictable intercellular interactions.

Previous reviews on microbial consortia have focused on either the top-down or bottom-up microbiome engineering approaches for environmental, public health, medical, or biotechnology applications (Duncker et al., 2021; Hu et al., 2022; Sauer & Marx, 2023; Zhou et al., 2024). However, there is a lack of comprehensive reviews that provide critical perspectives on both top-down and bottom-up approaches, and their integration to enable biomanufacturing from waste streams. This perspective paper first explores the promising potential of microbial consortia, highlighting their industrial and environmental applications utilizing diverse waste streams. Next, the paper elucidates recent advancements and knowledge gaps in top-down and bottom-up approaches with a focus on the design and assembly of synthetic microbial consortia, ecological engineering for process optimization, and metabolic engineering of microbial consortia. Finally, we also discuss the combination of the top-down and bottom-up approaches to maximize the potential of microbial consortia in different scenarios as well as metabolic modeling to predict and guide the microbial consortia design.

Microbial Consortia Enabled Biotechnologies for Diverse Waste Streams

Microbial consortia demonstrate remarkable diversity and metabolic capabilities to degrade a wide range of complex and heterogeneous biomass. Consequently, there have been several efforts to develop anaerobic and aerobic bioprocesses to harness microbial consortia to transform waste streams into a multitude of valuable products, such as polyhydroxyalkanoates (PHAs) (Venkateswar Reddy & Venkata Mohan, 2012; Valentino et al., 2018; Li et al., 2020; Pagliano et al., 2020), biohydrogen (Kotay & Das, 2010), biomethane (Yuan et al., 2012; Ghosh et al., 2020; Sahil et al., 2023; Wongfaed et al., 2023), alcohols (Lin, 2022), short-chain carboxylic acids (SCCAs) (Shahab et al., 2020a), and medium-chain carboxylic acids (MCCAs) (Gildemyn et al., 2017; Chi et al., 2018; Reddy et al., 2018; De Groof et al., 2019; Wang et al., 2023a), among others (Table 1). Such biotechnologies offer a sustainable alternative to conventional waste management and petrochemical based processes. For instance, as a potential source of biodegradable plastics, PHAs can be produced from glycerol, a common waste stream generated during biodiesel production (Alves et al., 2023). Furthermore, biohydrogen via microbial fermentation of waste biomass presents a sustainable and eco-friendly energy alternative with minimal environmental impact (Dzulkarnain et al., 2022). Conventional hydrogen production methods from biomass such as gasification and pyrolysis, require

Table 1. Different value-added products generated from diverse waste biomass using both top-down and bottom-up approaches on the laboratory scale.

Product	Waste source	Dominant microbial population or strains	Approach	Yield	Reference
Biogas (mainly methane)	Lignocellulosic biomass	*Bacteroidetes*, *Proteobacteria*, *Firmicutes*, *Spirochaetes*, and *Actinobacteria*	Top-down and bottom-up	0.14 to 0.39 L biogas/g VS	(Wongfaed et al., 2023)
	Agricultural residue	*Bacillus sp.*, *Delftia sp.*, *Pseudomonas sp.*, *Lysinibacillus fusiform*, *Arthrobacter nicotianae*, *Paenibaccilus ehimensis*, *Aspergillus sp.* and *Trichoderma sp.*	Top-down	0.19 L/g TS	(Sahil et al., 2023)
	Paper waste	*Clostridium straminisolvens* CSK1	Top-down	0.19–0.29 L CH_4/g VS	(Yuan et al., 2012)
	Municipal solid waste	*Arcobacter*, *Methanoculleus*, and *Aspergillus*	Top-down	0.59 L biogas/g VS	(Ghosh et al., 2020)
Carboxylic acid	Lignocellulosic biomass	*T. reesei*, *L. pentosus* *C. tyrobutyricum*	Bottom-up	0.35 g butyric acid/L	(Shahab et al., 2020a)
	Sludge waste	N/A*	Top-down	0.68 g COD/L.d	(Tayou et al., 2022)
	Food waste	*Clostridia*, *Sphingobacteriales*, *Desulfobacteraceae*, and *Bacillus*	Top-down and bottom-up	8.10 g caproic acid/L	(Reddy et al., 2018)
	Agricultural waste	*Clostridium thermocellum* ATCC 27405 and *Clostridium thermobutyricum* ATCC 49 875	Bottom-up	2.37 g butyric acid/L and 2.08 g lactic acid/L	(Chi et al., 2018)
1,3-propanediol	Glycerol	*Clostridiaceae* and *Peptostreptococcaceae*	Top-down	60.61–82.66 g/L	(Zhou et al., 2017)
PHA	Lignocellulosic biomass	*Bacteroidetes* and *Proteobacteria*	Top-down	2.64 g COD/L	(Li et al., 2020)
	Food waste	*Pseudomonas*, *Aeromonas*, and *Acinetobacter*	Top-down	2.12 g/L	(Venkateswar Reddy & Venkata Mohan, 2012)
	Dairy wastewater	*Cupriavidus necator* DSM 13513, 531 and 428	Bottom-up	0.52 g/L	(Pagliano et al., 2020)
	Municipal solid waste	*Proteobacteria* and *Cytophaga/Flexibacter/Bacteroidetes*	Top-down	0.18–0.49 g/L.h	(Valentino et al., 2018)
Hydrogen	Sewage sludge	*Enterobacter cloacae*, *Citrobacter freundii*, and *Bacillus coagulans*	Bottom-up	>0.35 L/g COD	(Kotay & Das, 2010)

N/A* refers to data not available.

high temperature and pressure and generate GHGs. Therefore, alternative biological methods that use microbial consortia such as dark fermentation (Dzulkarnain et al., 2022) have evolved, offering lower energy requirements, higher selectivity, and environmental benefits. Microbial consortia also offer a promising approach to valorize C1 gaseous waste streams (e.g., flue gas, biogas) containing CH_4, CO_2, and CO, into valuable products such as alcohol, fatty acids, and other chemicals (Diender et al., 2016; Jiang et al., 2021). Although C1 bioconversion is already used on an industrial scale (e.g., LanzaTech®), further advancements should focus on developing robust microbial consortia with ideal hosts that can efficiently utilize C1 compounds to achieve higher yields.

