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A comprehensive metabolic map for production of bio-based chemicals

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Production of industrial chemicals using renewable biomass feedstock is becoming increasingly important to address limited fossil resources, climate change and other environmental problems. To develop high-performance microbial cell factories, equivalent to chemical plants, microorganisms undergo systematic metabolic engineering to efficiently convert biomass-derived carbon sources into target chemicals. Over the past two decades, many engineered microorganisms capable of producing natural and non-natural chemicals have been developed. This Review details the current status of representative industrial chemicals that are produced through biological and/or chemical reactions. We present a comprehensive bio-based chemicals map that highlights the strategies and pathways of single or multiple biological reactions, chemical reactions and combinations thereof towards production of particular chemicals of interest. Future challenges are also discussed to enable production of even more diverse chemicals and more efficient production of chemicals from renewable feedstocks.

Petrochemical refinery processes using fossil oil or natural gas as a raw material have been used to make many chemicals and materials of everyday use. Due to our increasing concerns on depletion of fossil resources, climate change and other environmental problems, there has been much interest in producing chemicals and materials from renewable non-food biomass or even directly from CO_2. Over the past two decades, biological, chemical and combination methods have been employed for the production of an increasing number of chemicals. Recent years have witnessed new regulatory rules appearing to better protect our environment. For example, the European Commission announced a new rule on banning single-use plastics, including plastic cotton buds, cutlery, plates and straws, which have widely been contaminating Europe's beaches and seas. Governments and leading chemical manufacturers around the globe have already been taking actions to establish a recyclable plastic economy[1,2]. For example, Coca-Cola has been using PlantBottle, which is a brand of poly(ethylene terephthalate) (PET) comprising bio-based monoethylene glycol derived from bioethanol and petrochemical-derived terephthalic acid (TPA). Since its introduction to the market in 2009, more than 35 billion bottles (equivalent to more than 700,000 barrels of oil) have been distributed (Supplementary Table 1). More recently, coffee and beverage companies are participating in reducing single-use plastic cups and straws, and replacing them with paper- or renewable-based materials. These movements clearly suggest that production of bio-based chemicals and materials from renewable resources is becoming essential.

In industrial biotechnology, production of industrial chemicals and materials has been explored by using microorganisms as cell factories and renewable non-food biomass as a raw material in place of petroleum[3–6] (Supplementary Table 1 and Supplementary Fig. 1). In recent years, strain development has become increasingly more efficient and effective through metabolic engineering and, more recently, through systems metabolic engineering, which integrates metabolic engineering with tools and strategies of systems biology, synthetic biology and evolutionary engineering[7]. When performing metabolic engineering, the entire bioprocess should be optimized in an integrated manner by considering the upstream (strain development), midstream (fermentation) and downstream (recovery and purification) processes all together[4,5,8]. As metabolic engineering has become increasingly powerful, the number of microbially produced chemicals using biomass as a carbon source has substantially increased[9–12] (Supplementary Tables 1–3).

In current metabolic engineering, systematic design and construction of a biosynthetic pathway has become a routine job for initially constructing a microbial strain producing the desired product. An important, but often overlooked point is that it is not necessary to pursue only biological reactions for the production of target chemicals if more efficient alternative chemical reactions are available[13] and easily implementable without causing particular environmental harm (Table 1 and Fig. 1). Here, biological reactions are those operating in microorganisms through metabolic enzymes and transporters or reactions catalysed by single or multiple enzymes in vitro, whereas chemical reactions are those taking place through any non-biological means, including the use of catalysts, solvents, acids and/or heat. Consideration of chemical reactions in addition to biological reactions certainly opens up diverse opportunities for implementing strategies for highly efficient production of target chemicals.

Here, we thoroughly examine the current status of industrial chemicals produced using biological and/or chemical reactions. Industrial chemicals and their production routes are presented in the context of central carbon metabolic pathways as these key metabolites serve as precursors for the chemicals to be produced. As a result of this compilation study, all the relevant biological and chemical reactions are visually presented in a map, namely the bio-based

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Table 1 | Competitive strengths and limitations of biological, chemical and integrated processes

Method	Competitive strengths	Limitations
Biological	• Renewable and sustainable by using biomass as a feedstock • Environmentally friendly by using less harmful chemical reagents (for example, solvents and catalysts) • Highly regio- and stereoselective for the conversion of chemicals • Smaller number of unit operations needed in general • Possible to recycle enzymes and microbial cells through various forms of repetitive fermentation processes • Relatively safer due to milder reaction conditions (for example, pressure and temperature)	• Less reproducible due to more variables (for example, cell status and cultivation media) to optimize • Biological reactions working mainly on organic molecules • More sensitive to the toxicity of a target chemical to produce • Low production performance for certain chemical categories
Chemical	• More diverse types of chemicals producible • Much faster reaction rates in general • Much greater range of optimal conditions (for example, temperature and pressure) for catalysts	• Selectivity issue in the presence of isomers of a target chemical • Harsher reaction conditions (for example, high temperature and pressure) • Greater amount of toxic, non-degradable/recyclable by-products released • Greater number of unit operations in general
Integrated	• Possible to implement the most efficient (bio)synthetic pathway for a target chemical by combining competitive strengths of both biological and chemical processes	

chemicals map available in poster format as Supplementary Data 1. This comprehensive map is expected to serve as a blueprint for the visual and intuitive inspection of biological and/or chemical reactions for the production of industrial chemicals of interest from renewable resources. There are also excellent reviews, which complement the discussions in this Review, for metabolic engineering strategies[4,7,14,15], systems biology[16–18] and synthetic biology tools[19–22].

Bio-based chemicals map comprising biological and chemical reactions

The bio-based chemicals map provided in this Review shows a complex network of biological and chemical reactions that lead to the production of various industrial chemicals. The map presents both previously explored reactions (for example, already experimentally characterized reactions) and untouched reactions (for example, novel chemicals or reactions) of a biochemical space. The chemicals that have not been explored before now have better chances of production thanks to the state-of-the-art systems metabolic engineering tools and strategies. In a way, this bio-based chemicals map is analogous to comprehensive metabolic maps presented at a global scale, such as the Roche Biochemical Pathways (https://web.expasy.org/pathways/), which unequivocally served as a platform for providing knowledge and information on cellular metabolism. However, there has been no such extensive map in the context of industrial biotechnology, to the best of our knowledge. Hence, it is hoped that our bio-based chemicals map will similarly serve as a blueprint for developing further discussions and ideas on future chemical biosynthesis campaigns through providing biological and/or chemical synthetic pathways at a global scale. Figure 1 shows a summarized version of the production processes involving both biological and chemical reactions in the bio-based chemicals map. A detailed summary of the production processes using only biological reactions is available as Fig. 2 and Supplementary Tables 2,3. Supplementary Table 2 covers studies showing the best production performance for each chemical, whereas Supplementary Table 3 additionally covers relevant earlier breakthroughs.

