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PERSPECTIVE

Biomolecular Engineering

An argument for using anaerobes as microbial cell factories to advance synthetic biology and biomanufacturing

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Abstract

Anaerobes thrive in the absence of oxygen and are an untapped reservoir of biotechnological potential. Therefore, bioprospecting efforts focused on anaerobic microbial diversity could rapidly uncover new enzymes, pathways, and chassis organisms to drive biotechnology innovation. Despite their potential utility, anaerobic fermenters are viewed as inefficient from a biochemical perspective because their metabolisms produce fewer ATP (∼2) per molecule of glucose processed than heterotrophic respirers (∼32–38 ATP). While aerobes excel at ATP generation, they are often less efficient than anaerobes at processes that compete with ATP generation for cellular resources. This perspective highlights how anaerobic adaptations are advantageous for synthetic biology and biomanufacturing applications through the engineering of microbial cell factories. We further highlight emerging applications of anaerobic bioprocessing, including the use of anaerobic metabolisms for lignocellulosic bioprocessing, human and environmental health, and value-added bioproduction.

KEYWORDS
anaerobe, cellular engineering, metabolic engineering, microbial cell factory, microbial consortia, synthetic biology

1 | INTRODUCTION

For several decades, biotechnology and biomanufacturing efforts have focused on engineering and adapting model microbes that are fast-growing, easily cultivable, genetically tractable, and well-characterized. Common model chassis organisms *Escherichia coli* and *Saccharomyces cerevisiae* are natural choices for constructing cell circuits,[1,2] biosensors,[3,4] and engineering metabolic pathways for bio-based production.[5,6] As proposed biotechnological applications expand, other microbes and microbial communities that have not yet been adopted for bioproduction will serve as sources for specialized biosynthetic tools or serve as chassis systems themselves. For example, *Pseudomonas putida* is a prime example of a formerly non-model organism

Abbreviations: ATP, adenosine triphosphate; ETC, electron transport chain; TCA, tricarboxylic acid.

domesticated to carry out redox-intensive chemistry because of its native propensity for producing reducing cofactors.[7] The progression of organisms like *P. putida* or *Clostridium autoethanogenum* from non-model bacteria to synthetic biology and biomanufacturing chassis inspires and motivates the discovery and utilization of other valuable organisms, enzymes, and pathways from untapped microbes.[8]

Discovery, isolation, and domestication of new non-model organisms, enzymes, and pathways are extremely valuable, but mining anaerobic diversity—specifically microbes and microbial communities that thrive without oxygen—for biomanufacturing has been largely overlooked.[9,10] For example, anaerobes are often regarded as slow-growing, inefficient, and therefore perceived as not useful for bio-based production. This review challenges the traditional notion of anaerobes as metabolically inefficient, highlighting their attractive features, which strongly contrast with aerobic adaptations.[11–13] It argues that overlooking anaerobic diversity in biomanufacturing is a missed opportunity. Anaerobes inspire new synthetic biology strategies by demonstrating cost-saving adaptations, effective workload distribution, novel enzymes and metabolic pathways, and an array of unexplored secondary metabolites (Figure 1).

This perspective explores how microbial cell factories strategically invest in different metabolic processes, and it also introduces conceptual tools in cellular energetic costs to enrich discussions on cell efficiency. We compare and contrast anaerobic adaptations to glucose metabolism with those of model organisms before exploring anaerobic metabolic diversity as a tool to enhance cell factories' efficiency. Finally, we highlight the relevance of anaerobic metabolisms for biotechnology applications and discuss how to optimize metabolic configurations using Pareto optimality and by taking advantage of anaerobic metabolism and syntrophy. Anaerobic microbial diversity is vast, and synthetic biology applications like biorefining, synthesis of new-to-nature compounds, bioremediation, and bioproduction would benefit from further description and exploitation of anaerobic metabolism.

