Microbial Fermentation for the Sustainable Production of Fuels, Commodity Chemicals, and Materials

Aidan E. Cowan[1, 4, †], Sarah H. Klass[1, 5, †], Peter H. Winegar[1, 3, †], Jay D. Keasling[1, 2, 3, 5, 6, 7, *]

[1] Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA 94608, USA
[2] Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[3] QB3 Institute, University of California, Berkeley, Berkeley, CA 94720, USA
[4] Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720, USA
[5] Department of Chemical and Biomolecular Engineering and Department of Bioengineering, University of California, Berkeley, Berkeley, CA 94720, USA
[6] Center for Synthetic Biochemistry, Shenzhen Institutes for Advanced Technologies, Shenzhen 518055, P.R. China
[7] The Novo Nordisk Foundation Center for Biosustainability, Technical University Denmark, Kemitorvet, Building 220, Kongens Lyngby 2800, Denmark
* Corresponding Author
† Equal Contribution

ABSTRACT

Anthropogenic carbon emissions are driving rapid changes to the earth's climate, disrupting whole ecosystems and endangering the stability of human society. Innovations in engineered microbial fermentation enable the fossil resource-free production of fuels, commodity chemicals, and materials, thereby reducing the carbon emissions associated with these products. Microorganisms have been engineered to catabolize sustainable sources of carbon and energy (*i.e.*, plant biomass, plastic waste, and one-carbon feedstocks) and biosynthesize carbon-neutral or carbon-negative products. These engineering efforts exploit and optimize natural biological pathways or generate unnatural pathways which can biosynthesize chemicals that have not yet been accessed using synthetic chemistry. Recent advances in microbial fermentation seek not only to maximize the titer, rate, and yield of desired products, but also to tailor microbial catabolism to utilize inexpensive feedstocks. Ultimately, these advances aim to lower the cost of bioproduction so that microorganism-derived chemicals can be economically competitive with fossil-derived chemicals.

KEYWORDS

Synthetic Biology; Metabolic Engineering; Engineered Microorganisms; Microbial Fermentation; Plant Biomass; C1 Feedstocks; Plastic Waste; Biofuels; Biopolymers; Biomaterials

HIGHLIGHTS

- Carbon-neutral or carbon-negative production can be achieved using microbial biosynthesis from C1 feedstocks
- Waste plastics can be degraded and valorized using engineered microorganisms
- Small bioproduced alcohols can be catalytically upgraded into sustainable aviation fuels

- Engineered microorganisms can produce molecules that are currently inaccessible via synthetic chemistry
- Free radical polymerization reactions can be performed in microorganisms

INTRODUCTION

Carbon emissions from the use of fossil resources (*e.g.*, oil, coal, and natural gas) accelerate the rate of anthropogenic climate change, potentially beyond society's capability for adaptation (IPCC Sixth Assessment Report; URL: https://www.ipcc.ch/report/ar6/wg2/). Nevertheless, nearly all fuels, commodity chemicals, and materials are produced from fossil resources.[1] Current economic models maximize profit and growth by ignoring the future socioeconomic costs of fossil resource use, many of which are already being realized (NOAA NCEI U.S. Billion-Dollar Weather and Climate Disasters; URL: https://doi.org/10.25921/stkw-7w73). Furthermore, the uneven global distribution of fossil resource reserves leaves their control subject to political disputes and conflict. Dramatic action is urgently needed to transition the global economy away from its dependence upon fossil resources and towards circular uses of carbon. Herein, recent innovations in microbial fermentation technology are highlighted that achieve the efficient transformation of sustainable sources of carbon and energy (*i.e.*, plant biomass, plastic waste, and one-carbon feedstocks) into fuels, commodity chemicals, and materials (**Figure 1**). The combination of technological innovation and policy changes that allow the price of fossil resources to reflect their true socioeconomic cost will enable microbial fermentation-derived products to displace fossil-derived products in a perpetually sustainable bioeconomy.

The field of biomanufacturing is incredibly diverse due to the natural diversity of microorganisms and metabolisms; therefore, this concise review cannot be comprehensive. Notably, microbial production of methane for use as renewable natural gas, microbial production using methanotrophs, and microbial production using photosynthetic microorganisms that utilize light and atmospheric carbon as feedstocks are not described herein.

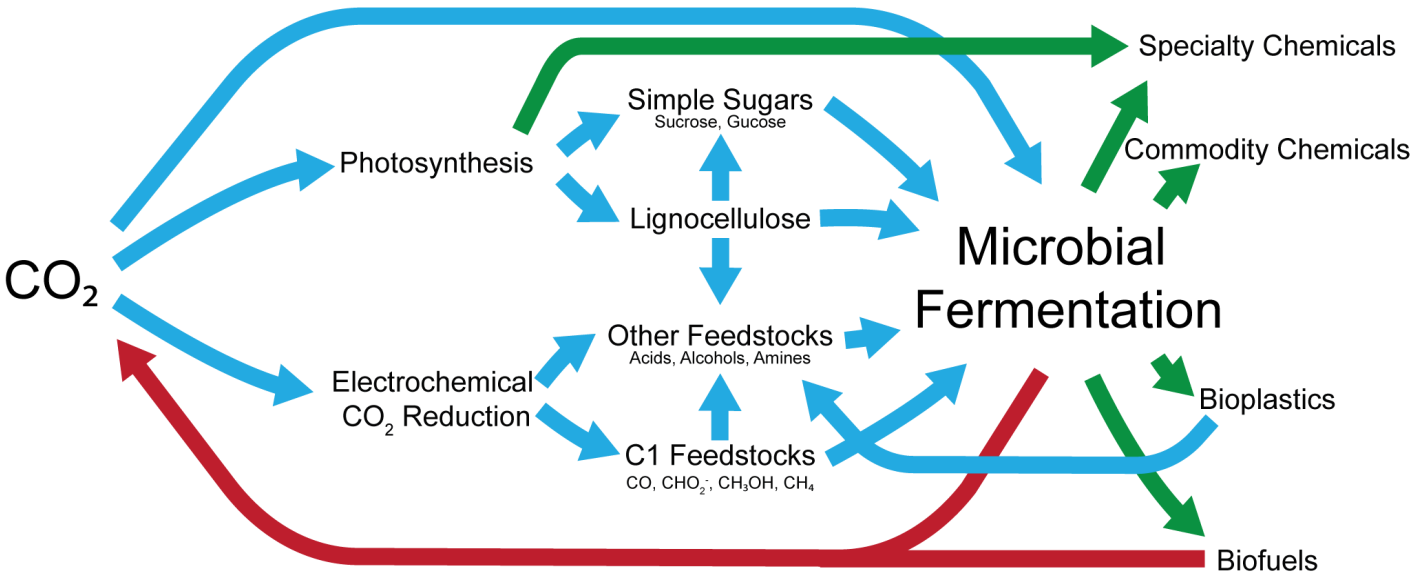

Figure 1. Carbon dioxide (left) is reduced using sustainable sources of energy (*i.e.*, light for photosynthesis or renewable electricity for electrochemical CO_2 reduction) to produce feedstocks. Engineered microorganisms convert these

[1] Levi PG, Cullen JM: **Mapping global flows of chemicals: from fossil fuel feedstocks to chemical products**. *Environ. Sci. Technol.* 2018, **52**:1725-1734.

feedstocks into desired products using fermentation (right). This system sequesters atmospheric carbon dioxide as chemicals and materials, enabling carbon-neutral or carbon-negative products. These circular uses of carbon are the foundation of a future sustainable bioeconomy. In this figure, red arrows represent the release of carbon dioxide within the system, blue arrows represent CO_2 fixation or transformation, and green arrows represent the bioproduction of chemicals.