Production processes using biological and/or chemical reactions

Designing a microbial cell factory for the production of a particular chemical has become possible more than ever, but at the same time is challenging, as we now have access to an even greater number of metabolic reactions than those currently covered by major metabolic databases such as MetaCyc, a curated database of metabolic pathways currently covering 14,971 reactions and 14,842 metabolites (MetaCyc v. 22.0; as of 1 September 2018)[23]. As 137,416 metabolic reactions have been predicted to take place in the biochemical space according to the ATLAS of Biochemistry[24], roughly 9.2% of metabolic reactions have been experimentally described, while the remaining 90.8% of reactions in the biochemical space await characterization. The pace of predicting novel metabolic reactions, for example through retrobiosynthesis[25–27] (Fig. 3a) or enzyme design[27] (Fig. 3b), has become much faster than experimental characterization and validation. For example, a set of novel biosynthetic pathways of flavonoid pinocembrin was computationally designed using retrobiosynthesis and experimentally constructed in *Escherichia coli* by expressing heterologous genes responsible for each reaction step from an endogenous metabolite to pinocembrin[28] (Fig. 3a). As another example, bacterial cytochrome *c* was evolved to catalyse formation of carbon–silicon[29] and carbon–boron bonds[30] (Fig. 3b). Therefore, it is a great challenge to decide whether biological, chemical or combined biological–chemical production strategies should be employed to explore this unexplored region of the biochemical space.

One great example is the collective effort towards bio-based production of PET, which has been mainly produced through petrochemical processes previously. As mentioned earlier, one of its two monomers, monoethylene glycol, was successfully produced by combined biological (for the production of bioethanol) and chemical (for the conversion of bioethanol to monoethylene glycol) methods[31]. Furthermore, monoethylene glycol can now be produced by direct fermentation of microorganisms through engineering the ribulose-1-phosphate pathway[32] or Dahms pathway[33], both of which utilize xylose, the second most abundant carbon source from lignocellulosic biomass (Fig. 4a). On the other hand, TPA, the other monomer of PET, is a chemical that has been thought rather difficult to produce by biological methods. This is why several research groups in industry and academia have pursued production of 2,5-furan dicarboxylic acid or 2,5-furan dicarboxylic acid dimethyl ester, to replace TPA[34]. For the same reason, 2-pyrone-4,6-dicarboxylic acid was recently produced from glucose using engineered *E. coli*, which can also replace TPA for the synthesis of more-environmentally friendly plastic alternatives to PET[35]. Recently, it was shown that TPA can also be biologically produced from *p*-xylene