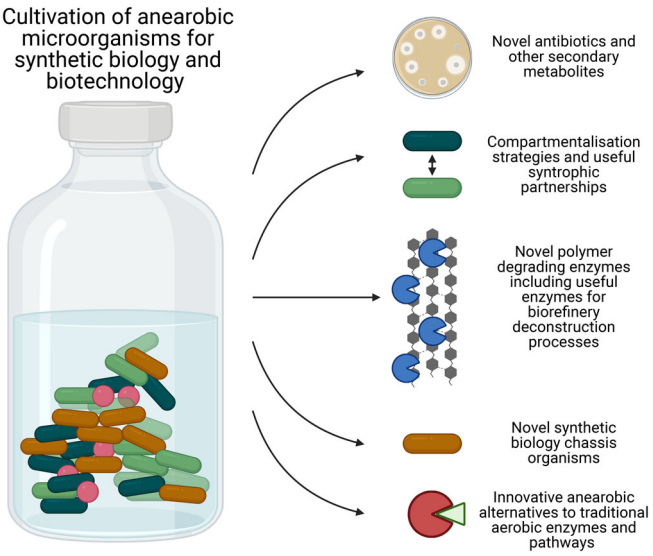

FIGURE 1 Anaerobic adaptations offer innovative solutions and tools for synthetic biology and microbial cell factories. Anaerobes produce novel antibiotics, chemical pathways, and enzymes that can be repurposed for biotechnology and bioproduction. Anaerobic chassis organisms offer advantages over traditional aerobic chassis in some cases, and anaerobes have a natural propensity to demonstrate compartmentalized metabolism, which can facilitate complex chemical transformations.

2 | CELL FACTORIES MUST BALANCE CATABOLISM AND ANABOLISM TO ENHANCE EFFICIENCY

Microbial cell factories carry out chemical transformations driven by their metabolism to produce target biomolecules, and engineering them can play a significant role in advancing biomanufacturing. Recent efforts have engineered traditional microbial platforms to benefit from some of the adaptations preferred by anaerobes.[14,15] As an example, *E. coli*, a fast-growing bacterium for which a panoply of engineering tools is already available, has been engineered to produce microbial butanol by expressing synthetic genes from *Clostridium acetobutylicum*, a feature typically found only in members of the *Clostridium* genus.[16] Here, we frame a discussion around the major factors that influence the efficiency of microbial bioproduction, where we focus on optimizing the production of a target biomolecule.

Due to the complex nature of biological cells, maximum efficiency in cell factories is a state that requires cell populations to survive as well as produce a target molecule. The combined costs and constraints of energy conservation (i.e., catabolism) and cellular component generation (i.e., anabolism) limit desired target biomolecule accumulation (i.e., secondary or recombinant metabolic processes) by cells. Therefore, an efficient cell factory will appropriately invest in catabolism and anabolism to maximize the production of the target molecule. Anaerobic microbes configure catabolism, anabolism, and secondary metabolic processes in different ways than aerobes, and learning from anaerobic adaptations will inform synthetic biology design schemes for efficient cell factories.

Microbial cell factories benefit from balancing cellular processes to maximize the yield of a target biomolecule. Much emphasis has been placed on how catabolism relates to cellular efficiency, and we first focus on the implications of catabolic tradeoffs for cell factory efficiency. We coin the term "volumetric cost" to describe the three-dimensional spatial demands that a biochemical pathway imposes on a cell, both in the cytoplasm and in cell membranes. In microbial cell factories (and all cells), the molecular costs of synthesizing biomolecules (protein, lipids, and cofactors) that facilitate catabolism and the volumetric cost incurred by these molecules are inseparable from the energy conserved per glucose equivalent.[17,18] Therefore, the number of ATP generated per molecule of glucose under-represents the input required, as proteins and other biomolecules necessary for energy conservation also consume resources. Investment in any specific metabolic pathway is therefore an opportunity cost decision for a cell since the returns from coding, expressing, and functionalizing biochemical pathways must provide returns that are worth the resources expended. This continued discussion refers to the "cellular cost" as the combined volumetric and molecular costs needed to operate a cell factory. Any metabolic pathway incurs complex cellular costs since each macromolecule differentially taxes cellular capacity and uses variable amounts of available molecular building blocks.