RENEWABLE SOURCES OF CARBON AND ENERGY

Renewable sources of carbon and energy are required at a significant scale (10s of billions of metric tons per year) for all fuels, commodity chemicals, and materials to be sustainably produced via microbial fermentation. Plant biomass, plastic waste, and one-carbon (C1) feedstocks are currently underutilized carbon and energy sources (**Figure 2**).

Plant Biomass Deconstruction:

The production of chemicals through the deconstruction and transformation of carbon fixed by plants is one of the more developed technologies to replace fossil resources. Over the past ~500 million years, plants have evolved to efficiently convert atmospheric carbon dioxide and sunlight into complex carbon molecules, including carbohydrates, proteins, lipids, vitamins, and lignin. Edible plants that accumulate rich stores of sucrose (*e.g.*, sugarcane, corn, and sugar beets) are “first generation” feedstocks that can be directly fermented by microorganisms to produce fuels and chemicals. However, the utilization of food crops for bioproduction requires the use of arable land that could otherwise be used to produce food. To address this challenge, intense research has focused on the deconstruction and valorization of non-edible plant biomass to generate “second generation” feedstocks for microbial fermentation (**Figure 2**, left).

The main component of plant biomass is lignocellulose, a complex assembly of hemicellulose, cellulose, and lignin. Hemicellulose is a biopolymer of C5 and C6 sugars, while cellulose is composed entirely of glucose. C5 and C6 sugars are excellent microbial feedstocks. Similarly, lignin is a biopolymer of aromatic compounds called monolignols, which can also be used as a feedstock or converted into other chemicals. Since lignocellulose is highly recalcitrant to degradation, it is often pretreated with a combination of mechanical shearing, heat, chemicals, acids/bases, and radiation prior to its use as a feedstock.[2] Pretreatment can help separate lignin from hemicellulose and cellulose, improving the accessibility of these biopolymers to depolymerization processes such as treatment with enzymes (*e.g.*, cellulases, laccases, and lignin peroxidases) and non-biological treatments. However, many pretreatment methods are expensive and difficult to scale. Accordingly, promising developments in the field of biomass deconstruction aim to decrease the cost of these processes. For example, the cost of biomass deconstruction can be decreased using ionic liquid pretreatment because they can be recycled, thereby reducing waste.[3] Alternatively, other efforts aim to decrease the reliance on pretreatments to prepare biomass for use as feedstocks. One such approach deconstructs plant biomass through simultaneous heat treatment and fermentation with thermophilic bacteria, which grow at the high temperatures needed to facilitate biomass deconstruction.[4] Recent discoveries in the pathways and mechanisms of lignin biosynthesis in plants could enable plants to be engineered to biosynthesize lignocellulose structures that are easier to

[2] Mankar AR, Pandey A, Modak A, Pant KK: **Pretreatment of lignocellulosic biomass: A review on recent advances**. *Bioresour. Technol.* 2021, **334**:125235.
[3] Lin X, Jiang K, Liu X, Han D, Zhang Q: **Review on development of ionic liquids in lignocellulosic biomass refining**. *J. Mol. Liq.* 2022, **359**:119326.
[4] Lynd LR, Beckham GT, Guss AM, Jayakody LN, Karp EM, Maranas C, McCormick RL, Amador-Noguez D, Bomble YJ, Davison BH, et al.: **Toward low-cost biological and hybrid biological/catalytic conversion of cellulosic biomass to fuels**. *Energy Environ. Sci.* 2022, **15**:938-990.

deconstruct.[5] Finally, engineering plants to produce high-value products that can be isolated from biomass during deconstruction can help offset the high capital costs associated with biomass deconstruction.[6] Together, these efforts are improving the economics of using plant biomass to generate “second-generation” feedstocks for microbial fermentation.

Plastic Waste Deconstruction:

The increasing production and accumulation of plastic waste has become a significant environmental concern in recent decades. Plastics derived from fossil resources, such as polyethylene terephthalate (PET), polyethylene (PE), and others, are extremely resistant to biodegradation. This leads to biopersistent macro- and micro-plastics that pollute terrestrial and aquatic ecosystems and harm the health of organisms that live there. There has been an intense search for innovative ways to valorize plastic waste into useful products that pose less environmental risk. Leveraging plastic waste as a microbial feedstock can help achieve the economically feasible production of target compounds or products (**Figure 2**, center).

The use of microorganisms to degrade plastic waste into its constituent monomers and then re-polymerize it into new plastics has gained particular interest over the past years. This was inspired by the discovery of a bacterium, *Ideonella sakaiensis*, that can deconstruct and metabolize PET,[7] one of the most commonly used plastics (62.3 million metric tons produced/year).[8] Since then, significant progress has been made in identifying enzymes and metabolic pathways that are involved in PET degradation.[9] Recent reports increased the activity and thermostability of PET hydrolases using methods such as bioinformatic searches of natural diversity,[10] rational engineering,[11] directed evolution,[12] [13] and machine learning-based engineering of enzymes.[14] Impressively, these aforementioned efforts have generated PET hydrolases that can depolymerize PET at industrially relevant scales with high yields and relatively mild conditions. Despite the significant progress in the microbial and enzymatic deconstruction of PET, many plastics composed solely of C–C bonds (*i.e.*,

---

[5] • Zhuo C, Wang X, Docampo-Palacios M, Sanders BC, Engle NL, Tschaplinski TJ, Hendry JI, Maranas CD, Chen F, Dixon RA: **Developmental changes in lignin composition are driven by both monolignol supply and laccase specificity**. *Sci. Adv.* 2022, **8**:eabm8145.
This paper elucidates factors that control the transition from the biosynthesis of guaiacyl lignin to the biosynthesis of catechyl lignin in the seed coat of *Cleome hassleriana*. Catechyl lignin has a more homogeneous polymer composition compared to guaiacyl lignin and, therefore, is easier to deconstruct. Future engineering efforts could use these discoveries to create plants with higher compositions of catechyl lignin to facilitate their deconstruction and use as a feedstock.

[6] Yang M, Baral NR, Simmons BA, Mortimer JC, Shih PM, Scown CD: **Accumulation of high-value bioproducts *in planta* can improve the economics of advanced biofuels**. *Proc. Natl. Acad. Sci. U. S. A.* 2020, **117**:8639-8648.

[7] Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K: **A bacterium that degrades and assimilates poly(ethylene terephthalate)**. *Science* 2016, **351**:1196-1199.

[8] Nicholson SR, Rorrer NA, Carpenter AC, Beckham GT: **Manufacturing energy and greenhouse gas emissions associated with plastics consumption**. *Joule* 2021, **5**:673-686.

[9] Han X, Liu W, Huang J-W, Ma J, Zheng Y, Ko T-P, Xu L, Cheng Y-S, Chen C-C, Guo R-T: **Structural insight into catalytic mechanism of PET hydrolase**. *Nat. Commun.* 2017, **8**:2106.

[10] Erickson E, Gado JE, Avilán L, Bratti F, Brizendine RK, Cox PA, Gill R, Graham R, Kim D-J, König G, et al.: **Sourcing thermotolerant poly(ethylene terephthalate) hydrolase scaffolds from natural diversity**. *Nat. Commun.* 2022, **13**:7850.