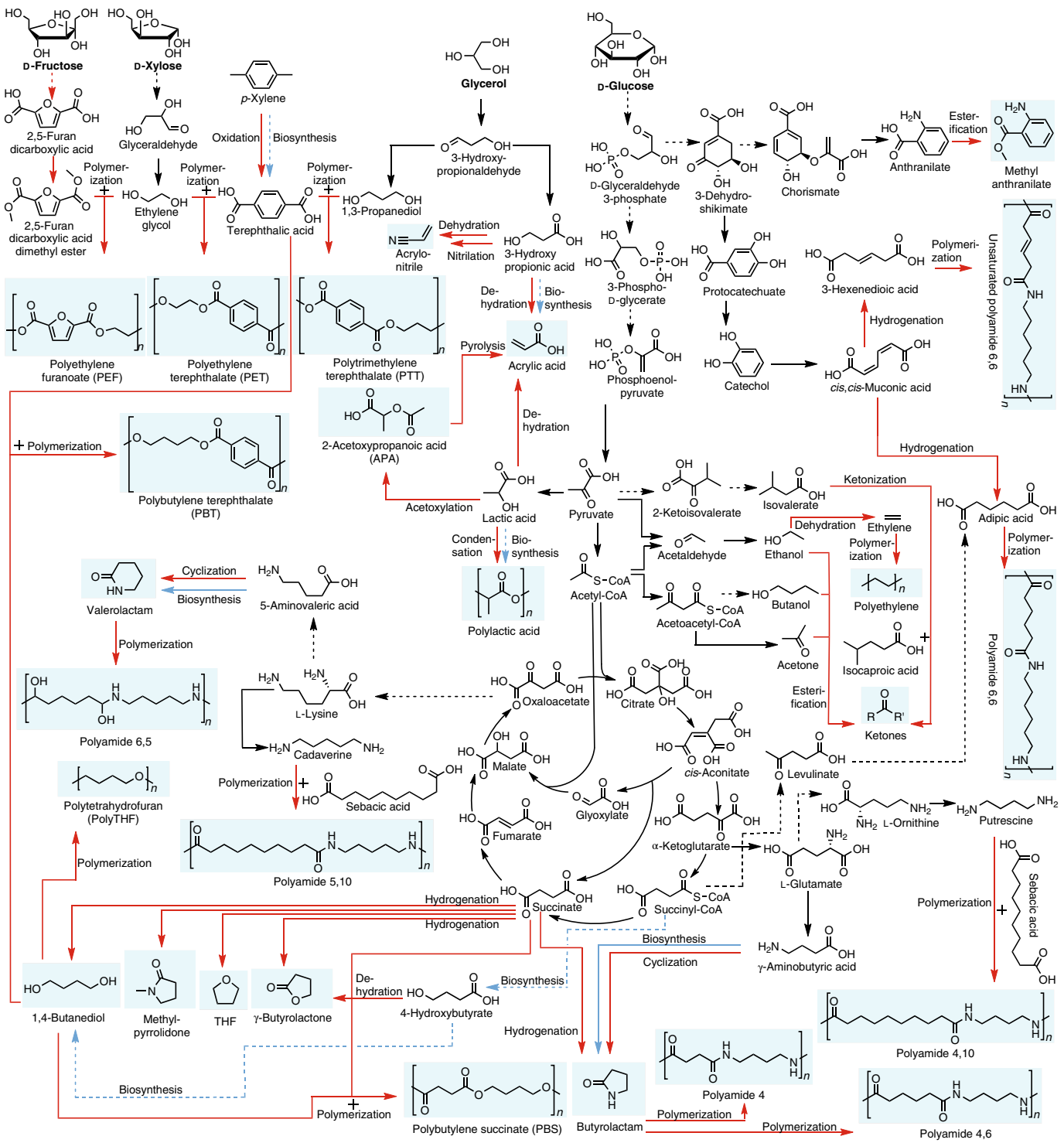

Fig. 1 | Industrial chemicals and materials produced using both biological and chemical methods. This simplified map covers 82 out of 435 chemicals and materials (boxed) presented in Supplementary Data 1. Black and red arrows represent biological and chemical reactions, respectively. Here, biological reactions refer to all those operating in microorganisms through metabolic enzymes and transporters or those catalysed by single or multiple enzymes in vitro. Chemical reactions refer to those that take place through any non-biological means, including the use of catalysts, solvents, acids and/or heat. Blue arrows represent metabolic reactions alternative to existing chemical reactions. Solid lines indicate single-step reactions while dotted lines represent multi-step reactions. Plus signs indicate polymerization of two different chemicals. The term 'polymerization' without the plus sign indicates polymerization of the same chemicals only. A full version of the bio-based chemicals map is available in poster format as Supplementary Data 1.

(Fig. 4b), which can be chemically produced from biomass[36]. This is a good example of combining chemical (biomass to *p*-xylene)[36] and biological (bioconversion of *p*-xylene to TPA)[37] methods for the production of a difficult-to-produce chemical. It is notable that two-phase partitioning fermentation of the engineered *E. coli* converted *p*-xylene to TPA with molar conversion yield of 96.7 mol% (ref. [37]; Fig. 4b), which is even higher than that (~95 mol%)[38] of the current chemical oxidation process (for example, the Amoco