A detailed evaluation of cellular costs is particularly relevant for glycolysis since various glycolytic pathways evolved different costs and ATP yields. Direct comparison of these glycolytic strategies could inform the rational design of efficient microbial cell factories.[17,18] Glycolysis is the first set of reactions by which enzymes break down glucose into three-carbon intermediates, and different glycolytic routes contrast with one another. The two most studied glycolytic pathways are the Embden–Myerhoff–Parnas (EMP) pathway and the Entner–Doudoroff (ED) pathway. These glycolytic routes are also the most commonly encoded by microbial genomes.[17] The EMP pathway is frequently employed by anaerobic microorganisms and yields a net of 2 ATP but requires a 3.5–5-fold increased allocation of cellular resources compared to the ED pathway.[17] The ED pathway yields only 1 ATP per glucose at a lower cost and enables rapid glucose processing due to increased thermodynamic favorability.[17,18]

Microbes have evolved those pathways based on their environments' demands, and these adaptations can inspire efficient cell factories. For many strict fermentative anaerobes, glycolysis represents their only energy conservation mechanism, and therefore they exhibit a bias towards the EMP pathway. Large fractions of aerobes and facultative anaerobes encode both EMP and ED (24% and 19%, respectively) or solely the ED pathway (21% and 10%, respectively)[17] due to its lower cost, greater thermodynamic favorability, and a lower burden on cellular volumetric capacity.[17–19] These cost-saving tradeoffs extend beyond glycolysis and have been demonstrated for certain amino acid biosynthetic pathways (e.g., isoleucine), indicating the importance of thermodynamics and material cost-saving in other aspects of metabolism. Anaerobic adaptations may therefore be preferred when a cell factory does not have high ATP requirements since cost-saving and glycolytic rate can both be increased by relying on the ED pathway.

The variable utility of catabolic end products is another factor that differentiates metabolic strategies for glucose processing and is critical to the design of efficient cell factories. Aerobic respiration drives the production of ATP through oxidation, and CO_2 is a major waste end product of aerobic respiration that must be re-converted into a usable form before it can be incorporated into new biomolecules. In contrast, pyruvate, the end product of glycolysis, directly contributes to the synthesis of amino acids, lipids, cofactors, and other biomolecules essential to cell function. Directly feeding pyruvate and other small carbon molecules, collectively named carbon skeletons in this manuscript, into anabolic processes could be particularly advantageous for maximizing efficiency in cell factories. In fact, even in the presence of oxygen, some cells process carbon equivalents through fermentative metabolism. During the rapid growth of aerobic organisms capable of complete glucose oxidation via the TCA cycle, many glucose equivalents are only partially oxidized even in the presence of a stoichiometric excess of oxygen.[20–24] Aerobic fermentation occurs in bacterial cells (overflow metabolism), yeast (the Crabtree effect), and human cancer cells (the Warburg effect) and disrupts the assumption that oxygen availability primarily governs cellular choice between respiration and fermentation.[11,25] These repeated observations in diverse biological systems allude to cellular metabolism's multifaceted nature and the need for organisms, including cell factories, to balance both catabolism and anabolism.[17–26] In cell factories specifically, artificial overinvestment in secondary metabolic processes for target biomolecule accumulation suggests that resource costs of anabolism and catabolism should be minimized to the extent possible without impacting cellular function and target biomolecule accumulation.

3 | THE BICYCLE ANALOGY: ANAEROBES "PEDAL" IN LOW GEAR, INCURRING LOWER COST AND RESERVING USEFUL CARBON

The analogy of a bicycle crank turning its rear cassette helps visualize differences between glucose processing ending with glycolysis versus complete oxidation of glucose for maximal ATP yield. In this analogy, the bicycle crank represents the processing of glucose while the rear cassette represents ATP production. One turn of the bicycle pedal and crank (gray rotational arrows, Figure 2) is equivalent to processing one molecule of glucose through cell factory catabolism, and one full rotation of the rear cassette (white rotational arrows, Figure 2) is equivalent to generating two molecules of ATP. The bicycle pedaled in high gear allows for 16 rotations of the rear cassette per turn of the crank, yielding 32 ATP (Figure 2A) analogous to respiratory catabolism. Conversely, in low gear, one turn of the crank is equal to only one rotation of the rear cassette, yielding 2 ATP (Figure 2B) analogous to glycolysis. Glycolytic metabolisms that do not wholly oxidize glucose (Figure 2B) must, therefore, turn the crank 16 more times to obtain the same amount of ATP as an organism employing respiratory catabolism (Figure 2A). So far, the analogy only represents variable ATP yield and does not address the potential benefits of incomplete glucose oxidation.