[11] Tournier V, Topham CM, Gilles A, David B, Folgoas C, Moya-Leclair E, Kamionka E, Desrousseaux ML, Texier H, Gavalda S, et al.: **An engineered PET depolymerase to break down and recycle plastic bottles**. *Nature* 2020, **580**:216-219.

[12] •• Bell EL, Smithson R, Kilbride S, Foster J, Hardy FJ, Ramachandran S, Tedstone AA, Haigh SJ, Garforth AA, Day PJR, et al.: **Directed evolution of an efficient and thermostable PET depolymerase**. *Nat. Catal.* 2022, **5**:673-681.
A high-throughput directed evolution platform was developed for engineering enzymes capable of PET degradation. This platform utilized HPLC detection of the release of PET monomers to identify functional enzymes within diverse libraries. Using selective pressures for high catalytic activity and elevated temperature stability, a thermostable PETase variant was engineered that can rapidly deconstruct semicrystalline PET at its glass transition temperature (Tg = 60–70 °C). Under optimal conditions, up to 31% of PET substrate could be depolymerized within 5 h at 60 °C.

[13] Shi L, Liu P, Tan Z, Zhao W, Gao J, Gu Q, Ma H, Liu H, Zhu L: **Complete depolymerization of PET wastes by an evolved PET hydrolase from directed evolution**. *Angew. Chem. Int. Ed.* 2023, **62**:e202218390.

[14] • Lu H, Diaz DJ, Czarnecki NJ, Zhu C, Kim W, Shroff R, Acosta DJ, Alexander BR, Cole HO, Zhang Y, et al.: **Machine learning-aided engineering of hydrolases for PET depolymerization**. *Nature* 2022, **604**:662-667.
A machine learning algorithm was used to engineer PETases with improved catalytic activity as well as temperature and pH stability over the wild-type (WT) PETase. By using three-dimensional (3D) self-supervised, convolutional neural network (CNN) to predict stabilizing mutations and data collected from iterative rounds of single point mutations, a single protein with five mutations compared to WT PETase was identified that could completely degrade 9 g of solid PET in less than 2 weeks at 50 °C.

PE) have proven more difficult to deconstruct. Some degree of enzymatic PE deconstruction, usually in the time frame of days to months, has been achieved using alkane hydroxylases[15] and ligninolytic enzymes that naturally cleave the C–C bonds in lignin biomass.[16] Improving the activity of these enzymes, especially ligninolytic enzymes, would facilitate both the degradation of recalcitrant plastics and plant biomass.[17] [18]

In addition to deconstructing plastic waste for re-polymerization, microorganisms have been engineered to degrade and valorize this carbon feedstock into valuable monomers for higher-value polymers. For example, *Pseudomonas putida* was engineered to convert PET into β-ketoadipate, a monomer used in the synthesis of nylon, through a tandem chemical deconstruction and biological upcycling approach.[19] In another example, PET waste was converted by an engineered and laboratory-evolved strain of *Pseudomonas umsongensis* capable of producing two types of bioplastics: a commercially relevant polyhydroxyalkanoate (PHA) and a novel poly(amide urethane).[20] A techno-economic analysis of enzymatic recycling of PET polymers highlights the importance of enzyme activity, enzyme production cost, and pretreatment costs (*e.g.*, feedstock and mechanical processing costs) on the economic feasibility of these processes.[21] Additional exciting progress in plastic upcycling and current state-of-the-art chemical and biological methods have been recently described in a comprehensive review.[22]

One-Carbon Feedstocks:

One-carbon (C1) feedstocks are single-carbon molecules (*e.g.*, methane, methanol, carbon monoxide, and formate) that can be used as microbial carbon and energy sources (**Figure 2**, right).[23] Many waste streams include C1 feedstocks, such as gas emitted from landfills (*i.e.*, methane) and flue gas from steel manufacturing (*i.e.*, hydrogen and carbon monoxide). Through electrocatalysis, renewable energy can be used to selectively reduce carbon dioxide to formate, methanol, and carbon monoxide. This process has become increasingly attractive as electricity costs decrease due to the adoption of wind and solar energy technology.[24] The metabolism of organisms that utilize these C1 compounds can be engineered for the bioproduction of carbon-neutral or carbon-negative products. Large and complex molecules can not be easily generated through electrocatalysis due to the low specificity of electrocatalysts which often generate mixtures of products.[24] Therefore, bioproduction using hybrid electrochemical-biological systems is attractive because they take advantage of the high energetic and Faradaic efficiency of the electrochemical reduction of carbon dioxide and the high selectivity of microbial biosynthesis.

---

[15] Jeon HJ, Kim MN: **Functional analysis of alkane hydroxylase system derived from *Pseudomonas aeruginosa* E7 for low molecular weight polyethylene biodegradation**. *Int. Biodeterior. Biodegrad*. 2015, **103**:141-146.

[16] Santo M, Weitsman R, Sivan A: **The role of the copper-binding enzyme – laccase – in the biodegradation of polyethylene by the actinomycete *Rhodococcus ruber***. *Int. Biodeterior. Biodegrad.* 2013, **84**:204-210.

[17] Chen C-C, Dai L, Ma L, Guo R-T: **Enzymatic degradation of plant biomass and synthetic polymers**. *Nat. Rev. Chem.* 2020, **4**:114-126.

[18] Zhang Y, Pedersen JN, Eser BE, Guo Z: **Biodegradation of polyethylene and polystyrene: From microbial deterioration to enzyme discovery**. *Biotechnol. Adv.* 2022, **60**:107991.

[19] Werner AZ, Clare R, Mand TD, Pardo I, Ramirez KJ, Haugen SJ, Bratti F, Dexter GN, Elmore JR, Huenemann JD, et al.: **Tandem chemical deconstruction and biological upcycling of poly(ethylene terephthalate) to β-ketoadipic acid by *Pseudomonas putida* KT2440**. *Metab. Eng.* 2021, **67**:250-261.

[20] Tiso T, Narancic T, Wei R, Pollet E, Beagan N, Schröder K, Honak A, Jiang M, Kenny ST, Wierckx N, et al.: **Towards bio-upcycling of polyethylene terephthalate**. *Metab. Eng.* 2021, **66**:167-178.

[21] Singh A, Rorrer NA, Nicholson SR, Erickson E, DesVeaux JS, Avelino AFT, Lamers P, Bhatt A, Zhang Y, Avery G, et al.: **Techno-economic, life-cycle, and socioeconomic impact analysis of enzymatic recycling of poly(ethylene terephthalate)**. *Joule* 2021, **5**:2479-2503.

[22] Ellis LD, Rorrer NA, Sullivan KP, Otto M, McGeehan JE, Román-Leshkov Y, Wierckx N, Beckham GT: **Chemical and biological catalysis for plastics recycling and upcycling**. *Nat. Catal.* 2021, **4**:539-556.

[23] Jiang W, Hernández Villamor D, Peng H, Chen J, Liu L, Haritos V, Ledesma-Amaro R: **Metabolic engineering strategies to enable microbial utilization of C1 feedstocks**. *Nat. Chem. Biol.* 2021, **17**:845-855.

[24] De Luna P, Hahn C, Higgins D, Jaffer SA, Jaramillo TF, Sargent EH: **What would it take for renewably powered electrosynthesis to displace petrochemical processes?** *Science* 2019, **364**:eaav3506.