Waste streams, such as domestic wastewater, digested sludge, food waste, brewery waste, dairy waste, etc. are mostly biodegradable and can be repurposed into valuable resources by microbial consortia systems. Conversely, substrates such as lignocellulosic biomass are difficult to degrade due to their inherent recalcitrance and structural heterogeneity, posing a significant obstacle to their widespread utilization (Gilmore et al., 2019; Shrestha et al., 2024). Currently, enzymes derived from fungi are the primary catalysts for lignocellulose hydrolysis (Shrestha et al., 2024). However, enzyme production yields low quantities, resulting in high production costs. This significantly impacts the overall process economics, with enzyme cost making up 20–40% of the total operational expenses (Naresh Kumar et al., 2019). As opposed to a single strain with limited enzymatic machinery, employing microbial consortia comprising microbes with a diverse array of cellulases, hemicellulases, and ligninases can enable the simultaneous and efficient breakdown of cellulose, hemicellulose, and lignin, respectively (Lopes et al., 2018; Shrestha et al., 2024). Using the consolidated bioprocessing concept, Shahab et al. (2018) integrated enzymatic hydrolysis with microbial fermentation using a synthetic fungal-bacterial consortium, where *Trichoderma reesei* produced cellulolytic enzymes to break down microcrystalline cellulose and beech wood, while the lactic acid bacteria fermented sugars to lactic acid, leading to increase in lactic acid production by 62.4% compared with single engineered microbes. In summary, microbial consortia enabled bioprocesses have made significant progress towards providing attractive

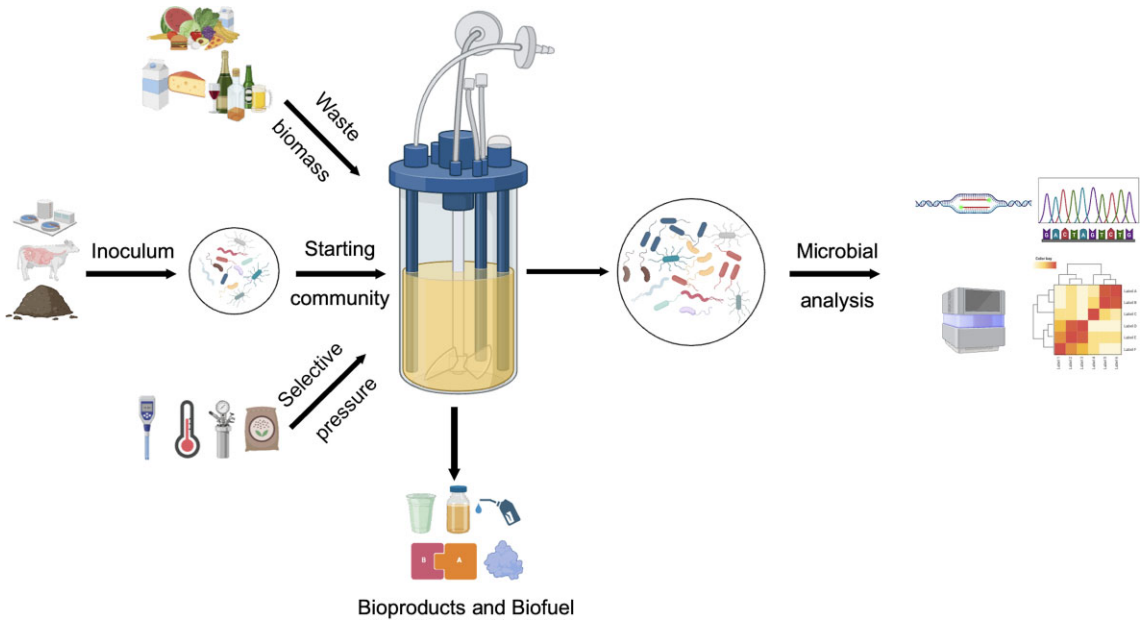

Fig. 1. The top-down design approach for bioconversion of diverse waste streams into value-added products. The inoculum can be derived from different natural and engineered microbial ecosystems such as wastewater treatment plants, rumen, compost, and soil. The mixed microbial consortia can be steered toward a targeted function by carefully manipulating environmental and operational parameters and the resulting microbial community can be characterized by various high-throughput molecular biology tools. Created with Biorender.com.

alternatives to petrochemical-based products, paving the way for resource recovery from waste biomass and a circular economy.

Top-down Design Approach to Engineer Naturally Occurring Microbial Consortia

The top-down approach is a classical approach that starts with selecting a suitable natural microbial community and providing selective pressure, for example, manipulating environmental conditions (pH, temperature), to shape the structure and function of the community toward the desired outcome (Fig. 1). The starting mixed microbial community used to seed a bioprocess is usually derived from a similar environment, thus requiring some basic understanding of the microbial ecology of the microbial community to guide the top-down design. For example, an adapted inoculum from an operational chain elongation bioreactor is suitable for starting a new MCCA-producing bioreactor as it shortens the start-up acclimation period. It uses ecological engineering approaches to reduce community complexity without prior knowledge about individual microbial populations, their interactions, or their functions (Lawson et al., 2019). By carefully manipulating the environmental conditions such as nutrients, organic loading rate, pH, oxygen levels, or temperature, the growth and activity of specific microbial populations within the community are encouraged, ultimately enriching the consortium with the most effective members (Lee et al., 2013; Cao et al., 2022). Microbial communities can be sourced from different natural and engineered microbial ecosystems including soil, wetlands, compost, herbivores gut, activated sludge, and anaerobic digesters. For instance, the rumen is an example of a natural microbial ecosystem that harbors diverse microbial populations (bacteria, methanogenic archaea, fungi, and protists), which can naturally break down recalcitrant lignocellulosic biomass (Hess et al., 2011; Gilmore et al., 2019; Liang et al., 2020). Several efforts have been made to harness rumen-derived mixed microbial communities or mimic such microbial ecosystems in engineered anaerobic systems, leveraging the physiochemical, and biological characteristics of rumen to achieve significant degradation of lignocellulose (Fonoll et al., 2021, 2024).