When a microbial cell factory stops the oxidation of glucose equivalents at pyruvate, it both reserves carbon skeletons for anabolism and incurs a lower cellular resource cost than respiratory catabolism. In Figure 2B, the cassette and crank gears are hollow to represent the reduced cost of synthesis and lower spatial cost achieved by reliance on partial glucose oxidation for ATP acquisition. Additionally, a reserve of carbon skeletons is not available if the TCA cycle fully oxidizes glucose. These additions to the bicycle analogy draw attention to the inherent value of directing carbon away from complete respiration. Producing ATP is only one useful output of glucose processing, and ignoring other valuable outputs and variable inputs misrepresents cellular efficiency. Cost-saving features of fermentative phenotypes ultimately suggest anabolic efficiency contrasts with the catabolic efficiency of glucose respirers. While these examples illustrate canonical respiration and fermentation, Figure 2 does not depict phenotypes with investments and ATP yields intermediate to these two archetypes. Non-canonical catabolisms that conserve energy with combined electron transport chains (ETC) and fermentation are particularly interesting and are discussed further.

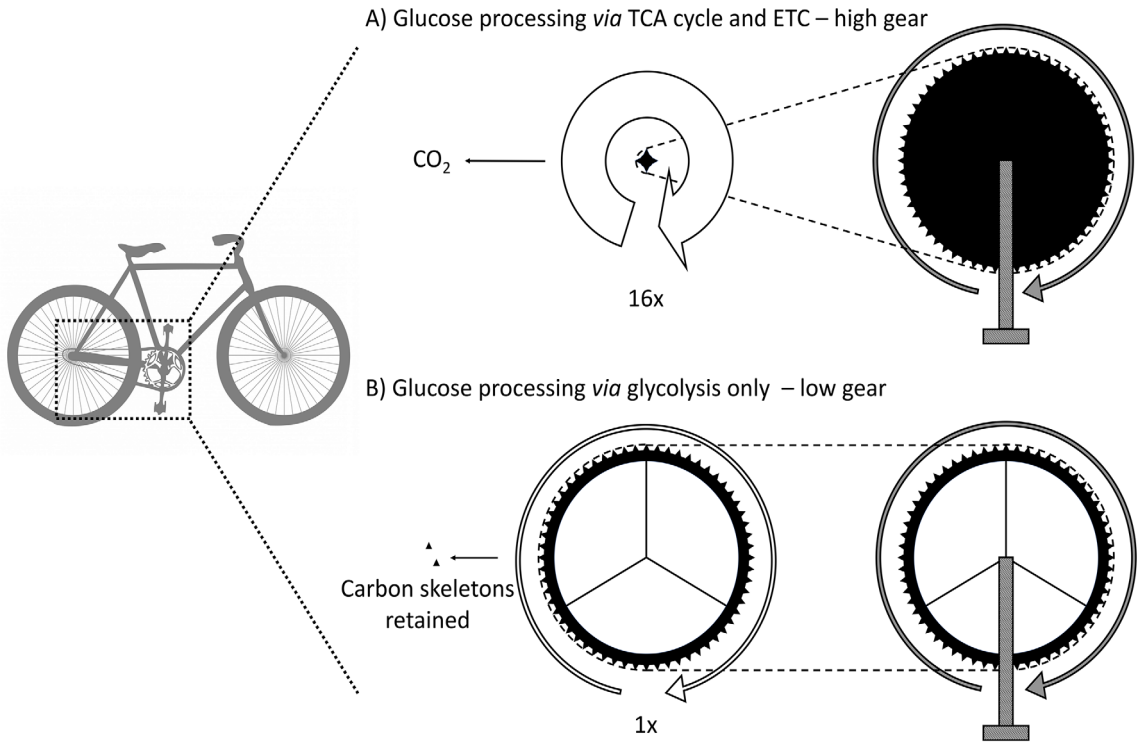

FIGURE 2 The bicycle analogy for cellular processing of glucose equivalents demonstrates contrasting fermentative and respiratory metabolic advantages. In the bicycle analogy, one 360 degrees rotation of the crank (gray circular arrows) represents processing one glucose molecule through cellular metabolism. Catabolic ATP yield is represented by one full rotation of the cassette (white circular arrows). Completely oxidizing a glucose molecule through TCA and coupling this process to ETC (Panel A) yields 16 times more ATP than processing the same glucose molecule through fermentative metabolism (Panel B). The benefits of relying on only glycolysis for energy generation are twofold. First, cells can repurpose incompletely oxidized carbon from fermentation as carbon skeletons to build cellular components (Panel B). Second, the cellular volumetric costs associated with fermentative metabolism are less and are represented by the hollow gears in Panel (B) versus the solid gears in Panel (A). Hollow gears require fewer carbon skeletons to create and occupy less cellular volume than solid gears.

4 | ANAEROBIC DIVERSITY IS A SOURCE OF INSPIRATION FOR NONSTANDARD METABOLIC CONFIGURATIONS