The utilization of waste carbon monoxide by acetogens, which rely upon the energy-efficient Wood-Ljungdahl pathway, is likely the most mature technology for the production of fuels and chemicals from C1 feedstocks. Notably, the company Lanzatech has used this technology to transform carbon monoxide into chemicals, mainly ethanol, using the bacteria *Clostridium autoethanogenum*.[25] Lanzatech has also recently engineered *C. autoethanogenum* to produce isopropanol and acetone from C1 feedstocks at an industrial pilot scale and at a high rate (~3 g/L/hr).[26] This is a significant advance because it demonstrates that acetogens can be engineered for the commercial production of compounds beyond ethanol, potentially paving the way toward the commercial production of higher-value chemicals.

A significant challenge associated with bioproduction using acetogens is that acetogens exhibit limited titers for compounds larger than four carbons.[4] Acetogenic metabolism is highly energetically constrained and has a limited ability to produce ATP relative to aerobic organisms, limiting their ability to produce complex molecules.[27] Recent advances have sought to engineer aerobic microorganisms, which are less thermodynamically constrained, to utilize C1 feedstocks for bioproduction. Natural aerobic autotrophs such as *Cupriavidus necator* or aerobic carboxydotrophic bacteria such as *Hydrogenophaga pseudoflava* hold promise for the conversion of C1 feedstocks into value-added products.[28] [29] In addition, common production hosts such as *E. coli* can be engineered for new C1 metabolisms. By engineering the Calvin cycle of *E. coli*, a strain was produced that assimilates all of its biomass from carbon dioxide using energy provided by formate.[30] In another promising example, the reductive glycine pathway in *E. coli* was engineered to achieve the fully formatotrophic or methylotrophic growth of *E. coli* along with the production of up to 0.108 g/L of lactate.[31] [32] However, engineering aerobic bacteria that utilize C1 feedstocks for bioproduction has yielded only limited titers to date. The metabolic constraints of growing microorganisms on C1 feedstocks could potentially be overcome using organisms that co-utilize C1 and traditional feedstocks or organisms that utilize C1

[25] Marcellin E, Behrendorff JB, Nagaraju S, DeTissera S, Segovia S, Palfreyman RW, Daniell J, Licona-Cassani C, Quek L-e, Speight R, et al.: **Low carbon fuels and commodity chemicals from waste gases – systematic approach to understand energy metabolism in a model acetogen**. *Green Chem.* 2016, **18**:3020-3028.

[26]•• Liew FE, Nogle R, Abdalla T, Rasor BJ, Canter C, Jensen RO, Wang L, Strutz J, Chirania P, De Tissera S, et al.: **Carbon-negative production of acetone and isopropanol by gas fermentation at industrial pilot scale**. *Nat. Biotechnol.* 2022, **40**:335-344.
In this paper, the authors achieve high-TRY production of two industrially important chemicals: acetone and isopropanol. The authors used multiomics analysis, kinetic modeling, and cell-free prototyping to shorten the time needed to engineer these organisms. This reduced reliance upon strain engineering, which can be difficult in their host *Clostridium autoethanogenum.* Furthermore, scaled up bioproduction to an industrial pilot plant and used life cycle analysis to support the claim that this system is carbon sequestering.

[27] Marcellin E, Behrendorff JB, Nagaraju S, DeTissera S, Segovia S, Palfreyman RW, Daniell J, Licona-Cassani C, Quek L-e, Speight R, et al.: **Low carbon fuels and commodity chemicals from waste gases – systematic approach to understand energy metabolism in a model acetogen**. *Green Chem.* 2016, **18**:3020-3028.

[28] Panich J, Fong B, Singer SW: **Metabolic engineering of *Cupriavidus necator* H16 for sustainable biofuels from CO_2**. *Trends Biotechnol.* 2021, **39**:412-424.

[29] Grenz S, Baumann PT, Rückert C, Nebel BA, Siebert D, Schwentner A, Eikmanns BJ, Hauer B, Kalinowski J, Takors R, et al.: **Exploiting *Hydrogenophaga pseudoflava* for aerobic syngas-based production of chemicals**. *Metab. Eng.* 2019, **55**:220-230.

[30] Gleizer S, Ben-Nissan R, Bar-On YM, Antonovsky N, Noor E, Zohar Y, Jona G, Krieger E, Shamshoum M, Bar-Even A, et al.: **Conversion of *Escherichia coli* to generate all biomass carbon from CO_2**. *Cell* 2019, **179**:1255-1263.e1212.

[31]•• Kim S, Lindner SN, Aslan S, Yishai O, Wenk S, Schann K, Bar-Even A: **Growth of *E. coli* on formate and methanol via the reductive glycine pathway**. *Nat. Chem. Biol.* 2020, **16**:538-545.
This paper describes the implementation of the reductive glycine pathway in *E. coli*, a synthetic pathway for the assimilation of formate. The reductive glycine pathway is highly energy efficient and is a promising pathway for the utilization of formate as a microbial feedstock. In order to integrate the reductive glycine pathway into *E. coli*, the authors took a modular approach using the pathway to complement multiple auxotrophies before realizing their ultimate goal of supporting cell growth entirely on formate. Adaptive laboratory evolution allowed for optimization of their strain and the realization of a sub-8 hour doubling time.

[32] Kim S, Giraldo N, Rainaldi V, Machens F, Collas F, Kubis A, Kensy F, Bar-Even A, Lindner SN: **Optimizing *E. coli* as a formatotrophic platform for bioproduction via the reductive glycine pathway**. *Front. Bioeng. Biotechnol.* 2023, **11**:1091899.

feedstocks strictly for bioproduction and traditional feedstocks for cell growth, potentially leading to increases in titers.[33]