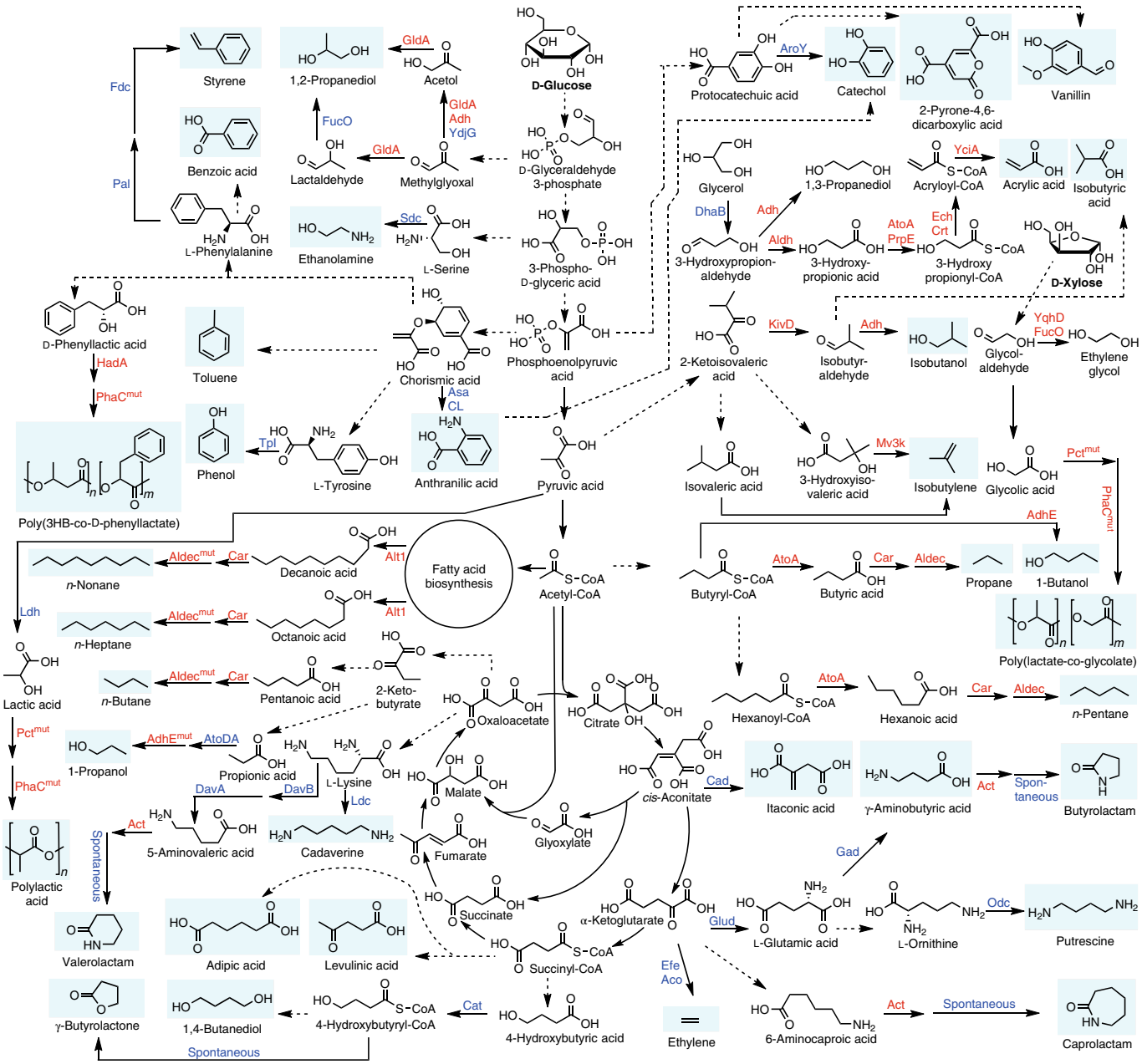

Fig. 2 | Industrial chemicals and materials produced using only biological reactions. This simplified map covers 92 out of 435 chemicals and materials (boxed) presented in the poster in Supplementary Data 1. Solid and dotted arrows indicate single and multiple reaction steps, respectively. The abbreviated enzyme names indicate enzymes with a single substrate specificity (blue) and those with a broad substrate range (red). Glucose is used as a representative carbon source towards the production of industrially relevant chemicals and materials. Xylose is another carbon source that is also often considered for the production of ethylene glycerol and poly(lactate-co-glycolate). The fatty acid biosynthetic pathway is presented as a circle to indicate a series of fatty acid chain elongation reactions. The presented abbreviated enzyme names are: Aco, aminocyclopropanecarboxylic acid oxidase; Act, β-alanine CoA transferase; Adh, alcohol dehydrogenase; AdhE, alcohol aldehyde dehydrogenase; Aldec, aldehyde decarbonylase; Aldh, aldehyde dehydrogenase; Alt1, acyl-acyl carrier protein thioesterase I; AroY, protocatechuic acid decarboxylase; Asa, anthranilic acid synthase; AtoA, acetate CoA-transferase; AtoDA, acetyl-CoA:acetoacetyl-CoA synthase; Cad, *cis*-aconitic acid decarboxylase; Car, carboxylic acid reductase; Cat, 4-hydroxybutyric acid CoA transferase; CL, chorismic acid lyase; Crt, 3-hydroxybutyryl-CoA dehydratase; DavA, δ-aminovaleramidase; DavB, L-lysine 2-monooxygenase; DhaB, glycerol dehydratase; Ech, enoyl-CoA hydratase; Efe, 2-oxoglutarate dioxygenase; Fdc, ferulic acid decarboxylase; FucO, lactaldehyde reductase; Gad, glutamic acid decarboxylase; GldA, glycerol dehydrogenase; Glud, glutamic acid dehydrogenase; HadA, isocaprenoyl-CoA:2-hydroxyisocaproate CoA-transferase; KivD, α-keto isovaleric acid decarboxylase; Ldc, L-lysine decarboxylase; Ldh, lactic acid dehydrogenase; Mv3k, mevalonic acid 3-kinase; Odc, ornithine decarboxylase; Pal, phenylalanine ammonia lyase; Pct, propionyl-CoA transferase; PhaC, poly(3-hydroxyalkanoate) polymerase; PrpE, propionate-CoA ligase; Sdc, serine decarboxylase; Tpl, tyrosine phenol-lyase; YciA, acyl-CoA thioester hydrolase; YdjG, methylglyoxal reductase; and YqhD, glycolaldehyde reductase. A full version of the bio-based chemicals map is available in poster format as Supplementary Data 1.

process). In the future, it is likely that an engineered microorganism capable of fermentatively producing TPA directly from glucose will be developed.

Biological production of polylactic acid (PLA) is another relevant example where biological reactions play an increasingly greater role; PLA is currently one of the most popular bio-based plastics in

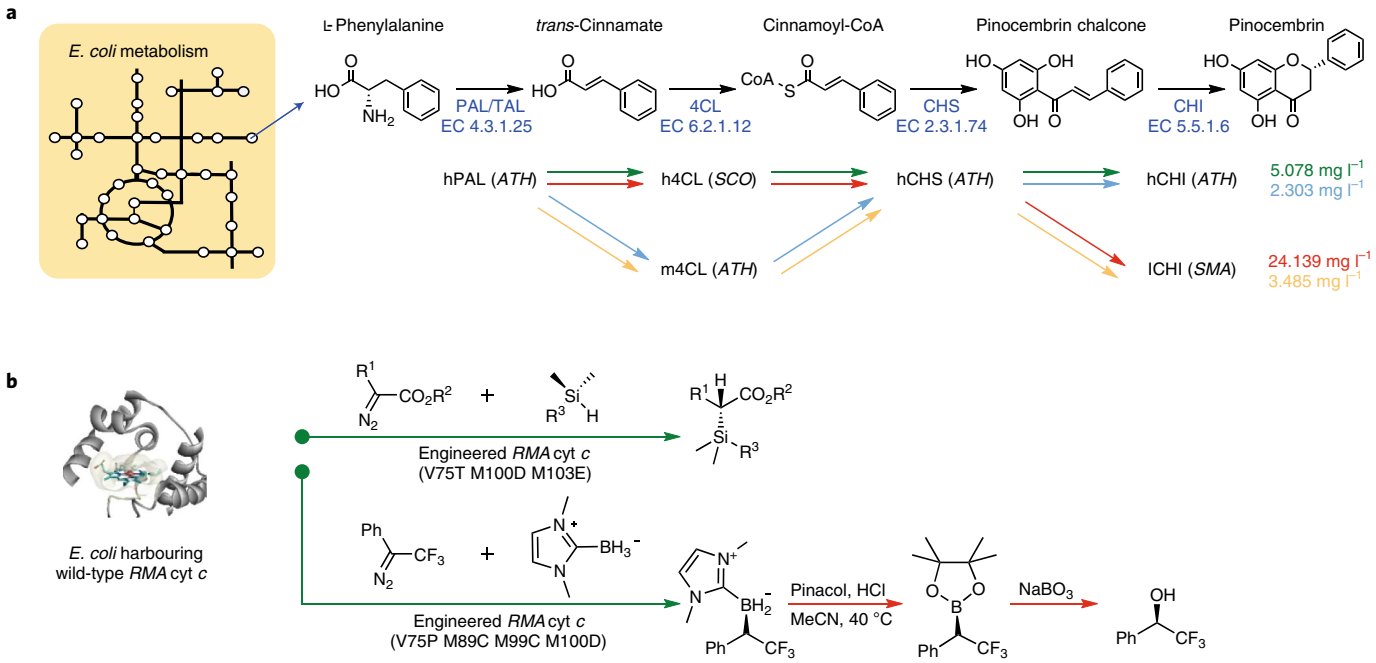

Fig. 3 | Novel biosynthetic pathways by retrobiosynthesis and enzyme engineering. a, Design of a biosynthetic pathway of pinocembrin, a plant flavonoid, in *E. coli* using a retrobiosynthesis tool RetroPath[28]. Retrobiosynthesis uses biochemical reaction rules to design a biosynthetic pathway that bridges a target chemical to a starting metabolite through a series of metabolic reactions. The novel biosynthetic pathway was experimentally validated by using heterologous genes responsible for each reaction step. Genes responsible for the second and fourth reaction steps were adopted from two different organisms each, which gave four different gene combinations for the biosynthesis of pinocembrin. Pinocembrin production titres from each gene combination are indicated with different colours below the structure of pinocembrin. Enzyme names are: 4CL, 4-coumaroyl-CoA ligase; CHI, chalcone isomerase; CHS, chalcone synthase; and PAL/TAL, phenylalanine/tyrosine ammonia lyase. Organism names are: *ATH*, *Arabidopsis thaliana*; *SCO*, *Streptomyces coelicolor*; *SMA*, *Streptomyces maritimus*. **b**, Engineering of cytochrome *c* from *Rhodothermus marinus* (*RMA* cyt *c*) to catalyse the formation carbon–silicon[29] and carbon–boron bonds[30] in *E. coli*. An engineered *RMA* cyt *c* (V75T M100D M103E) catalyses the formation of 20 different silicon-containing products from a single mutant protein, which represents an example of enzyme promiscuity. Organoboron products produced by another engineered *RMA* cyt *c* (V75P M89C M99C M100D) can be converted to further diverse boron-containing derivatives using subsequent chemical processes. The shown structure is a motif found in compounds useful for the treatment of cancer and neurodegenerative diseases. Green and red arrows indicate biological and chemical synthesis, respectively.

use with annual production of about 180,000 metric tonnes worldwide[39] (Supplementary Table 1). Currently, PLA is produced in two-step processes; low-pH yeast fermentation for lactic acid production followed by a series of chemical processes involving formation of a lactide dimer and subsequent ring-opening polymerization of lactide to produce PLA[40]. This is a great success story of combining biological and chemical methods to produce an industrial polymer. However, PLA production has become even better through the development of metabolically engineered *E. coli* capable of producing PLA by one-step direct fermentation from glucose[41] (Fig. 4c; see below for the expansion of this technology for the production of even more diverse non-natural polymers).