Anaerobes exhibit a large diversity of metabolic strategies, making them excellent models to enhance cell factories' efficiency. These organisms balance anabolism, catabolism, and secondary metabolic processes in many unique configurations, often offering higher ATP yield than canonical fermentation but lower associated catabolic investments than aerobic glucose respiration.[27] Unlike canonical fermentation, which relies solely on energy conservation via substrate-level phosphorylation, many anaerobes, including many eukaryotic ones, extend basic fermentative metabolism by using electrochemical gradients coupled to oxidative phosphorylation to conserve energy.[27–29] Respiration is a catabolic process in which the terminal electron acceptor is acquired exogenously by the cell, whereas a fermentative process uses endogenously generated molecules as the terminal acceptor. Therefore, both definitions allow for energy conservation via ETC, and anaerobic fermenters can generate proton gradients using malate dismutation, fumarate reduction, or proton reduction.[27,29] When harnessed for biomanufacturing, these catabolisms are analogous to modular cell factory power sources, each offering different costs and energy outputs.

Microbial diversity, including anaerobic adaptations, could also inspire efficiency in non-glucose cell factory tasks that, despite their low flux, can be just as crucial to cell factory efficiency as central metabolism. Fine-tuning investment in cellular tasks like cofactor and non-carbon elemental acquisition might increase synthetic biological processes' efficiencies. Like central metabolism, investment in each of these processes should be optimized so that over- and under-investment does not impede the cell factory's efficiency. Future discussions of cell factory efficiency could incorporate a holistic view of cells, where each of these auxiliary processes could be optimized to minimize investment while maximizing target biomolecule production.

5 | PARETO OPTIMALITY CONCEPTUALIZES METABOLIC TRADE-OFFS FOR SYNTHETIC BIOLOGY TASKS

A Pareto optimality discussion for cell factories and other synthetic biology applications helps conceptualize which organisms, enzymes, and pathways are desirable for deployment in microbial cell factories. A Pareto optimality function describes the states in which further investment in one process cannot occur without reduced investment in another (Figure 3A). This concept is applicable to cell factories'