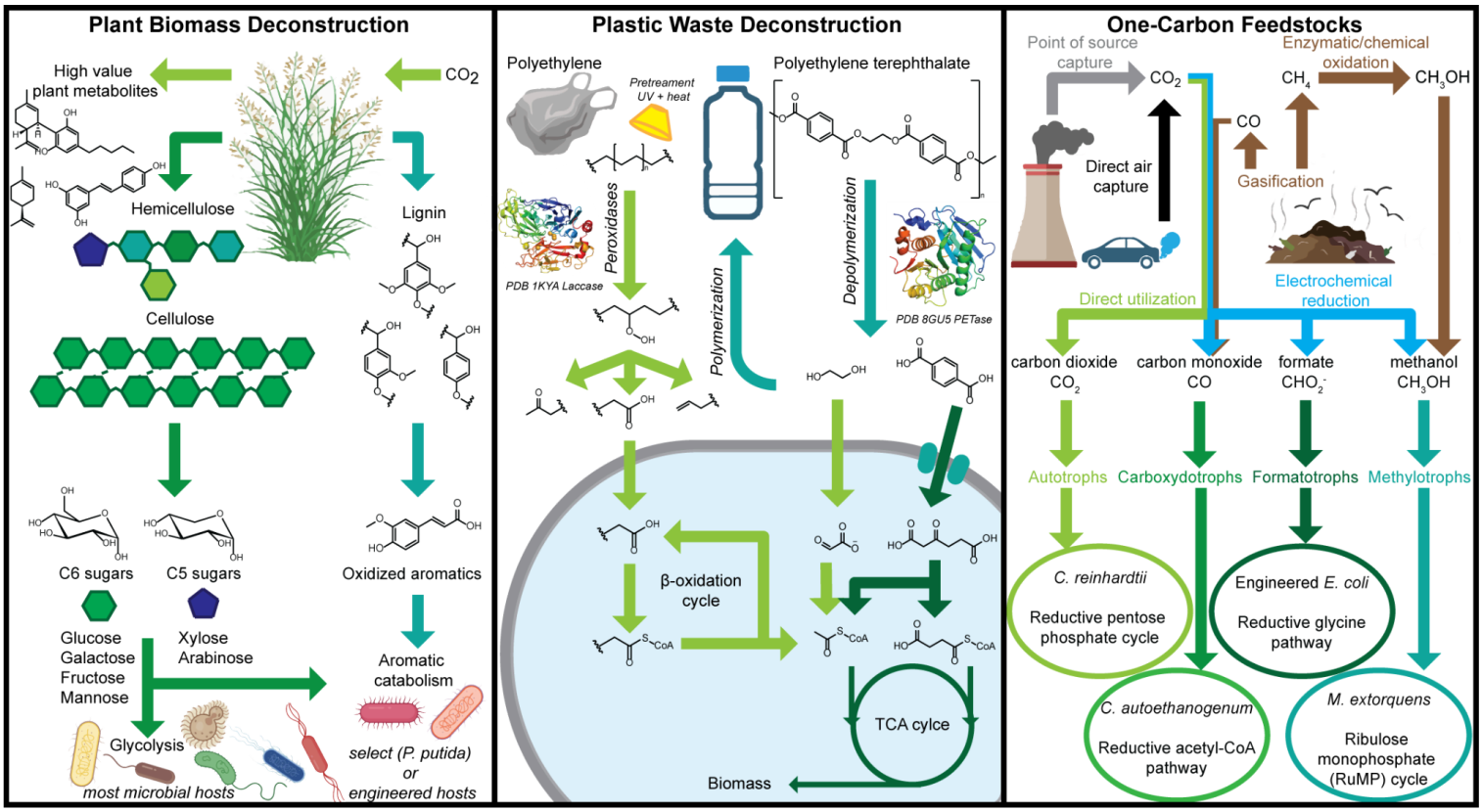

Figure 2. a. Plant biomass is deconstructed via pretreatments into the biopolymers hemicellulose, cellulose, and lignin. Subsequently, these biopolymers can be depolymerized into sugars and monolignols that are feedstocks for many hosts for microbial fermentation. **b.** Plastic waste (*e.g.*, polyethylene terephthalate or polyethylene) is deconstructed enzymatically into monomers that can be re-polymerized or used as microbial feedstocks. **c.** One-carbon feedstocks such as methane and carbon monoxide can be harvested from waste streams or generated by the electrochemical reduction of carbon dioxide. These simple carbon molecules can be used as a source of carbon and energy by diverse clades of organisms using various metabolic pathways.

FUELS, COMMODITY CHEMICALS, AND MATERIALS PRODUCED BY ENGINEERED MICROORGANISMS

Recent advances in the microbial production of fuels, industrially relevant small molecules, and polymers focus on improving titers, rates, and yields (TRYs) towards theoretical limits while minimizing economic and environmental costs. These works aim to maximize the efficiency of carbon and energy use,[34] measure economic costs with techno-economic analyses,[35] and quantify environmental impacts with life cycle analyses.[36]

Biosynthesis of Fuels:

Biofuel production has been an area of intense research and development in the past two decades, reflecting the urgency with which society must transition its sources of energy. The microbial

[33] Chou A, Lee SH, Zhu F, Clomburg JM, Gonzalez R: **An orthogonal metabolic framework for one-carbon utilization**. *Nat. Metab.* 2021, **3**:1385-1399.
[34] Henard CA, Freed EF, Guarnieri MT: **Phosphoketolase pathway engineering for carbon-efficient biocatalysis**. *Curr. Opin. Biotechnol.* 2015, **36**:183-188.
[35] Lynch MD: **The bioprocess TEA calculator: An online technoeconomic analysis tool to evaluate the commercial competitiveness of potential bioprocesses**. *Metab. Eng.* 2021, **65**:42-51.
[36] Curran MA: **Life Cycle Assessment: a review of the methodology and its application to sustainability**. *Curr. Opin. Chem. Eng.* 2013, **2**:273-277.

production of a diversity of fuel molecules, such as terpenes, fatty acid methyl esters, alcohols, and alkanes, has been reviewed extensively.[37] [38] These molecules could be used to decarbonize sectors such as remote construction and long-haul shipping. Recent efforts aim to decrease production costs and tailor biofuel properties to replace fossil fuels in sectors that are difficult to decarbonize through electrification or the use of liquid hydrogen or ammonia (**Figure 3**).

Long-haul aviation requires higher energy density storage than can be provided by batteries or liquid hydrogen. Therefore, the future decarbonization of long-haul flights requires sustainable aviation fuel (SAF), which can maintain the properties of conventional jet fuel without the associated carbon footprint. To be compatible with existing airplane engines, SAF must be composed of long-chain alkane molecules (C8–C18) with no heteroatoms. SAF must also remain a liquid across the wide range of temperatures (-50–40 °C) and pressures (0.3–1 bar) that are experienced during flight.[38] While the direct microbial production of SAF that meets these requirements is desirable, current efforts exhibit low productivity for alkane biosynthesis, resulting in challenging economics for commercialization. Exciting recent progress in SAF bioproduction utilizes well-established, high-TRY microbial biosynthesis of small alcohols (*e.g.*, ethanol) that are then catalytically upgraded into SAF. This process relies upon the catalytic dehydration of small alcohols into ethylene or other alkenes and the subsequent oligomerization of these alkenes into higher molecular weight alkanes.[39] Using this strategy, companies including LanzaJet, GEVO, and Vertimass report the conversion of bioethanol into SAF with proprietary techniques. The high-titer conversion of sorghum biomass to isoprenol and subsequent upgrading to the SAF 1,4-dimethylcyclooctane (DMCO) was described along with techno-economic analysis supporting a cost-competitive production scheme.[40] These technologies are moving towards economic parity with fossil jet fuel, facilitating the potential replacement of fossil jet fuels with SAF.

While biofuels are economically uncompetitive in applications that can be electrified or powered with liquid hydrogen or ammonia, biofuels are attractive for specialty applications such as racing, military, or rocketry. In these markets, biofuels are not cost-prohibitive because they constitute a marginal fraction of the overall cost. In addition, biological production enables the generation of new fuel molecules with improved properties compared to traditional fossil fuels. For example, *Streptomyces coelicolor* was engineered to produce polycyclopropanated fatty acid methyl esters, molecules that have higher energy density compared to fossil fuels.[41] Similarly, hybrid biological-chemical approaches can be used to produce cyclopropanated terpene molecules which

---

[37] Liu Y, Cruz-Morales P, Zargar A, Belcher MS, Pang B, Englund E, Dan Q, Yin K, Keasling JD: **Biofuels for a sustainable future**. *Cell* 2021, **184**:1636-1647.

[38] Keasling J, Garcia Martin H, Lee TS, Mukhopadhyay A, Singer SW, Sundstrom E: **Microbial production of advanced biofuels**. *Nat. Rev. Microbiol.* 2021, **19**:701-715.

[39] Eagan NM, Kumbhalkar MD, Buchanan JS, Dumesic JA, Huber GW: **Chemistries and processes for the conversion of ethanol into middle-distillate fuels**. *Nat. Rev. Chem.* 2019, **3**:223-249.

[40] • Baral NR, Yang M, Harvey BG, Simmons BA, Mukhopadhyay A, Lee TS, Scown CD: **Production cost and carbon footprint of biomass-derived dimethylcyclooctane as a high-performance jet fuel blendstock**. *ACS Sustain. Chem. Eng.* 2021, **9**:11872-11882.
This paper describes the bioproduction of a cyclic sustainable aviation fuel, dimethylcyclooctane (DMCO), by upgrading a small alcohol, isoprenol, that can be produced at high titer. Cyclic molecules are an important component of current jet fuels and this molecule could become an essential component in future bio-derived aviation fuel. This paper also uses techno economic analysis to identify future scenarios where the production of DMCO could compete economically with fossil-derived fuels.