Enrichment is one of the most commonly used top-down approaches, involving repeated dilution cycles under controlled conditions to selectively promote the growth of microbial populations with desired functionalities over others for improved system output. This is often followed by the study of the mechanistic contributions of different microbial populations and optimization to enhance process efficiency (Lawson et al., 2019). For example, shifting an AD community from methane production—the most energetically favorable pathway under anaerobic conditions—to MCCA production via chain elongation requires inhibiting methanogenic archaea. Successful approaches to achieving this include maintaining low pH (Agler et al., 2014), adding chemical methanogenic inhibitors (Shrestha et al., 2023), decreasing the solids retention time to wash out slow-growing methanogens (Grootscholten et al., 2013), or heat-shock pretreatment of the inoculum (Cai et al., 2023). Other studies have also highlighted how altering operational conditions such as pH can affect diverse fermentative products including SCCAs product profile and concentration (Wang et al., 2014), lactate production during food waste fermentation (Rhee & Pack, 1980; Tang et al., 2017), and alcohol (De Souza Moraes et al., 2019).

Top-down microbiome engineering can decrease the system complexity as manipulating the environmental parameters can selectively retain the microbial populations that perform target processes, without requiring in-depth knowledge of individual microbial populations and all their cellular interactions (Chang et al., 2021). The diverse microbial populations in a mixed culture are integral for valorizing complex and heterogeneous biomass waste due to their broad metabolic activities. Furthermore, the functional redundancy in mixed microbial communities provides

resilience against external disturbances making it more stable compared to a monoculture system. However, the top-down approach still suffers from some limitations. First, there is less control over the initial microbial composition, and the presence or absence of certain microbial functions can significantly influence the outcome leading to unpredictable output (Arora et al., 2020). Therefore, the system cannot be optimized on a molecular scale. Second, the unknown microbial interactions and wide metabolic diversity can lead to undesirable competitive reactions thus lowering the product yield (Kucek et al., 2016; Shrestha et al., 2023). Lastly, microbial compositional analysis has been possible with advanced high-throughput sequencing technologies, however, unraveling the complexity and linking the taxonomic identity with function is still a challenge. Third, the mixed microbial consortia studies rely on the relative abundance metric during microbial characterization whereby less abundant but functionally significant microbial populations may be overlooked. A previous study on murine gut microbiome and their diet determined the absolute abundance of individual microbial taxa in the microbiome by combining the precision of digital PCR with high throughput 16S rRNA gene sequencing (Barlow et al., 2020). A similar approach can possibly be implemented in other complex microbial consortia studies. Lastly, mixed culture studies use correlation results between the dominant microbial population and the target function to identify the functional microbial populations (Carr et al., 2019). To address such limitations, some mixed culture studies have combined DNA-, RNA-, and protein-stable isotope probing with amplicon sequencing or shotgun sequencing to identify functional populations, *in-situ* interactions, and exchanges within complex populations involved in defined metabolic processes (Aoyagi et al., 2020; McDaniel et al., 2023; Poulsen et al., 2023). Bioorthogonal noncanonical amino acid tagging coupled with fluorescence-activated cell sorting is another recent approach to identifying functionally active populations in a complex consortium (Madill et al., 2021).

Bottom-up Design Approach to Develop Synthetic Microbial Consortia

Complementary to the top-down approach, the bottom-up microbiome engineering approach refers to assembling naturally occurring or engineered microorganisms with desired functions (Göpfrich et al., 2018; Massot et al., 2022) into well-defined synthetic microbial consortia. We use the term synthetic microbial consortia here to refer to consortia made up by assembling strains that may or may not coexist naturally. The genetic, metabolic, and physiological traits of the individual microorganisms [e.g., mutant strains (Mee et al., 2014) or environmental isolates (Friedman et al., 2017)] used to assemble such synthetic consortia are typically characterized. Beyond this, it also necessitates a comprehensive understanding of the possible interactions between potential consortia partners (Weisenberger & Deans, 2018; Hirschi et al., 2022). Synthetic biology and system biology have significantly enhanced traditional biological methodologies by enabling the mimicking and engineering of specific cellular traits. We discuss below approaches and tools to improve the bottom-up approach, mostly focusing on designing and assembling the microbial consortia, microbial consortia optimization via ecological engineering, and metabolic engineering.

Strategies for Rationally Designing and Assembling Synthetic Microbial Consortia

Designing and assembling synthetic microbial consortia for waste valorization involves strategically combining microbial strains to carry out complex tasks than individual strains can achieve alone. Various bottom-up approaches have been employed, including enrichment or community reduction (Lee et al., 2013), combinatorial evaluation (Kapoore et al., 2022), and computational model-based design (Haruta & Yamamoto, 2018; Ibrahim et al., 2021). Community reduction involves selectively enriching desirable microbial species while reducing the undesired ones, thereby enhancing the specificity and efficiency of the microbial consortia. It allows for the determination of species that should be retained based on their contributions (Eng & Borenstein, 2019). In combinatorial evaluation, well-characterized microbial species are selected and paired in different combinations to optimize biomass bioconversion (Fig. 2A). This method, although labor-intensive, enables the exploration of a wide range of species combinations, including engineered and native ones, to achieve a desirable outcome (Ravikrishnan et al., 2020). While pairwise combination and testing provide valuable insights into intercellular interactions, it can be a time-consuming process. To overcome this bottleneck, novel high-throughput culturing and phenotypic screening technologies have been developed (Kehe et al., 2019; Burmeister & Grünberger, 2020; Jiang et al., 2022). The kChip device was developed to enable the simultaneous assembly and testing of 10^5 pairwise strains, significantly increasing the efficiency of interaction analysis (Kehe et al., 2019). Lastly, in the model-guided design, genomic and metabolic data is used to simulate the interactions between microbes for designing a consortium thus reducing the reliance on the laborious trial-and-error wet lab-based methods (discussed in detail in Section "Applying Metabolic Modeling to Enhance the Bioproduction Capabilities of Microbial Consortia").

In the bottom-up strategy, the initial composition of a consortium is carefully designed. It is a modular process that allows for controlled manipulation of the initial composition by adding or removing specific species and examining how it affects the rest of the consortium (Sanchez-Gorostiaga et al., 2019). The abiotic (pH, temperature, nutrient) and biotic (inoculation ratio or the timing of inoculation) factors are optimized to assemble such consortium. The structure, stability, and interaction outcomes of these synthetic communities under different conditions can further be studied in detail by using various systems biology approaches (e.g., proteomics and metabolomics). For instance, metaproteomics is a powerful high-throughput approach to getting insights into community composition by quantifying the protein contribution of each member (Chen et al., 2023; Wang et al., 2023b). Additionally, exometabolomics can be applied to individual members and the entire consortium to profile their metabolic niches (e.g., metabolite production and depletion) (De Raad et al., 2022). This information can be used to predict potential interactions like resource competition or cross-feeding in the consortia (Wang et al., 2022).