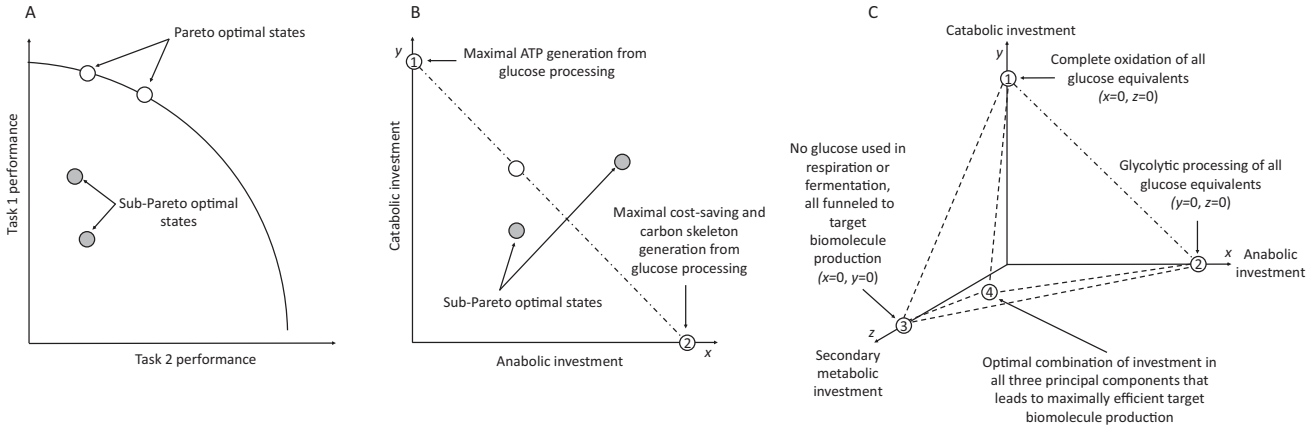

FIGURE 3 A qualitative Pareto framework for glucose processing conceptualizes cell factory tradeoffs. Panel (A) depicts a generalized Pareto space where axes represent two generic tasks that compete for the same pool of resources. In a cellular context, tasks compete for molecular building blocks, cellular solvent capacity, and other resources. The Pareto front represents states where one of the two tasks cannot be improved without sacrificing the competing task's performance. Therefore, all combinations of Tasks 1 and 2 located on the Pareto front are efficient in different contexts. Panel (B) is a simplified representation of a Pareto front between two cellular tasks, namely energy conservation via respiration (1) and glucose processing via fermentation (2). The axes of Panel (B) represent principal components of cellular investment in catabolism (y) and cellular investment in anabolism (x). The Pareto function's true shape could be determined by assessing principal component combinations with no over- or under-investment in either task. Panel (C) adds secondary metabolic investment (z) to the axes from Panel (B), making Panel (C) the relevant phenotype space for this discussion. In addition to archetypal organisms (1) and (2) from Panel (B), which invest exclusively in the anabolism or catabolism of glucose, Panel (C) includes archetypal organism (3), which invests solely in secondary metabolite production. Archetypal organism (4) represents an efficient cell factory that exists in this phenotype space. Organism (4) is an ideal combination of investments in the three principal components where investment maximizes target biomolecule production for the cell factory.

efficiency because a Pareto front represents various efficient states where all inputs are used without over-investment or under-investment in cellular tasks (Figure 3B). Pareto optimality analysis has been applied to biological systems to provide insight on glycolytic pathway tradeoffs, microbial metabolism, and macro-organism evolutionary adaptation.[18,30,31]

A three-dimensional Pareto space with axes representing anabolic investment, catabolic investment, and secondary metabolic investment can demonstrate the contrasting efficiencies required for different synthetic biology tasks such as biomolecule production by a cell factory versus bioremediation (Figure 3C). In both cases, the process's goal is to transform chemicals, but the valuable output is different in each case. In a cell factory, the useful output is narrowly defined as the yield of a target molecule of interest, but in the case of bioremediation, the maximal yield of a specific chemical is, most often, not tied directly to the efficiency of the process. It seems likely that a metabolic configuration optimized for cell factory processes will be positioned on the Pareto front nearer the axis representing secondary metabolic investment. In contrast, organisms for bioremediation may invest more extensively in anabolic and catabolic processes without compromising efficiency, placing them in spaces on the Pareto front that are distal to those occupied by cell factories. Further observations of microbial metabolic diversity will help determine the full breadth of efficient metabolic configurations possible and, in return, these possible configurations will help define the shape of the Pareto front.

6 | DIVISION OF LABOR AND SECONDARY METABOLITE PRODUCTION ARE ATTRACTIVE FEATURES OF ANAEROBES

In nature, microbial ecological interactions shape communities' structures. Teams of anaerobic microbes in guts and other environments are known for their propensity to divide chemical tasks between different organisms.[32] In cooperative microbial communities, various cell types carry out complementary tasks that would otherwise compete for resources or be biochemically incompatible through intermediate cross-reactivity or enzyme promiscuity.[19] Therefore, these consortia are more efficient than individual populations at performing complex functions. The evolution of division of labor between cell types as a means of spatial efficiency is an important principle that synthetic biology and biomanufacturing applications should consider.[33] In many anaerobic microbial communities, labor divisions give rise to extensive microbial diversity and syntrophic relationships, or cross-feeding partnerships, among microbes.[34] Additional insight into biochemical divisions of labor by natural microbial partnerships will help future efforts to divide tasks deliberately among cell types within synthetic microbial consortia.[35]