[41] •• Cruz-Morales P, Yin K, Landera A, Cort JR, Young RP, Kyle JE, Bertrand R, Iavarone AT, Acharya S, Cowan A, et al.: **Biosynthesis of polycyclopropanated high energy biofuels.** *Joule* 2022, **6**:1590-1605.
Herein, a high energy density biofuel, a polycyclopropanated fatty acid methyl ester, is produced using an engineered microorganism. This molecule has high amounts of ring strain because it contains many cyclopropane functional groups, resulting in its higher energy density compared to conventional fossil fuels.

have high energy density and can be used in high-performance fuels.[42] Together, these studies leverage the high specificity of enzymes to access fuels with properties that are superior to traditional fossil fuels.

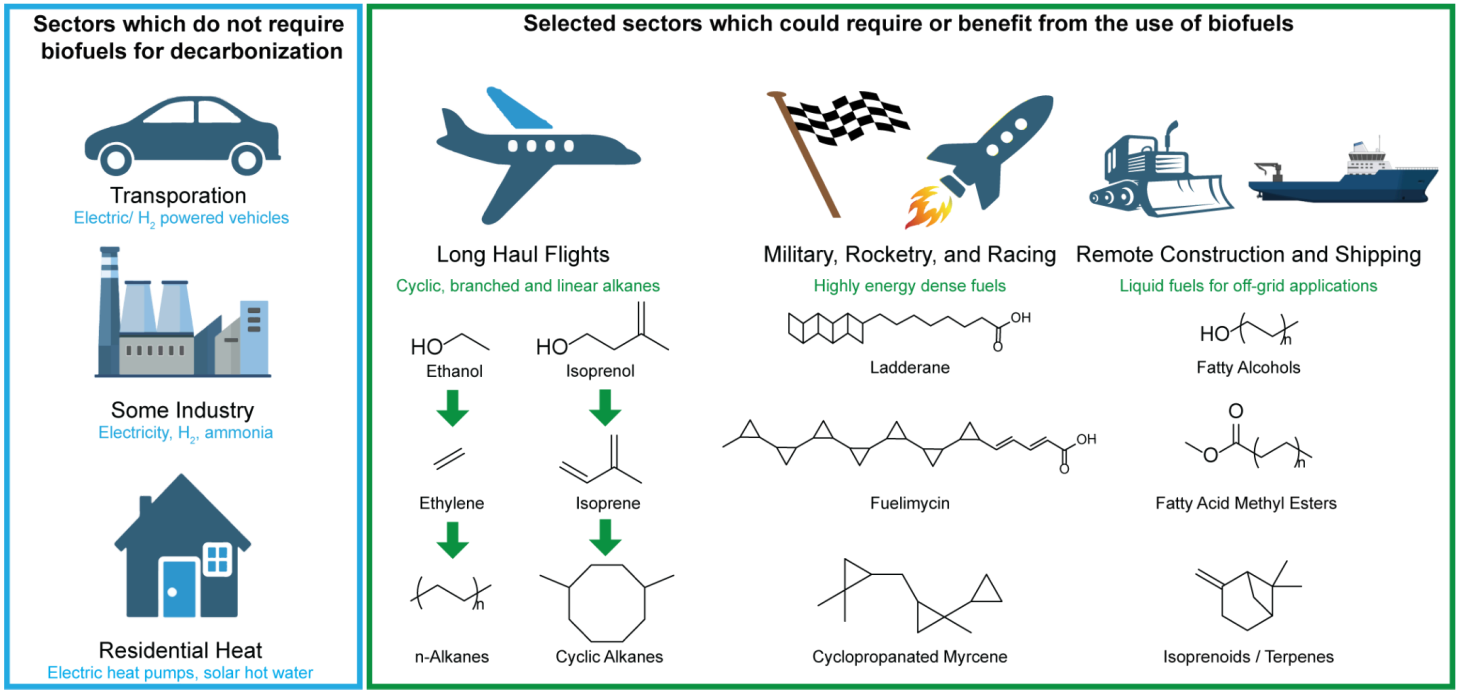

Figure 3. Biofuels are a key component in decarbonization. While many sectors (*e.g.*, transportation, some industry, and residential heat) can be decarbonized through non-biological technologies, other sectors (*e.g.*, long haul flights, racing, rocketry, remote construction, and shipping) require the use of biofuels for decarbonization. These sectors can benefit from specialty biofuels that have properties that are superior to traditional fossil fuels.

Biosynthesis of Commodity Chemicals:

Engineered microorganisms can directly produce a wide range of important molecules, such as styrene, phenol, lactic acid, lactones, lactams, dicarboxylic acids, diols, diamines, hydroxy acids, and amino acids (**Figure 4**).[43] Alternatively, microorganisms have been engineered to produce platform chemicals like ethanol, which can subsequently be converted via synthetic chemistry into industrially relevant molecules (*e.g.*, ethylene, propylene, butylene, acrylates, and acrylamides).[44] In addition, microorganisms can be engineered into "platform strains" that generate diverse sets of molecules by modularly combining interchangeable enzymes with defined reactivity and promiscuous substrate scopes. Many microbial strains have been effectively employed to produce various acids,[45]

[42] Woodroffe J-D, Lupton DV, Garrison MD, Nagel EM, Siirila MJ, Harvey BG: **Synthesis and fuel properties of high-energy density cyclopropanated monoterpenes**. *Fuel Process. Technol.* 2021, **222**:106952.
[43] Chung H, Yang JE, Ha JY, Chae TU, Shin JH, Gustavsson M, Lee SY: **Bio-based production of monomers and polymers by metabolically engineered microorganisms**. *Curr. Opin. Biotechnol.* 2015, **36**:73-84.
[44] Hayes G, Laurel M, MacKinnon D, Zhao T, Houck HA, Becer CR: **Polymers without petrochemicals: sustainable routes to conventional monomers**. *Chem. Rev.* 2023, **123**:2609-2734.
[45] Cheong S, Clomburg JM, Gonzalez R: **Energy- and carbon-efficient synthesis of functionalized small molecules in bacteria using non-decarboxylative Claisen condensation reactions**. *Nat. Biotechnol.* 2016, **34**:556-561.

alcohols,[46]-[47] lactones,[48] lactams,[49]-[50] esters,[51] and amines[52] with diverse structures. Importantly, microbial biosynthesis can access chemicals with multiple stereocenters and diverse sets of functional groups that are challenging to access via synthetic chemistry.