The above examples, however, are not yet universally or easily applicable for the production of more diverse chemicals and materials of interest. There are biological reactions that are not economically or technically feasible, or there are no known enzymes for catalysing the intended conversion reactions. In the former case, it is usually more efficient to deploy chemical reactions unless enzymes and pathways leading to the production of a desired chemical are improved to satisfy the conversion rate and yield. As a representative example, the final steps in converting biologically produced artemisinic acid to artemisinin, an antimalarial drug, involve a series of efficient chemical reactions because enzymes responsible for the conversion between the two chemicals are unknown[42] (Fig. 4d). In the latter case, new enzymes and pathways need to be experimentally identified or developed to realize biological production. For example, 1,6-hexanediamine is an industrially important monomer for the manufacture of polyamide 6,6 and polyamide 6,10 (Fig. 4e). Although biological reaction steps for 1,6-hexanediamine have been proposed in silico[43], the enzymes and pathways have not yet been experimentally validated to be efficient enough to replace the existing chemical production process (Fig. 4e). Further developments are needed in such areas. Nonetheless, there have been a number of great examples of the production of chemicals and materials from renewable biomass using biological, chemical or combined methods. These examples are classified and organized with respect to their key starting intermediate metabolites (Supplementary Data 1).

Chemicals derived from intermediates of central carbon metabolic pathways

Major industrial chemicals that can be derived from the key precursor metabolites of glycolysis, pentose phosphate pathway and TCA cycle[44] are presented in our comprehensive poster (Supplementary Data 1 and Supplementary Note 1) and explained below. Emphasis was given to two categories: direct biological production of chemicals and chemical conversion of biologically produced chemicals. Also, description on physicochemical treatments of fermentable sugars derived from lignocellulosic biomass (Supplementary Data 1a) is available in Supplementary Note 2.

From glyceraldehyde-3-phosphate and 3-phospho-D-glycerate of glycolysis. In the glycolytic pathway, glyceraldehyde-3-phosphate

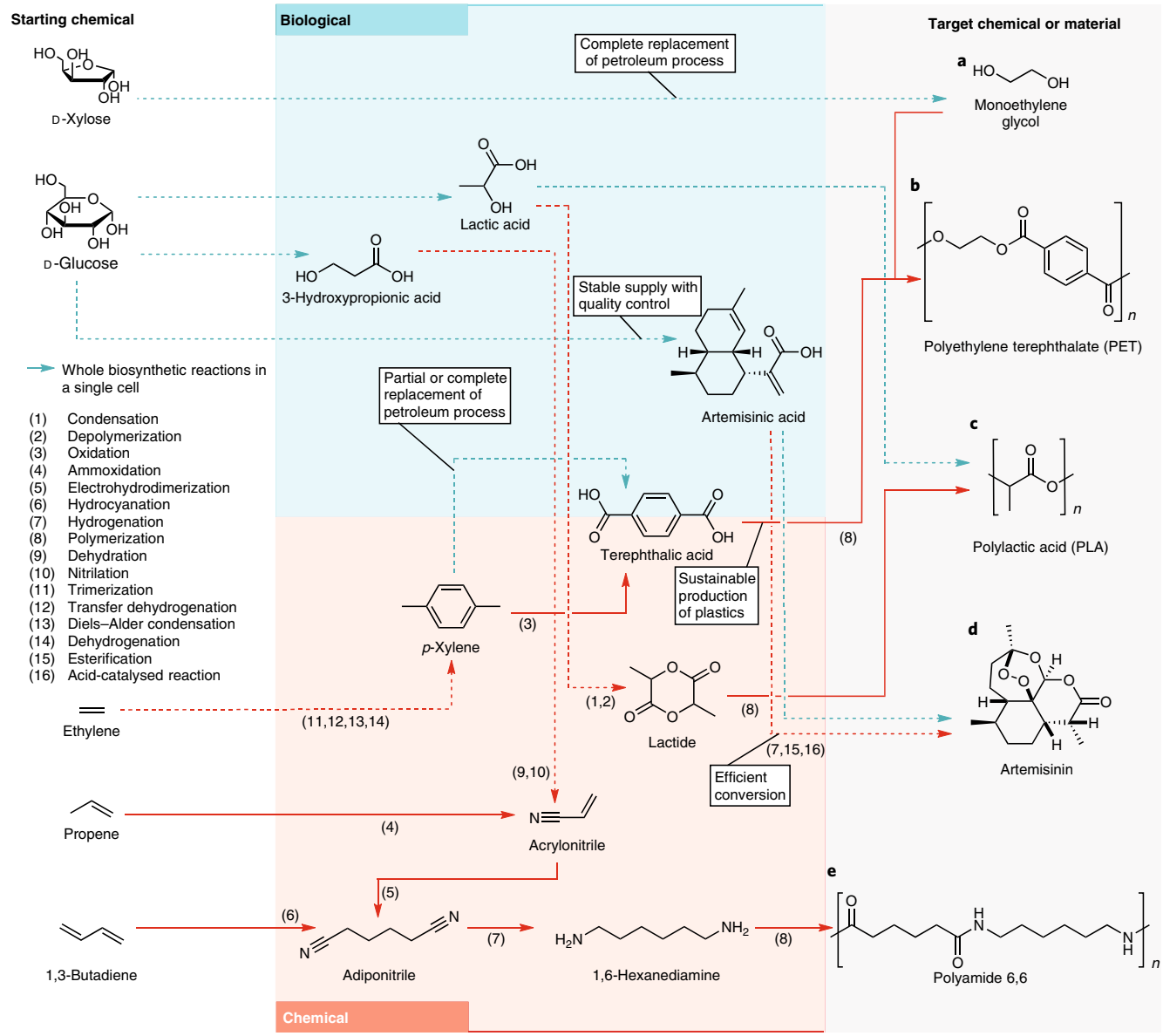

Fig. 4 | Production processes using biological and/or chemical reactions. Five representative industrial chemicals and materials are presented, which can be produced using biological, chemical and combined biological and chemical methods. **a**, Monoethylene glycol can be produced from xylose, the second most abundant carbon source from lignocellulosic biomass, using only biological reactions. **b**, Terephthalic acid (TPA) can now be produced from *p*-xylene using biological reactions in place of existing chemical reactions. TPA is polymerized with monoethylene glycol to produce PET. **c**, PLA has been produced using combined biological and chemical methods where its monomer lactic acid is biologically produced first, and chemically polymerized to PLA. PLA can now be produced from glucose using a single biological process. **d**, Production process of artemisinin uses combined biological and chemical methods where artemisinic acid, a precursor of artemisinin, is biologically produced from glucose and subsequently converted to artemisinin using chemical means. **e**, Polyamide 6,6 production is currently feasible only using chemical reactions as enzymes do not exist to yield 1,6-hexanediamine.