The design of microbial synthetic communities usually selects microbial partners in two different ways.[36] The most common one is a top-down approach, where researchers reduce system complexity by identifying key community players. However, this approach often faces challenges in disentangling symbiotic or cooperative partnerships due to the presence of non-intervening species, often resulting

in a lack of stability. Alternatively, a bottom-up approach builds complexity by associating partners that may not originate from the same environment but present well-defined genetic backgrounds essential to performing various steps of a complex task. Bottom-up approaches can help to fully characterize the degree of cooperation between partners in microbial communities. Since obligate mutualism increases stability by preventing competitive exclusion, and since culturing interdependent organisms outside of their natural setting often leads to culture failure or instabilities, engineering consortia from the bottom up can help overcome failure in laboratory settings and understand the emergence of collaboration in microbial communities. For example, Kane et al. constructed synthetic anaerobic communities by engineering sets of *Shewanella oneidensis* and *Geobacter sulfurreducens* (two bacterial species that had no naturally evolved metabolic interactions) and monitored the emergence of cooperation as they increased the degree of metabolic dependency between partners.[37] Some bottom-up model systems have already replicated natural partnerships' performance advantages, increasing titer, rate, and yield in model systems.[38,39]

In a recent study, Peng et al.[40] created a toolkit for engineering small communities of syntrophic *Saccharomyces*. Using an ensemble modeling approach, the authors identified initial population ratios and metabolite production rates as key interactions for co-culture dynamics. They then engineered different modules of *Saccharomyces* strains with "receivers" and "donors" capable of overproducing different intermediate metabolites and experimentally combined those modules to demonstrate the approach's suitability for producing high-value secondary metabolites like aromatic resveratrol.[40]

However, bottom-up approaches are often limited to 2–3 member consortia. To overcome the requirement for similar culture conditions, engineering microbial consortia can be coupled with process engineering to create niches by physically segregating a higher number of partners in bioreactors. Using this approach, Sahab et al. created a lactate platform for the production of short-chain fatty acids using highly diverse microbes.[41]

Anaerobes' potential to synthesize novel and useful secondary metabolites is currently a renewed area of interest.[9,10,42,43] Anaerobes likely synthesize unique natural products or produce known chemicals through alternative reactions that could be useful to medicine, industry, and agriculture. Additionally, since anaerobic catabolism tends to reduce volumetric costs and molecular building block demands, as previously discussed, some anaerobes might be efficient production platforms for secondary metabolites. Anaerobic microbes specializing in secondary metabolite production can inspire biotechnology by illuminating previously undiscovered target molecules and providing new enzymes, pathways, and chassis organisms (Figure 1).

7 | EMERGING APPLICATIONS FOR ANAEROBES: FROM LIGNOCELLULOSE TO GUT MICROBIOME HEALTH

The most successful bioprocessing platforms currently in use provide evidence that anaerobic metabolisms produce functional cell factories. Wastewater treatment, food preservation, and anaerobic digestion are typical biotechnological applications of anaerobic consortia that maintain naturally occurring syntrophic partnerships.[44] Soy sauce, alcoholic beverages, yogurt, cheese, and preserved meats are all examples of bioproducts that leverage fermentative microbial communities to achieve desired end products and specific flavor profiles.[45–48] Lactate, ethanol, and acetate are the bulk of these systems' carbon outputs, while complex carbon compounds produced by microbial participants impart flavor profiles. The importance of microbially produced volatile organics can be confirmed quantitatively via careful experimentation with mass spectrometry.[45–49]

Anaerobic metabolisms also have direct impacts on human health. Many large aerobic organisms, like mammals, depend on fermentative metabolism by anaerobic consortia in their guts.[50,51] Recent studies suggest that healthy guts exclude oxygen, whereas guts in disease states harbor more oxygen-tolerant organisms.[50,52] Further, some findings implicate the disruption of short-chain fatty acid transport by human gut epithelial cells in gastrointestinal diseases such as inflammatory bowel diseases, Crohn's disease, and ulcerative colitis.[53–55] Improved understandings of gut microbiomes, their metabolisms, and their interplay with mammalian cells have the potential to enable targeted engineering of gut communities, which in turn might allow for therapies and treatments to improve human health outcomes.[56,57]