The bioproduction of platform chemicals that are chemical precursors to many high-value chemicals is economically attractive. The platform chemical 3-hydroxypropionic acid (3HP) is a precursor to propylene glycol, acrylic acid, and other industrially important chemicals and is one of the top 12 value-added chemicals identified by the United States Department of Energy.[53] Compared to current synthetic methods to produce 3-HP, microbial production of 3-HP is more sustainable, utilizes less expensive starting materials, and generates less toxic waste.[54] Recently, *Halomonas bluephagenesis* has been engineered to convert 1,3-propanediol into 3-HP at a titer of 154 g/L, rate of 2.4 g/L h, and overall yield of 0.93 g/g.[55] *H. bluephagenesis* is an exciting microbial host because it can grow in media containing high salt concentrations and basic pH that are generally unsuitable for other organisms, enabling open air cultivation. Advancements in the microbial bioproduction of 3-HP exemplify the success achieved for the bioproduction of many other platform chemicals (*e.g.*, diamines, amino acids, and dicarboxylic acids), which have been recently reviewed elsewhere.[56]

Production by engineered microorganisms can be used to access diverse sets of chemicals. In a recent example, an enzymatic cascade was introduced into engineered *E. coli* strains to produce ten structurally distinct C3–C5 diols, six of which had not been previously accessed through microbial production.[47] Similarly, a platform strain of *E. coli* harnessed promiscuous enzymatic cascades to convert target amino acid precursors from the growth medium into ten structurally distinct short-chain primary amines (SCPAs).[52] Alternatively, platform strains can be established that utilize complex monomer substrate mixtures, such as those derived from lignin. In this vein, a single *E. coli* strain was engineered to produce adipic acid (*i.e.*, a major industrial chemical used in the production of nylon, plastics, and polyurethane foams) and branched-chain adipic acid analogs (AAAs).[57] While the production titers of AAAs in this example were lower than previously reported,[58] branched chain AAAs

---

[46] Kang A, George KW, Wang G, Baidoo E, Keasling JD, Lee TS: **Isopentenyl diphosphate (IPP)-bypass mevalonate pathways for isopentenol production**. *Metab. Eng.* 2016, **34**, 25-35.

[47] Liu Y, Wang W, Zeng A-P: **Biosynthesizing structurally diverse diols via a general route combining oxidative and reductive formations of OH-groups**. *Nat. Commun.* 2022, **13**:1595.

[48] Marella, ER, Dahlin J, Dam MI, ter Horst J, Christensen HB, Sudarsan S, Wang G, Holkenbrink C, Borodina, I: **A single-host fermentation process for the production of flavor lactones from non-hydroxylated fatty acids**. *Metab. Eng.* 2020, **61:**427-436.

[49] Zhao X, Wu Y, Feng T, Shen J, Lu H, Zhang Y, Chou HH, Luo X, Keasling JD: **Dynamic upregulation of the rate-limiting enzyme for valerolactam biosynthesis in *Corynebacterium glutamicum***. *Metab. Eng.* 2023, **77**:89-99.

[50] Chae TU, Ko Y-S, Hwang K-S, Lee SY: **Metabolic engineering of *Escherichia coli* for the production of four-, five- and six-carbon lactams**. *Metab. Eng.* 2017, **41**:82-91.

[51] Rodriguez GM, Tashiro Y, Atsumi S: **Expanding ester biosynthesis in *Escherichia coli***. *Nat. Chem. Biol.* 2014, **10**:259-265.

[52] •• Kim DI, Chae TU, Kim HU, Jang WD, Lee SY: **Microbial production of multiple short-chain primary amines via retrobiosynthesis**. *Nat. Commun.* 2021, **12**:173.
Through extensive strain engineering, ten short chain primary amines (SPCAs) were synthesized in *E. coli*. Further optimization of one strain resulted in the production of isobutylamine at 10.67 g/L, 0.36 g/L/h, and an overall yield of 0.057 g/g from glucose.

[53] Werpy T, Petersen G: **Top value added chemicals from biomass: volume I--results of screening for potential candidates from sugars and synthesis gas**. Edited by: National Renewable Energy Lab., Golden, CO (US); 2004.

[54] Della Pina C, Falletta E, Rossi M: **A green approach to chemical building blocks. The case of 3-hydroxypropanoic acid**. *Green Chem.* 2011, **13**:1624-1632.

[55] •• Jiang X-R, Yan X, Yu L-P, Liu X-Y, Chen G-Q: **Hyperproduction of 3-hydroxypropionate by *Halomonas bluephagenesis***. *Nat. Commun.* 2021, **12**:1513.
Halomonas bluephagenesis was engineered to produce 3-hydroxypropionate (3-HP) from 1,3-propanediol with very high titer (154 g/L), rate (2.4 g/L/h) and yield (0.93g/g). Since the 1,3-propanediol used in this work was generated through fermentation of corn sugar, this 3-HP was fully derived from plant biomass.

[56] Son J, Sohn YJ, Baritugo K-A, Jo SY, Song HM, Park SJ: **Recent advances in microbial production of diamines, aminocarboxylic acids, and diacids as potential platform chemicals and bio-based polyamides monomers**. *Biotechnol. Adv.* 2023, **62**:108070.

[57] Kruyer NS, Wauldron N, Bommarius AS, Peralta-Yahya P: **Fully biological production of adipic acid analogs from branched catechols**. *Sci. Rep.* 2020, **10**:1-8.

[58] Zhao M, Huang D, Zhang X, Koffas MAG, Zhou J, Deng Y: **Metabolic engineering of *Escherichia coli* for producing adipic acid through the reverse adipate-degradation pathway**. *Metab. Eng.* 2018, **47**:254-262.

could be used to access materials with novel properties while also valorizing lignin substrates. Together, platform strains show promise for the rapid diversification of molecules that can be microbially produced.

Biosynthesis of Materials:

Nearly all of the 8.3 billion metric tons of plastics produced to date were created from fossil resources.[59] The sustainable production of plastics can be achieved if components of these plastics can be produced by engineered microorganisms (**Figure 4**). Currently, the most widely used biosynthesized monomer is lactic acid (LA), which can be polymerized into polylactic acid (PLA).[60] While synthetically produced LA is a racemic mixture, microorganisms can produce nearly enantiopure L-LA, allowing for improved plastic properties. Companies, including NatureWorks, have commercialized this strategy to produce PLA. Another target for microbial bioproduction are monomers which can be polymerized into the environmentally biodegradable bioplastic polyhydroxyalkanoates (PHAs). PHAs also find use in biomedical applications where degradation within the body is required.[61] In another approach, microorganisms can biosynthesize whole polymers, decreasing the cost of downstream plastic manufacturing. For example, engineered microorganisms can produce PLA[62], and >90 genera of native bacteria have been reported to produce different PHAs that contain >150 monomeric structures.[63] Impressively, microorganisms can be engineered to increase polymer yields to ~90% of the theoretical yield and polymer content to ~90% wt% of cell mass.[64] Recent efforts have introduced additional polymerization mechanisms (*i.e.*, light-mediated free radical polymerization) into engineered microorganisms.[65] [66] However, additional efforts are required to produce these polymers without introducing synthetic components. Together, technologies for the biosynthesis of monomers and polymers have been used to establish a ~$13 billion/year global market for bioplastics (Precedence Research on Bioplastics; URL: https://www.precedenceresearch.com/press-release/bioplastics-market). While this comprises around 2% of the ~$750 billion/year global market for plastics, the bioplastics market is growing at 3.6× the rate of the plastics market (Precedence Research on Polymers; URL: https://www.precedenceresearch.com/polymers-market). In particular, bioplastics are becoming increasingly economically competitive in applications where specialty polymers are required, such as 3D printing, compostable food storage, textiles, and electronics.[67]