and 3-phospho-D-glycerate serve as important precursors for the biosynthesis of various industrial chemicals: 1,2-propanediol[45,46], 1,3-propanediol[47], 3-hydroxypropionic acid[48,49], acrylic acid[50] and 1-propanol[51] from glyceraldehyde-3-phosphate; and ethanolamine and 5-aminolevulinic acid[52] from 3-phospho-D-glycerate (Supplementary Data 1b).

1,2-Propanediol and 1-propanol. 1,2-Propanediol, also known as propylene glycol, can be biologically produced. For example, *Clostridium thermosaccharolyticum* producing 9.0 g l^{-1} of 1,2-propanediol with a yield of 0.20 g g^{-1} glucose and productivity of 0.36 g l^{-1} h^{-1} was reported a long time ago[45] (Fig. 2, Supplementary Tables 2,3). More recently, *Corynebacterium glutamicum* was metabolically engineered for 1,2-propanediol production by expressing heterologous genes encoding methylglyoxal synthase, glycerol dehydrogenase and alcohol dehydrogenase from *E. coli*[46] (Supplementary Data 1b). Also, using this 1,2-propanediol-producing pathway, further engineering was performed to produce 1-propanol in *C. glutamicum*[46] and *E. coli*[51].

1,3-Propanediol. 1,3-Propanediol is an important monomer of polytrimethylene terephthalate (PTT) with TPA being the other. Various microbial strains belonging to *Clostridia, Enterobacter, Escherichia, Klebsiella*, *Lactobacilli* and *Saccharomyces* have been engineered to

produce 1,3-propanediol. Among these, a metabolically engineered *E. coli* strain capable of producing 135 g l^{-1} of 1,3-propanediol with a productivity of 3.5 g l^{-1} h^{-1} has been successfully commercialized[47] (Supplementary Table 1). In this strain, glucose is converted to glycerol via glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatase, both from *Saccharomyces cerevisiae*, and glycerol to 3-hydroxypropanal mediated by glycerol dehydratase and glycerol reactivase, both from *Klebsiella pneumoniae*[47]. Finally, 1,3-propanediol is produced from 3-hydroxypropanal using 1,3-propanediol oxidoreductase (Supplementary Data 1b).

3-Hydroxypropionic acid, acrylonitrile and further derivatives. 3-Hydroxypropionic acid is a precursor of several important industrial chemicals including acrylonitrile, acrylic acid and methylacrylic acid (Supplementary Data 1b). As 3-hydroxypropionic acid shares a biosynthetic pathway with 1,3-propanediol, similar metabolic engineering strategies have been applied to its production. 3-Hydroxypropionic acid can be produced by conversion of 3-hydroxypropanal using aldehyde dehydrogenase. This has been implemented in *C. glutamicum* by the introduction of *K. pneumoniae* 1,2-propanediol dehydratase and its reactivase, and *Cupriavidus necator* mutant aldehyde dehydrogenase[53]. The engineered *C. glutamicum* strain was able to produce 62.6 g l^{-1} of 3-hydroxypropionic acid with a yield of 0.51 g g^{-1} using glucose as a carbon source[48]. So far, the highest titre (83.8 g l^{-1}) of 3-hydroxypropionic acid was achieved using the engineered *K. pneumoniae* and glycerol as a carbon source[49]. 3-Hydroxypropionic acid has been attracting much attention as a precursor for acrylic acid (see 'Acrylic acid'). It can also be further converted by a chemical process into acrylonitrile, a major monomer of polyacrylonitrile-based carbon fibres; recently, over 90% molar yield of acrylonitrile was achieved by dehydration and nitrilation of microbially produced 3-hydroxypropionic acid with ammonia over an inexpensive TiO_2 solid acid catalyst[54]. This integrative process also prevented the generation of cyanide, a main by-product from a well-established chemical process utilizing propylene ammoxidation[54]. Acrylonitrile can be further converted into adiponitrile, a precursor for 1,6-hexanediamine through electrohydrodimerization[55] (Fig. 4e). Amination, oxidation and hydrogenation of 3-hydroxypropionic acid can further lead to the generation of 3-hydroxypropylamine, malonic acid and 1,3-propanediol, respectively[31] (Supplementary Data 1b).

Acrylic acid. Recently, an *E. coli* strain capable of producing 3-hydroxypropionic acid was further engineered to produce 0.12 g l^{-1} of acrylic acid from glucose[50] (Fig. 2, Supplementary Tables 2,3). The titre achieved clearly suggests that the biological method is much inferior to an existing chemical process such as oxidation of propylene for the synthesis of acrylic acid[56]. In addition to the low titre achieved, acrylic acid toxicity to the cells will remain a big challenge for biological production. Thus, it is notable that there was an attempt to produce acrylic acid by combined biological and chemical methods. 3-Hydroxypropionic acid produced biologically by *Lactobacillus reuteri* was dehydrated to acrylic acid over TiO_2 catalyst with a molar yield of over 95% (ref. [57]). Because 3-hydroxypropionic acid can be efficiently produced by fermentation, this combined method of chemically converting 3-hydroxypropionic acid to acrylic acid is much more competitive than one-step fermentative production of acrylic acid. Acrylic acid can also be produced from dehydration of lactic acid[58], but this chemical process is not as efficient as the dehydration of 3-hydroxypropionic acid. Esterification of chemically or biologically generated acrylic acid further generates methylacrylic acid[55] (Supplementary Data 1b).

From pyruvate in glycolysis. Pyruvate also serves as an important precursor metabolite for the production of a variety of commodity chemicals, including lactic acid[59], ethanol[60], 2,3-butanediol[61,62] and isobutanol[63] (Supplementary Data 1c,d).