Synthetic microbial consortia have been so far used for syngas waste and lignocellulosic biomass conversion (Shahab et al., 2020a; Diender et al., 2021; Cai et al., 2022). By leveraging the concept of metabolic division of labor (DOL), researchers can design a consortium to achieve specific waste valorization goals. In DOL, each member performs a specific task in a complex conversion process, reducing the metabolic burden by dividing the labor across the consortium members (Fig. 2B and C) (Roell et al., 2019).

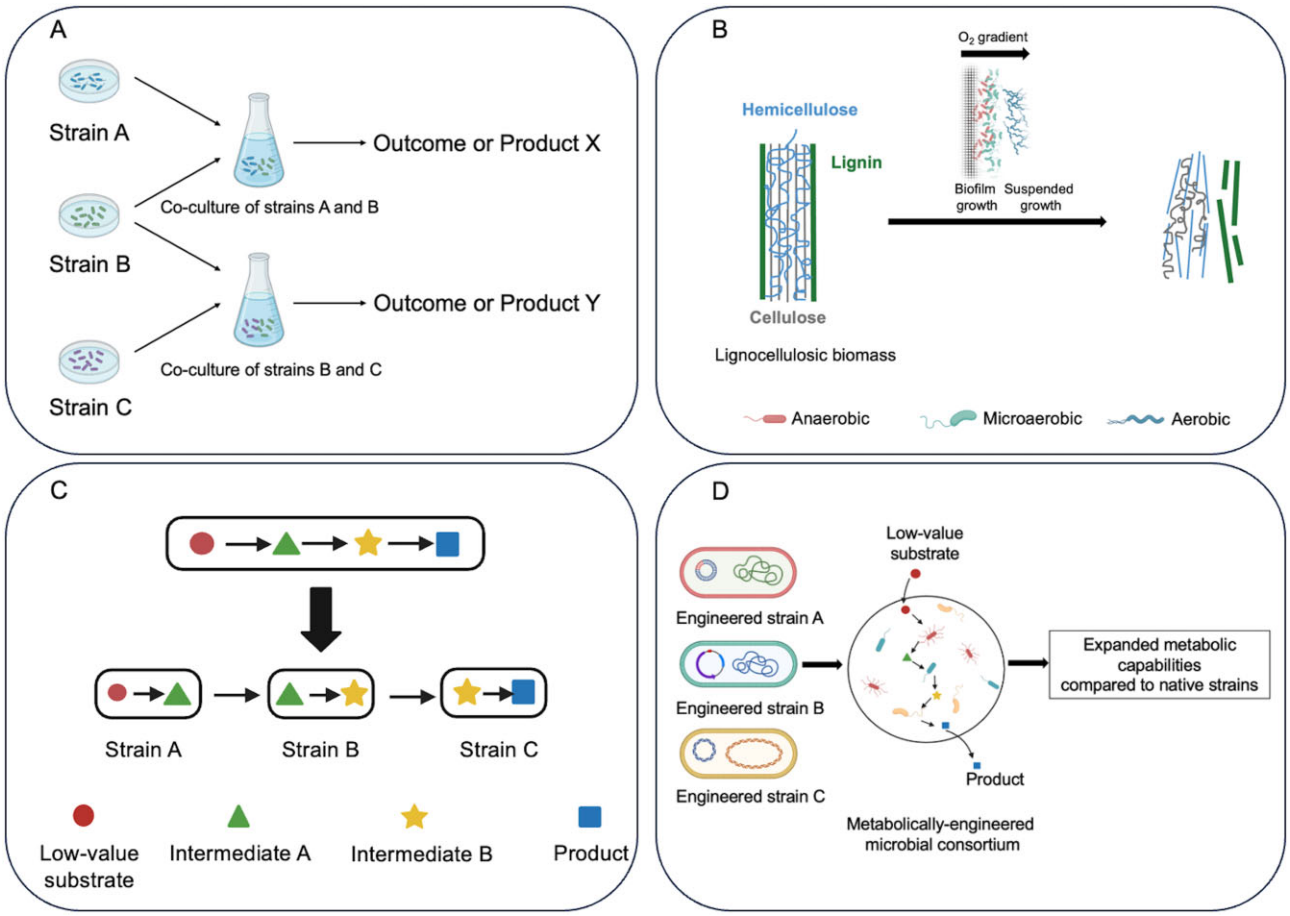

Fig. 2. Bottom-up approaches showing: (A) Assembly of well-characterized strains into different co-cultures depending on the target outcome; (B) leveraging biofilm-based systems to spatially organize strains occupying different niches to accomplish consolidated bioprocessing of lignocellulosic biomass; (C) promoting division of labor by dividing a complex metabolic pathway into multiple modules, in which the strains A, B, and C are assembled into a consortium; and (D) metabolic engineering to improve strain performance and hence the metabolic potential of the resulting consortium. Created with Biorender.com.

Competition between the consortium members can also be avoided by promoting metabolite cross-feeding via DOL and making them interdependent, a strategy helpful for maintaining stability. For example, simultaneous lignin depolymerization and upgrading is challenging to achieve in a monoculture system compared to microbial consortia, due to its complex structure and toxic lignin-derived compounds (Shrestha et al., 2024). A co-culture system consisting of *Sphingobium* and *Rhodococcus opacus* was able to effectively break down lignin via DOL, with each strain handling separate steps during conversion of lignin-derived dimers and aromatic monomers to *cis, cis*-muconate, and gallate (Cai et al., 2022). Another study successfully designed a three-member consortium of *T. reesei, Lactiplantibacillus pentosus,* and *Clostridium tyrobutyricum* for efficient conversion of lignocellulose-derived components through DOL. The hydrolytic enzymes from aerobic fungus deconstructed the cellulose into constituent sugars which were subsequently converted by the facultative anaerobic bacterium to lactic acid and finally fermented to valuable SCCAs by the anaerobic lactate-fermenting bacterium (Shahab et al., 2020a). Tsoi et al. (2018) developed a mathematical model to assess 24 metabolic pathways and analyzed the metabolic burden on host populations and the transport of enzymes, revealing the criteria for defining conditions favoring DOL. These examples highlight the capability of bottom-up design in achieving desired outcomes through DOL.