---

[59] Geyer R, Jambeck JR, Law KL: **Production, use, and fate of all plastics ever made**. *Sci. Adv.* 2017, **3**:e1700782.
[60] Moradali MF, Rehm BHA: **Bacterial biopolymers: from pathogenesis to advanced materials**. *Nat. Rev. Microbiol.* 2020, **18**:195-210.
[61] Zheng Y, Chen J-C, Ma Y-M, Chen G-Q: **Engineering biosynthesis of polyhydroxyalkanoates (PHA) for diversity and cost reduction.** *Metab. Eng.* 2020, **58**:82-93.
[62] Choi SY, Cho IJ, Lee Y, Park S, Lee SY: **Biocatalytic synthesis of polylactate and its copolymers by engineered microorganisms**. In *Methods Enzymology*. Edited by Bruns N, Loos K: Academic Press; 2019:125-162.
[63] Nduko JM, Taguchi S: **Microbial production of biodegradable lactate-based polymers and oligomeric building blocks from renewable and waste resources**. *Front. Bioeng. Biotechnol.* 2021, **8**:618077.
[64] Tao G-B, Pu N, Wang M-R, Li Z-J: **Hyper production of polyhydroxyalkanoates by a novel bacterium *Salinivibrio* sp. TGB11**. *Biochem. Eng. J.* 2022, **185**:108538.
[65] • Zhou Z, Maxeiner K, Ng DYW, Weil T: **Polymer chemistry in living cells**. *Acc. Chem. Res.* 2022, **55**:2998-3009.
This review highlights recent progress to produce synthetic polymers on the surface of and within living cells. Many groups have achieved the *in vivo* synthesis of polymers with distinct morphologies, including hydrogels, nanoparticles, and nanofibers.
[66] •• Geng J, Li W, Zhang Y, Thottappillil N, Clavadetscher J, Lilienkampf A, Bradley M: **Radical polymerization inside living cells**. *Nat. Chem.* 2019, **11**:578-586.
In this manuscript, a light-mediated free radical polymerization was performed within living cells. Biocompatible acrylic monomers, methacrylic monomers, and photoinitiators were uptaken into cells and polymerized upon irradiation with light (λ = 365 nm). Polymerization within cells impacted the ordering of actin filaments, highlighting potential future applications of polymer synthesis in intracellular engineering.
[67] Naser AZ, Deiab I, Darras BM: **Poly (lactic acid)(PLA) and polyhydroxyalkanoates (PHAs), green alternatives to petroleum-based plastics: a review**. *RSC Adv.* 2021, **11**:17151-17196.

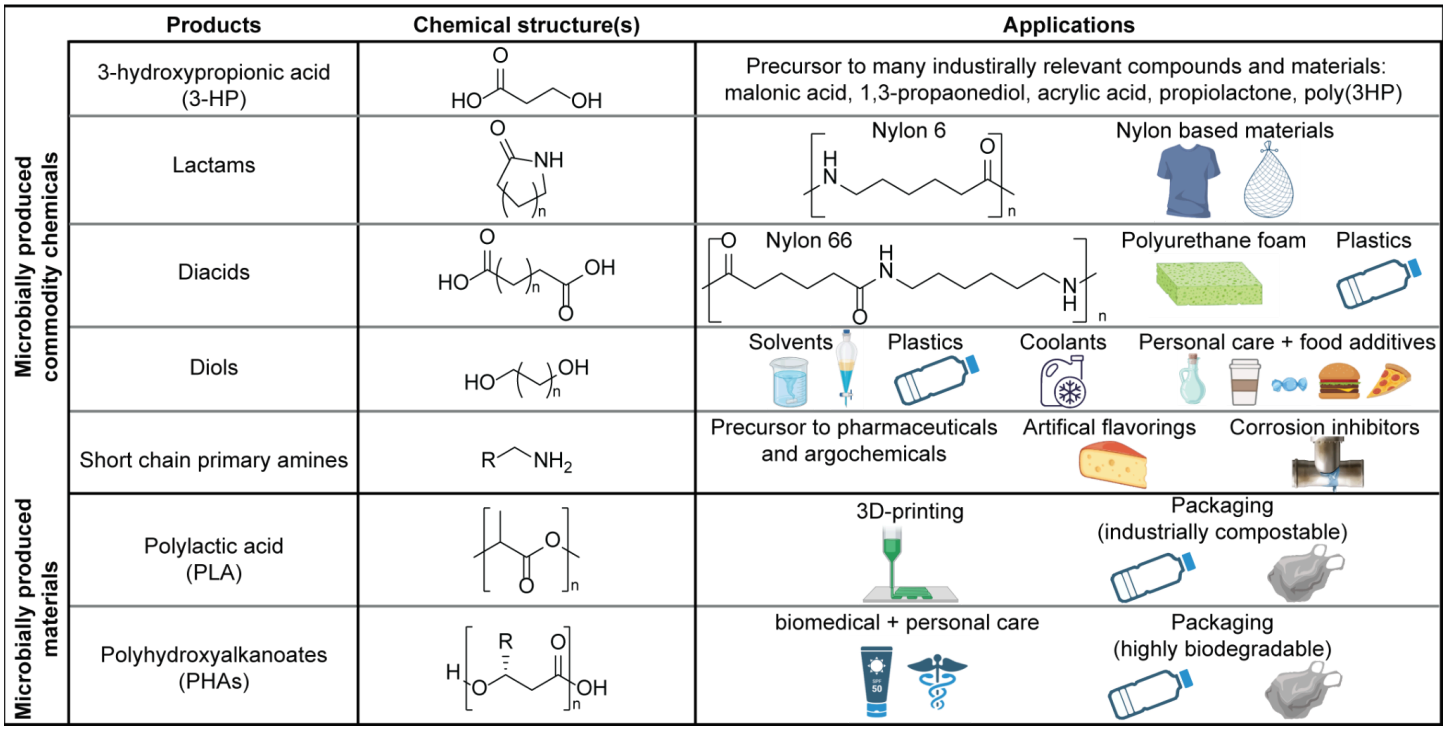

Figure 4. The names, chemical structures, and applications are listed for selected commodity chemicals and materials that can be produced using engineered microorganisms.

CONCLUSION AND OUTLOOK

Innovations in microbial fermentation continue to lower the cost of bioproduction and enable a sustainable bioeconomy with circular carbon use. Recent advances have decreased the cost of carbon and energy feedstocks and increased the efficiency of microbial conversion of these feedstocks to products. Bioproduction will continue to displace traditional chemical processes based on fossil resources in sectors with no other decarbonization alternatives or where the high selectivity of enzymes can be used to access chemicals that are difficult to produce using synthetic chemistry. Government and public support is required to make the significant infrastructure investments required for the rapid and widespread adoption of sustainable bioprocesses. To realize a bioeconomy with circular carbon use, the true cost of fossil resource use must be incorporated into the economics of chemical markets. In addition, bioprocesses will need to undergo rigorous life cycle assessments and be integrated into larger energy systems that are themselves decarbonized. Through the creative use of sustainable sources of carbon and energy at every step, bioprocesses have the potential to sequester carbon that has already been emitted into the atmosphere. Decarbonizing the global economy is a monumental task; however, accomplishing this goal is imperative to the future health of the biosphere and the stability of human society.

CONFLICT OF INTEREST STATEMENT

J.D.K. has financial interests in Amyris, Ansa Biotechnologies, Apertor Pharma, Berkeley Yeast, Demetrix, Lygos, Maple Bio, Napigen, ResVita Bio, and Zero Acre Farms. The other authors declare no competing interests.

ACKNOWLEDGEMENTS

This work was supported by the US Department of Energy (DOE) Joint BioEnergy Institute (https://www.jbei.org), supported by DOE, Office of Science, Office of Biological and Environmental

Research, under contract DEAC02-05CH11231 between DOE and Lawrence Berkeley National Laboratory, the DOE Distinguished Scientist Fellow Program, the Philomathia Foundation, National Institutes of Health grant R01 AT010593-02, and National Science Foundation grant 2036849.

DATA AVAILABILITY

No data were generated for this review article.