Lactic acid, 1-propanol, PLA, PLGA and other derivatives. Lactic acid (2-hydroxypropionic acid) is an important organic acid as a food additive, solvent and a monomer for various polymers (Supplementary Data 1c). Microbial production of lactic acid, a representative industrial fermentation product, has reached near the theoretical maximum yield. For example, fermentation of *Bacillus* sp. WL-S20 led to the production of 225 g l^{-1} of lactic acid with a yield of 99.3% (g g^{-1} glucose) and a productivity of 1.04 g l^{-1} h^{-1} (ref. [59]). Also, various fermentation strategies have been attempted in order to increase lactic acid productivity[64]. For example, fermentation of *Lactobacillus rhamnosus* using a two-stage bioreactor with membrane cell recycling produced 92 g l^{-1} of lactic acid with a productivity of 57 g l^{-1} h^{-1} (ref. [65]). Lactic acid can also be converted to other derivatives or used as a monomer of industrially important polymers such as PLA. For example, lactic acid can be converted to propionic acid and then to 1-propanol using a bifunctional Pt/Nb_2O_5 catalyst that is responsible for the two-step conversions[66]. Since the pK_a value of lactic acid is 3.86, purification of lactic acid produced by fermentation requires acidification after the fermentation. During such a recovery process, large amounts of by-products such as gypsum are generated. To avoid this problem and reduce the purification cost, low-pH-tolerant yeast strains were engineered[67].

As briefly described above, two-step processes have been employed for the production of PLA because there had been no known enzymes and pathways for PLA biosynthesis. The above-mentioned low pH-tolerant yeast fermentation process is used for lactic acid production, followed by chemical processes involving lactide (dimer of lactate) formation and ring opening polymerization[40]. As mentioned earlier, however, metabolically engineered bacterial strains were developed for the one-step direct fermentative production of PLA[41] and also more recently poly(lactate-co-glycolate) (PLGA)[68] (Supplementary Table 1). PLGA is a Food and Drug Administration (FDA)-approved polymer useful for various medical applications, thanks to its biodegradability and biocompatibility. To accomplish production of non-natural polymers PLA and PLGA by engineered bacteria, two enzymes were evolved to establish a novel biosynthetic pathway. First, propionyl-CoA transferase was engineered to more efficiently accept lactic acid as a substrate and convert it to lactyl-CoA. Second, polyhydroxyalkanoate (PHA) synthase was evolved to polymerize lactyl-CoA as a substrate. When the two genes of these evolved enzymes were expressed in *E. coli* with additional optimization of metabolic fluxes in other parts of metabolic pathways, PLA and other lactate-containing polyesters could be produced by one-step direct fermentation from glucose. More recently, the same engineering scheme plus establishment of flux-controllable pathways producing glycolic and lactic acids, led to the development of *E. coli* strains capable of PLGA and other lactate- and glycolate-containing polyesters having diverse material properties[68,69]. One such example is a terpolyester, poly(lactate-co-glycolate-co-4-hydroxybutyrate) showing an elongation at break of 769%, which is considerably higher than that of PLGA (9%), and therefore might be useful as a flexible biomedical polymer[68].

Ethanol, ethylene and monoethylene glycol. Ethanol is the most abundantly produced fermentation product for its use in alcoholic beverages and as biofuel from renewable biomass (Supplementary Data 1d). As with other industrial bioproducts, improving the host strain's tolerance to a high concentration of ethanol is critical. Due to the complex mechanisms behind the product tolerance, rational random approaches[4] (for example, random mutagenesis under rationally constrained screening or selection condition, genome shuffling[70] and global transcription machinery engineering, gTME[71]) have often been undertaken to improve the production

host's tolerance to a wide range of chemicals. For example, genome shuffling was successfully applied to a newly isolated *S. cerevisiae* strain SM-3 to enhance its ethanol productivity, thermotolerance and ethanol tolerance by exposing protoplasts to ultraviolet radiation and implementing a recursive protoplast fusion[70]. In another study, gTME was applied to *S. cerevisiae* to substantially improve its osmotolerance and ethanol tolerance through mutagenesis of a global transcription factor, Spt15p (ref. [71]). Microbial production of ethanol has reached its near theoretical yield using *S. cerevisiae*[60] (Supplementary Tables 2,3), which allows the use of ethanol as a starting material for the synthesis of various derivative chemicals of industrial importance by chemical methods. Ethylene is one such chemical that can be produced by catalytic dehydration of biologically produced ethanol in place of the convectional steam cracking of hydrocarbon feedstocks[72]. Ethylene can be further converted to polymers and other chemicals such as polyethylene and monoethylene glycol for the PET production as mentioned above. Recently, such green polyethylene has been commercialized to make plastic bottles, containers and bottle caps.

2,3-Butanediol, butanone and 1,3-butadiene. 2,3-Butanediol is another industrial chemical of importance, which has been used as a plasticizer, pharmaceutical ingredient and agricultural chemical. It can be produced by several different microorganisms, including *Klebsiella* spp., *Enterobacter aerogenes* and *Pseudomonas chlororaphis*, and is produced from pyruvate as a key intermediate metabolite (Supplementary Data 1c). For example, a newly isolated strain of *K. pneumoniae*, SDM, produced 150 g l^{-1} of 2,3-butanediol through its fed-batch fermentation using an optimized medium and without any gene manipulation[61]. Heterologous production hosts also showed competitive production performances, such as an engineered *S. cerevisiae* producing 154.3 g l^{-1} of 2,3-butanediol with a yield of 0.404 g g^{-1} glucose (80.7% of its theoretical yield)[62]. Among these high-performance strains, *Klebsiella* spp. have been more widely adopted as 2,3-butanediol producers, mainly due to their competitive advantages in natural high-performance production of 2,3-butanediol without any metabolic engineering: fast growth rate, simple cultivation conditions and a wide range of utilizable carbon sources[61]. However, it should be noted that some *Klebsiella* species are opportunistic pathogens and thus the genes involved in pathogenicity should be removed from the genome for developing final industrial fermentation strains. Microbially produced 2,3-butanediol can also be chemically converted to several derivative chemicals such as butanone (also known as methyl ethyl ketone) and 1,3-butadiene, which have various industrial applications such as solvent, welding agent, synthetic rubber and polymers. Both butanone and 1,3-butadiene can be produced through dehydration of 2,3-butanediol[73]. 1,3-Butadiene can also be generated from 1,3-butanediol[74] or 1,4-butanediol[75] as a starting chemical through different chemical dehydration reactions. Furthermore, 1,3-butanediol was recently produced by metabolically engineered *E. coli* from glucose[76] and also from syngas or other gaseous carbon sources including CO and/or CO_2 in addition to methanol[77] (Supplementary Data 1c).