Anaerobes also have an important role to play in decreasing reliance on fossil fuels. Lignocellulosic biomass is an abundant, inexpensive, and renewable resource derived from plant material containing over 200 valuable chemical derivatives.[58] Its complex structure comprises cellulose (a polysaccharide with internal β-1,4-glycosidic bonds complex structure) and hemicellulose (a polysaccharide with randomly branched sugars and uronic acids). Both are surrounded by lignin, a polymer of irregular aromatic alcohols. The presence of lignin is a barrier to lignocellulose hydrolysis as it prevents the necessary enzymes from accessing and hydrolyzing the inner polysaccharides. To facilitate lignin breakdown, biomass is often physically or chemically pre-treated. This additional step represents about 20% of the total project cost and poses barriers to low-cost and efficient biomass conversion that could be removed with biological pretreatments.[59] However, biological pretreatment is currently limited because lignin deconstruction typically occurs under aerobic conditions, while fermentation is done anaerobically.

Recent compelling evidence from nuclear magnetic resonance (NMR), mass spectrometry, and RNA sequencing demonstrates that anaerobic fungi found in large herbivores can break down unpretreated lignin under anaerobic conditions that are completely devoid of oxygen.[60,61] This discovery could allow minimizing or skipping the costly, energy-intensive, and environmentally unfriendly pre-treatment steps[62] currently required, which often produce inhibitors with negative impacts on anaerobic digestion.[63] Moreover, anaerobic fungi could be leveraged in large-scale production systems, as many anaerobic gut fungi have an extensive rhizoid organized in a network that maximizes contact surface with lignocellulosic fibers, which can be advantageous for processing large volumes of biomass. The entanglement of rhizoid and fiber allows for efficient and targeted enzyme distribution.[64,65]

The discovery of anaerobic lignin deconstruction[60,61] is opening new avenues to explore anaerobes as potential synthetic polymer degraders, offering alternative options to the current laborious, costly, and environmentally unfriendly methods of plastic waste management, such as incineration and recycling. For example, anaerobic fungi can modify lignin by targeting β-aryl-ether (β—O—4 bonds) units and phenyl coumarins (β—5 bonds); they might also be able to target synthetic polymers that possess similar aromatic compounds.[66] In this way, anaerobic metabolisms may also have a role to play in the biodegradation and design of new polymers.[67] As society seeks alternative solutions to petroleum-based plastics, biopolymers such as polylactic acid (PLA), polyhydroxybutyrate (PHB), and bio-based polyamide have been developed.[66,67] However, their limited mechanical strength and water barrier properties make them less competitive than petroleum-based plastics.[68] Therefore, incorporating lignin into bioplastics to improve their mechanical properties but also to accelerate their breakdown[69] is a promising area of research where anaerobic lignin degraders could play a key role.

8 | CONCLUSIONS

Anaerobes specialize in different ways compared to aerobic organisms, and their adaptations could enhance specific synthetic biology applications like microbial cell factories for scalable biomanufacturing. Aerobic glucose-respiring cells excel at conserving free energy as ATP, making this metabolic configuration the most catabolically efficient currently described. However, anaerobic metabolisms gain underappreciated benefits by conserving molecular building blocks and reducing volumetric costs associated with core metabolic pathways. Therefore, in contrast to aerobes' catabolic efficiency, anaerobes tend toward anabolic efficiency and volumetric cost reduction, making them particularly well suited for microbial cell factories where target molecule production pathways tax molecular building blocks and cell volume. Anaerobic microbes also possess the native ability to produce secondary metabolites and to divide tasks among different cell types cooperatively; both divisions of labor and complex biomolecule production are valuable traits for synthetic biology applications. Anaerobes are currently an underused source of enzymes, pathways, chassis, and inspiration for synthetic biology, and increased investment in their development will rapidly expand synthetic biology toolkits.

AUTHOR CONTRIBUTIONS

Thomas S. Lankiewicz: Conceptualization; writing – original draft; writing – review and editing; investigation. **Nathalie H. Elisabeth:** Writing – review and editing; investigation. **David L. Valentine:** Writing – review and editing; supervision; project administration. **Michelle A. O'Malley:** Supervision; project administration; writing – review and editing; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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