To improve the ability to predict community functions, it is critical to study interactions and how these interactions shape the dynamics and functions of the whole community. However, more commonly, the collective activity of a consortium may not be equal to the sum of individual members' activities. This is because members in a consortium engage in various interactions, such as mutualism, competition, commensalism, amensalism, predation, neutralism, and facilitation (Johnston et al., 2020; Duncker et al., 2021; Wang et al., 2022) that may give rise to emergent community-level functions that deviate from the summation of individual behaviors. For example, Sanchez-Gorostiaga et al. (2019) showed that a simple function (starch hydrolysis rate) of synthetic consortia composed of starch-degrading bacteria was not additive due to higher-order interactions when there were more species added to the consortia. Another study by Bell et al. (2005) that constructed several consortia with random combinations from 72 bacterial species showed that both community composition and synergistic interactions among the consortium members can significantly influence the function of the ecosystem.

Overall, the modularity of synthetic microbial consortia designed via the bottom-up strategy can be leveraged to enhance substrate utilization and diversify the product spectrum. Unlike the complex and multitude of interactions in mixed microbial consortia, the interspecies interactions in the synthetic microbial

consortia are much simplified avoiding undesirable side reactions. A better understanding of community interactions will further help link the composition to the overall activity of the community and stabilize a synthetic microbial consortium for the long term. Besides using model organisms, employing non-traditional yet naturally occurring microorganisms will help broaden the capability of synthetic consortia. The computational approach, such as artificial intelligence and machine learning, can help predict the optimal consortia composition and metabolic pathways based on known genomics, facilitating the design and assembly of microbial consortia and identifying the promising potential that could be difficult to predict manually.

Engineering the Ecology of Synthetic Microbial Consortia to Optimize Bioprocesses

Using synthetic microbial consortia in processes using unsterilized waste streams as feedstocks might be challenging. Competition with indigenous microbial communities present in the waste can impact the microbial community established in the existing bioprocess (Shrestha et al., 2022). This highlights the need to engineer the ecological stability of synthetic microbial consortia to build resilient, optimizable processes for long-term, stable, continuous waste biomass valorization. Here, ecological engineering of (synthetic) microbial consortia refers to interventions that alter the ecological interactions within the consortia to deliver improved process outcomes, for example, productivity, stability, etc. Consequently, the importance of introducing ecological engineering principles lies in balancing the relationship between environmental variation and the community's functional response to improve the performance of synthetic microbial consortia (Song et al., 2015).

Bio-augmentation—in which one or more defined organisms with functions of interest are added to a microbiome to improve or alter its functionality—can be seen as a bridge between top-down and bottom-up microbial consortium design (described in detail in Section "A Combined Top-down and Bottom-up Strategy for Engineering High-Performance Microbial Consortia") (Lawson et al., 2019; Liu et al., 2024). This approach has been proposed and applied for several decades, and, while successful in some cases, a common issue across contexts is the loss of the added organism over time (Mikesell & Boyd, 1988; Albright et al., 2022; Shan et al., 2023). Adding synthetic consortia with functional features of interest could potentially improve their retention, but rational rules for their design can be challenging to identify (Carr et al., 2023; Louie et al., 2023). Two lignocellulolytic microbial consortia, CL and YL, that were enriched from the rumen content of Tibetan yak and Holstein cow, respectively, were augmented with *Lactobacillus plantarum* (LP) to enhance the fermentation of alfalfa silage. The addition of these consortia to alfalfa silages improved lactic acid production, with the combined YLP (YL augmented with LP) showing the highest lactic acid content of 76.72 g/kg DM and conversion of water-soluble carbohydrates into lactic acid reaching 184.03% (Li et al., 2022). Although this approach has promise as an ecological engineering strategy, it often requires laborious identification of suitable organisms or consortia that remain in the system in the long term.

Taking inspiration from both natural and engineered environments, cell immobilization in self-formed biofilms or synthetic biofilm-like matrices may be a powerful alternative approach to engineer both ecology and stability in bottom-up synthetic microbial consortia (Fig. 2B). Biofilms are aggregates of microbes embedded in an extracellular biopolymeric matrix (Flemming et al., 2016). These structures provide a range of benefits, including protection from stressors in the bulk phase and spatial niche formation as a result of diffusion limitations (Flemming et al., 2016). These properties make biofilms interesting tools for biotechnological applications. For instance, granular biofilms are widely applied in complex microbial community-catalyzed environmental biotechnology, including in AD of wastes, nutrient removal from wastewater, and waste bioproduction technologies (Beun et al., 1999; Carvajal-Arroyo et al., 2019; Imajo et al., 2004; Mills et al., 2024). Critically, the ease of retaining these millimeter-scale, fast-settling, spherical biofilms allows for achieving higher biomass densities, and consequently improves process rates up to an order-of-magnitude compared to planktonic systems (Carvajal-Arroyo et al., 2019; Mills et al., 2024). Developing synthetic granular biofilm consortia could therefore potentially improve both process rates and ecological stability, key properties for real-world applications. To date, however, no such process exists. Recently developed pure culture granular biofilm approaches could provide a template to begin building functional synthetic granular biofilm consortia (Adav & Lee, 2008; An et al., 2021). Such work could be complemented with developments in understanding member-dependent aggregation processes in synthetic microbial consortia (Doloman et al., 2024). Alternatively, if none of the consortium members are (controllable) biofilm-formers, hydrogel-based synthetic biofilm matrices can provide a robust alternative. Hydrogels are water-retaining polymer structures with similar properties as natural (granular) biofilms, but offering a higher degree of control over physicochemical properties and consortium composition (Seviour et al., 2009; Johnston et al., 2020; Candry et al., 2022). Moreover, they are sufficiently robust for long-term application in bioprocesses (Li et al., 2023). Pairing these synthetic biofilms with biomass-valorizing synthetic microbial consortia could yield a new generation of bioprocess technologies.

Spatial niche formation is another opportunity to control functionality & ecological stability in synthetic microbial consortia biofilm-based bioprocesses (Shahab et al., 2020b). Spatial niches allow the pairing of organisms that could not coexist in well-mixed bioreactor systems. For instance, a synthetic consortium membrane biofilm reactor combined (i) an aerobic, lignocellulose-degrading fungus growing on an air-exchange membrane with (ii) anaerobic, fermentative bacteria in the anoxic bulk liquid phase of the bioreactor (Shahab et al., 2018, 2020a). Light, temperature, or other chemical gradients have also been proposed to engineer functional synthetic consortia (Shahab et al., 2020b). Alternatively, hydrogel-based synthetic biofilms can also sustain chemical gradients to combine chemically incompatible members. For instance, an aerobic N-cycling strain was paired with an anaerobic N-cycling complex community in hydrogel-based synthetic biofilms to remove nitrogen from real wastewater, with the aerobe consuming O_2 and creating an anoxic niche for the anaerobe (Gottshall et al., 2021; Li et al., 2023). Similar principles could be used to create novel biomass-valorizing synthetic consortia. How the multimodal interactions (i.e., cross-feeding and spatial niche formation) in such a consortium affect ecological stability remains to be tested experimentally. Overall, there are clear opportunities to leverage cell immobilization strategies to stabilize and optimize synthetic microbial consortia in the context of bioproduction from complex biomass.

Applying Metabolic Engineering Approaches to Manipulate and Expand the Functional Potential of Synthetic Microbial Consortia

Microorganisms in the native state may not be optimized to produce the target product at high titer, rate, and yield from the desired waste feedstock. Several synthetic biology techniques including genome editing, adaptive laboratory evolution (ALE), and engineered mutualism among others can be utilized to modify microbes and microbial consortia to improve their metabolism for optimal substrate valorization (Fig. 2D) (Lindemann et al., 2016). Furthermore, utilizing some complex wastes might require designing novel biosynthetic pathways. Existing information on enzymes, reactions, and the desired host organism can be used to design new pathways (Boob et al., 2024). Databases like BLAST (sequence alignment) can aid in identifying potential enzymes for desired reactions (Altschul et al., 1990). However, this knowledge-based approach relies on existing pathways, which limits the potential pathways that can be created. Retrobiosynthesis solves this issue by designing pathways backward from the available substrate and target molecule (Boob et al., 2024). This approach has been utilized to achieve de novo production of dencichine, a plant-derived chemical with pharmacological applications, from glycerol using *Escherichia coli* (Li et al., 2022). Recently, machine learning algorithms like RXN (Probst et al., 2022) and XGBoost (Schwander et al., 2016) are revolutionizing the identification and design of novel metabolic pathways and enzymatic reactions. These algorithms predict novel pathways and optimize existing ones (e.g., CETCH cycle) by analyzing sequence data without the reliance on known reactions (Singh et al., 2023). This paves the way for engineering microbes to utilize waste feedstocks through entirely new pathways (Aggarwal et al., 2023).

Engineering a single strain with complex biosynthetic pathways can put a metabolic burden and cause poor growth and low product yield. By artificially engineering a consortium, it is possible to spatially segregate a metabolic pathway into modules, reducing the metabolic burden on one species as well as taking advantage of their high robustness and resilience against environmental fluctuations (Zhou et al., 2015). For example, Li et al. (2019) developed a three-strain co-culture of *E. coli* to produce rosmarinic acid, via a diverging-converging pathway that is difficult to balance in one species, dividing the pathway into modules optimized for each strain. This approach yielded 172 mg/L of rosmarinic acid, a 38-fold increase over monoculture. Metabolite cross-feeding can be used to improve consortium stability, where one member consumes an intermediate toxic to the other consortium member. For example, Cha et al. (2021) applied this approach to a co-culture consisting of wild-type and engineered strains for C1 (carbon monoxide) conversion that is limited in monoculture due to CO toxicity and the accumulation of inhibitory byproducts. The engineered *E. coli* utilized acetate, a byproduct of CO metabolism toxic to *Eubacterium limosum*, consequently leading to upcycling of CO to itaconic acid. Similarly, in a consortium designed for the degradation of model lignocellulosic components, the breakdown of vanillic acid by *Pseudomonas putida* produced formaldehyde at concentrations that inhibited the growth of other species (Lee et al., 2021). An engineered *Methylorubrum extorquens* was introduced into the consortia to consume the formaldehyde, decreasing the formaldehyde levels to below the minimum inhibitory concentration. While much effort has been focused on the conversion of model compounds, similar metabolic engineering approaches could be expanded to diverse waste biomass.

As detailed in Table 2, metabolic engineering strategies like ALE is used to cultivate microorganism or microbial consortia under controlled laboratory conditions for extended periods with specific selective pressures or environmental stresses. This allows the microbes to adapt and evolve new traits beneficial for survival and growth in that particular environment (Aggarwal et al., 2023). An algal-bacteria consortium was adapted to grow on landfill leachate, which otherwise is inhibitory to the algal growth, leading to more than doubling growth of the dominant *Chlorella vulgaris* (Okurowska et al., 2021). Metagenomic analyses revealed that the adapted microbiome had an increased total organic carbon degradation vs., the non-adapted consortia. Similarly, ALE can be applied to adapt a consortium to toxic lignin degradation products such as aromatics and phenolics that can otherwise result in low growth and product titers during lignin bioconversion. Therefore, ALE may be a valuable tool to improve growth in the presence of an inhibitory compound, community interactions between species in a consortium, increasing the efficiency of a construction crew of different microbes, breaking down and building up different molecules to convert waste to a desired product (Kawai et al., 2022).

Signaling molecules involved in quorum sensing can act as switches to manipulate gene transcription and control population size (Brophy & Voigt, 2014; Diao et al., 2021). In a consortium, signaling molecules can be exchanged between members where one species reacts to the action of another. Chen et al. (2015) engineered two *E. coli* strains to produce and respond to intercellular signaling molecules, which coupled positive and negative feedback loops to control their population densities. An activator strain produced a signaling molecule that increased the transcription of key genes in both strains, while a repressor strain produced a different molecule that would slow gene expression. Combining quorum sensing with a population control circuit resulted in approximately 30 times more control compared to monoculture, with the consortia producing around 0.15 g/L of tyrosine (VanArsdale et al., 2022). Complex "biocomputing circuits" to control communication via signaling molecules between different species in a consortium are achievable, opening doors to control gene expression and increase robustness against fluctuating environmental conditions or varying composition of waste feedstocks (Du et al., 2020). Additionally, *in situ* engineering offers exciting possibilities for biomanufacturing eliminating the need for laboratory domestication. However, there is a lack of genetic tools available for genetic modification within a consortium. Genome editing techniques are emerging to selectively edit closely related strains in a complex consortium (Rubin et al., 2021) which provides a possibility to engineer other complex microbial communities for waste valorization. Lastly, as most metabolic engineering studies use simple model compounds, further investigation utilizing real waste is needed to fully leverage the potential of metabolic engineering.

A Combined Top-down and Bottom-up Strategy for Engineering High-Performance Microbial Consortia

Microbial consortia can be constructed via a top-down or a bottom-up approach as discussed above or by a combination of both approaches. The top-down approach is particularly valuable when a naturally occurring microbial community with the desired functionality is readily available. This approach is relatively straightforward as it utilizes naturally occurring microbial

Table 2. Microbial consortia engineering using metabolic engineering strategies to improve bioprocesses

Microbial consortia	Substrate	Metabolic engineering strategies/Improvement/mutation	Outcome	Reference
Algal-bacterial consortium (Chlorella Vulgaris -Pseudomonas)	Landfill Leachate	Blocking of lactate, ethanol, and acetate synthesis pathways and introducing *Lactococcus lactis* pyruvate carboxylase to drive succinate generation via adaptive laboratory evolution.	The adapted consortium showed a 2.9-fold increase in the growth rate of *C. vulgaris* compared with the original algal-bacterial consortium before the adaptation, nitrate production reduced by 97.6%, and 93.66% of total organic carbon was degraded.	(Okurowska et al., 2021)
Pseudomonas putida KT2440, Bacillus coagulans NL01	Corn stover hydrolysate	Deletion of glucose dehydrogenase (*gcd*) and glucose transporter (*gts*ABCD) genes in *P. putida* to block sugar metabolism.	*Pseudomonas putida* metabolized and removed various inhibitors (e.g. 100% conversion of furan aldehydes, ~90% removal of most monoaromatic compounds). *Bacillus coagulans* fermented the detoxified hydrolysate, yielding 35.8 g/L lactic acid.	(Zou et al., 2021)
Pseudomonas putida KTΔABZF (p2-a-J) and Escherichia coli Δ4D (ACP-SCLAC)	Lignocellulosic hydrolysate	Overexpression of *acs* and *phaJ* genes; knockout *phaZ, yqeF, paaJ,* and *tct*A genes and expression of *SCLAC* gene using T3 and Tac promoters.	The engineered consortium achieved a maximum titer of 3.98 g/L mcl-PHA using glucose and octanoic acid, and 1.02 g/L mcl-PHA using lignocellulosic hydrolysate.	(Qin et al., 2022)
Engineered strains of *Escherichia coli* and *Bacillus subtilis*	Watermelon rinds and okara (soybean waste)	Expression of *alsS, kivD, ilvC, ilvD,* and *yqhD* genes in *E. coli* and *leuDH, kivD,* and *yqhD* genes in *B. subtilis*; optimization of *E. coli* to *B. subtilis* ratio.	Highest overall biofuel production of 1.1 g/L with 80% being isobutanol using a 4:1 ratio of *E. coli* to *B. subtilis* when carbohydrate levels were high.	(Kim et al., 2020)
Clostridium cellulovorans and Clostridium beijerinckii.	Alkali-extracted, deshelled corn cobs (AECC)	In *C. cellulovorans*, *ack* and *ldh* were knocked out, *buk* was overexpressed, and *hyd* was down-regulated using CRISPRi. In *C. beijerinckii*, *ctfAB* was engineered for organic acids reassimilation, and *xylR* and *xylT* for pentose utilization.	The engineered consortia decomposed 83.2 g/L of AECC and produced 22.1 g/L of solvents, including acetone, butanol, and ethanol, approximating the titer achieved from starchy feedstock.	(Wen et al., 2017)
E. coli DH1 ΔadhE, E. coli MG1655, E. coli MG1655 ΔfadE	Switch grass	Heterologous expression of cellulase, xylanase, beta-glucosidase, and xylobiosidase for biomass hydrolysis; integration of synthetic pathways for gasoline, diesel, and jet fuel substitutes.	Achieved 0.5 mg/L extracellular enzyme activity for xylanase and cellulase, 8% total sugar hydrolysis from IL-treated switchgrass, and biofuel yields at 80% of theoretical yield from estimated sugar release.	(Bokinsky et al., 2011)

communities, but it offers less control over the final composition. This may limit applications requiring defined microbial populations or understanding individual member contributions (Cao et al., 2022). The bottom-up approach starts by carefully characterizing and selecting individual microbes with desired traits and well-studied metabolic pathways. These chosen microbes are then combined and tested for their ability to function together. However, a large proportion of microbes remain unculturable with current methods which restricts strain selection for bottom-up approaches (Bodor et al., 2020). Another challenge in the bottom-up approach is the unpredictable interactions that occur within the consortium which makes it necessary to consider factors like interspecies communication, metabolite exchange, and potential competition for resources when designing a consortium, some of which were discussed in Section "Bottom-up Design Approach to Develop Synthetic Microbial Consortia".

While both the top-down and bottom-up approaches have limitations, integrating them offers a promising way to overcome some of these constraints. By synergistically harnessing the strengths of both methodologies, novel solutions for biomass valorization can be achieved. One such approach, exemplified by the work of Díaz-García et al. (2021), utilized a sequential combination of dilution-to-stimulation (i.e., enrichment) and dilution-to-extinction techniques to enrich a minimal and efficient lignocellulolytic consortium from soil microbiome. This approach progressively enriched key lignocellulose degraders

while eliminating less efficient members, ultimately resulting in a two-species consortium (*Pseudomonas* sp. and *Paenibacillus* sp.) capable of efficient lignocellulose breakdown. Top-down (domestication of activated sludge) and bottom-up (self-assembly with *Psychrobacter aquimaris*) approaches were integrated to create an ammonium-assimilating microbiome for wastewater treatment (Zhang et al., 2021). While domestication failed due to salinity-induced competition and disaggregation, the self-assembly approach yielded success. By co-culturing *P. aquimaris* with environmental microbial population, they enriched for efficient ammonium-assimilating bacteria, achieving 88.4% nitrogen removal. Combining top-down and bottom-up approaches in microbial consortia design presents an important strategy that can expedite development compared with using these methods individually (Lin, 2022). This integrated approach allows researchers to utilize existing, functional communities identified through top-down enrichment as a starting point, reducing the time required to identify potential functional strains. Moreover, it provides more precise control over the final consortium composition compared to relying solely on top-down methods (Khandelwal et al., 2013). By introducing specifically selected microbes, researchers can adjust functionalities and potentially uncover novel metabolic pathways within the consortia. However, the combinatorial strategy also poses challenges. Understanding the intricate interactions between the natural microbial community and synthetically assembled microbes is complex and can affect the overall stability and function of the consortium (Jiang et al., 2023). Further research is necessary to optimize selective pressure during enrichment to maintain the desired community structure, function, and long-term stability while adding new members. Advanced techniques such as metagenomics and metabolomics combined with computational modeling can provide deeper insights into these complex interactions. Future research directions could explore the integration of machine learning algorithms to predict and optimize consortia design based on desired functionalities.

Applying Metabolic Modeling to Enhance the Bioproduction Capabilities of Microbial Consortia

Metabolic modeling can be used to get insight into the microbial interactions, predict and optimize the titer, rate, and yield, and guide rational design and assembly of novel synthetic microbial consortia. Genome-scale metabolic models, also referred to as GEMs and M-models, are network-based representations of metabolism in which nodes are metabolites and edges are reactions that link the metabolites (Passi et al., 2021). M-models are knowledge bases of the available genetic and biochemical information for the target organism that enables linking the genotype with the phenotype in a high-throughput manner. For example, M-models of microorganisms with lignocellulose degradation capacities have been used to explore metabolic bottlenecks (Balagurunathan et al., 2012), to propose metabolic engineering strategies for biosynthesis from cellulose (Gonzalez & Antoniewicz, 2017), to hypothesize cross-feeding interactions (Lee et al., 2021), and to contextualize multi-omics data (Kundu et al., 2019; Kim et al., 2021), resulting in deeper insights into pathways and interactions for lignocellulosic biomass valorization.

M-models can be used to study microbial communities in graph-based and Flux Balance Analysis (FBA) frameworks. Graph-based methods have been classified as reverse ecology that enables the study of evolutionary traits (Borenstein et al., 2008; Janga & Babu, 2008; Soyer, 2012). These methods use hundreds of M-models to unravel topological information encoded in a large stoichiometric matrix that identifies potential interactions within a microbial community (Zelezniak et al., 2015; Ravikrishnan et al., 2018, 2020). On the other hand, FBA uses a stoichiometric matrix to define a linear programming problem subject to mass balance and thermodynamic constraints. To simulate FBA-based interactions, M-models are combined into community models (CM-models) by creating a shared metabolite compartment to represent the metabolic exchange capabilities of each community member, as well as the extracellular space shared by all members (Khandelwal et al., 2013; Fang et al., 2020; Passi et al., 2021).

A series of manually curated CM-models showed that some partnerships enabled different titers in bioproduction capabilities. For example, CM-models guided strain selection by pairing the phototroph and sucrose-secreting *Synechococcus elongatus* with heterotrophic production chassis such as *Bacillus subtilis*, *E. coli* K-12, *E. coli* W, or *Yarrowia lipolytica*. Model simulations showed that only partnerships containing *B. subtilis* and *E. coli* K-12 could produce butanol, ethanol, formaldehyde, methanol, and succinate. Microbial interactions were validated using targeted metabolomics and RNA sequencing (Zuñiga et al., 2020). The accuracy of CM-model simulations heavily relies on the quality of M-models and available experimental metabolite transport capabilities (Zuniga et al., 2021). However, draft reconstructions can be automatically generated (Karlsen et al., 2018; Machado et al., 2018; Wang et al., 2018; Heinken et al., 2021; Seaver et al., 2021; Zimmermann et al., 2021; Karp et al., 2022; Jenior et al., 2023), accelerating the model reconstruction which often reduces the accuracy of the predictions (Bernstein et al., 2021; Scott et al., 2023; Joseph et al., 2024).

Despite limitations, recent draft reconstruction tools are consistently used to study metabolic interactions in microbial consortia with up to hundreds of members (Levy & Borenstein, 2013; Henry et al., 2016; Machado et al., 2021; De Bernardini et al., 2022; Heinken et al., 2023; Zampieri et al., 2023). Remarkable progress has been made in the development of algorithms to account for the growth maximization of both consortia and individual members. For example, OptCom (Zomorrodi & Maranas, 2012), d-OptCom (Zomorrodi et al., 2014), and NEcom (Cai et al., 2021) allow us to predict the growth rate of several organisms at the same time and in some cases their resource allocation (Kim et al., 2022). Omics- and growth constraint-based approaches such as MICOM and SteadyCom predict trade-offs of cooperative interactions by constraining relative abundances within the consortia (Diener et al., 2020). This approach reduces the simulation of meaningless FBA due to the use of automatically generated models, in which non-growing members can contribute to the production of metabolites in the shared extracellular environment.

Overall, genome-scale metabolic modeling holds great potential to understand complex microbial consortia for biomass valorization. For example, in the case of the synthetic consortia consisting of *P. putida*, a lignocellulose degrader; *Cellulomonas fimi*, a cellulose degrader; *Y. lipolytica*, an oleaginous yeast, and *M. extorquens*, a methylotroph capable of using formaldehyde (Lee et al., 2021). Thus, for a successful understanding and modulation of metabolism, it is critical to expand the metabolic modeling tools to be specifically responsive to microbial consortia designed for waste valorization.

Conclusions

There have been unprecedented efforts to harness microbial consortia to develop several biotechnologies for biomanufacturing. This review specifically highlights the potential of microbial consortia and discusses the top-down and bottom-up engineering design approaches including the challenges and future recommendations. The top-down strategy still remains the most commonly used approach for waste valorization due to its relative ease of implementation. The bottom-up approach holds promise, but successful implementation will require developing techniques for stable consortia assembly, process optimization using ecological principles, and metabolic engineering to develop stable synthetic microbial consortia. There has been significant progress in engineering individual strains for biomanufacturing, however, less focus has been placed on consortium engineering. While models exist for single microbial metabolism, there is a need for models that can predict and identify the metabolic features governing interactions and long-term stability within microbial consortia. Besides focusing on the technical advancements, the economics should also be considered as more resources are needed for developing synthetic microbial consortia using the bottom-up approach compared to the traditional top-down approach. For instance, it might be economically beneficial to invest in developing and improving synthetic consortia for bioprocesses that produce high-value specialty chemicals. Future research should also explore integrating undefined natural microbial consortium (top-down) with defined synthetic consortium (bottom-up) for accomplishing efficient conversion of complex waste biomass. An interdisciplinary approach of combining bioprocess engineering, multi-omics analysis, metabolic engineering, system biology, and metabolic modeling is needed for a deeper understanding and optimization of microbial consortia-driven biomanufacturing for a sustainable future.

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Conflict of interest

The authors declare no competing commercial or financial interests.

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