Isobutanol, isobutylene and p*-xylene.* 2-Ketoisovalerate, a metabolite four enzymatic steps away from pyruvate, serves as a precursor for the formation of L-valine and L-leucine as well as isobutanol (Supplementary Data 1c). Isobutanol is an important industrial solvent and a gasoline additive, which can be biologically produced from 2-ketoisovalerate through two-step enzymatic conversion involving 2-ketoisovalerate decarboxylase and alcohol dehydrogenase[63]. By increasing 2-ketoisovalerate formation through the removal of competing pathways and replacing an endogenous acetolactate synthase with a heterologous enzyme of higher catalytic efficiency, an engineered *E. coli* strain could produce 22 g l^{-1} of isobutanol in flask culture. Fermentation of this engineered *E. coli* strain in a bioreactor with in situ product removal led to the enhanced production of isobutanol up to 50.8 g l^{-1} (ref. [78]). Dehydration of isobutanol by a chemical process allows synthesis of isobutylene, which is an important precursor of fuel additives such as methyl *tert*-butyl ether (MTBE) and ethyl *tert*-butyl ether (ETBE)[79]. Isobutylene can be further converted to *p*-xylene, a precursor of TPA discussed above, through a chemical process involving dehydration, oligomerization and dehydrocyclization[80].

From acetyl-CoA, which connects glycolysis and the TCA cycle. Acetyl-CoA is one of the versatile metabolites in a metabolic network and is formed from several different metabolites such as pyruvic, acetic and fatty acids. It is also a starting metabolite of major metabolic pathways such as the TCA cycle, biosynthesis of fatty acids, isoprenoids and polyketides, and formation of malate through glyoxylate shunt. Furthermore, acetyl-CoA is involved in many different CoA transfer reactions. Thus, acetyl-CoA can be an important metabolite for the biosynthesis of a variety of industrial chemicals, polymers and biofuels[15] (Supplementary Data 1d,e).

Acetone, butanol, ethanol and their derivatives. Acetone–butanol–ethanol (ABE) fermentation, one of the oldest (>100 years) industrial fermentation processes[81], uses acetyl-CoA as a starting material in *Clostridium* species (Supplementary Data 1d). From ABE, butanol can be used as a great biofuel, fuel additive and industrial solvent, but microbial production of butanol has been limited to about 20 g l^{-1} due to butanol toxicity[82]. Due to the lack of a solution to increase butanol tolerance, fermentation has been performed by coupling with various in situ recovery techniques, such as adsorption, gas stripping, pervaporization and liquid–liquid extraction[83] to increase the butanol production performance (Supplementary Tables 2,3). Another problem in clostridial ABE fermentation is that acetone cannot be used as a biofuel, while butanol and ethanol can be used as a fuel mixture. In one study, the *Clostridium beijerinckii* gene encoding a primary/secondary alcohol dehydrogenase responsible for converting acetone to isopropanol was expressed in an engineered *Clostridium acetobutylicum* to produce only biofuels comprising isopropanol, butanol and ethanol (IBE)[84]. In another study, biofuels were produced by catalytic conversion of ABE produced by fermentation of *C. acetobutylicum*. ABE was first extracted from the fermentation broth, and was subjected to Pd/C alkylation to produce ketones with varied carbon lengths suitable as fuel[85] (Supplementary Data 1d).

Fatty acids, fatty alcohols and fatty acid-derived biofuels. Fatty acids and their various derivatives are important sources of not only chemicals and polymers, but also biofuels such as gasoline, diesel and jet-fuel[86]. With initial successes in microbial production of bioethanol and biodiesel[87], a more diverse range of fuels has been produced using biological methods. This movement is well aligned with a roadmap of the International Energy Agency (IEA) in Paris, which aims at utilizing biofuels up to 27% of global transport fuels by 2050[88]. So far, *E. coli*[89] and *S. cerevisiae*[90] have played as major hosts for producing fatty acids and their derivatives through engineering of a fatty acid biosynthetic pathway and/or a (reverse) β-oxidation pathway[91]. For example, various engineered *E. coli* strains were developed that could produce 6.6 g l^{-1} of long-chain fatty acids (for example, mainly C_{16} and C_{18})[92], 0.42 g l^{-1} of fatty alcohols (for example, C_6 to C_{10} alcohols)[92], 0.30–2.80 mg l^{-1} of selective short-chain alkanes (for example, C_3, C_4, C_5, C_7 and C_9)[93], 0.58 g l^{-1} of gasoline-range alkanes (for example, C_9, C_{12}, C_{13} and C_{14})[94] and 8.6 g l^{-1} of total fatty acids[89] (Supplementary Data 1e). Establishment of synthetic metabolic pathways through the introduction of heterologous enzymes in production hosts also allowed production of various fatty acid derivatives with industrial value[87,95], such as non-natural hydrocarbons or alka(e)nes[96], fatty alcohols[97]

and fatty esters[98] (including fatty acid methyl esters, FAMEs[99], and fatty acid ethyl esters, FAEEs[100]) (Supplementary Data 1e). For example, FAEEs, diesel fuel alternatives of high potential, were produced at 1.5 and 0.52 g l^{-1} by using metabolically engineered *E. coli* and *S. cerevisiae*, respectively, where wax-ester synthase was introduced to promote condensation of acyl-CoAs and ethanol[101,102] (Supplementary Table 3). FAEEs are known to have high energy density and low toxicity to the host strain.

However, the titres, yields and productivities achieved with these engineered *E. coli* and *S. cerevisiae* have been rather low; for example, the highest titre of fatty acids ranging from C_{10} to C_{18} reported so far was 10.4 g l^{-1} using an engineered *S. cerevisiae* strain[103]. Thus, another strategy of employing host strains capable of naturally accumulating large amounts of lipids (mainly triacylglycerols) was pursued. Such oleaginous microorganisms employed for the production of fatty acid-based biofuels include *Rhodococcus opacus*[104], *Rhodosporidium toruloides*[105] and *Yarrowia lipolytica*[99]. One of the best performances was achieved with metabolically engineered *Y. lipolytica*, which could produce 98.9 g l^{-1} of lipids (measured as FAMEs) with a yield of 0.27 g g^{-1} glucose and a productivity of 1.30 g l^{-1} h^{-1} (ref. [99]) (Supplementary Data 1e). Here, $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase, the pyruvate–oxaloacetate–malate cycle and the non-oxidative glycolytic pathway were introduced to increase the levels of NADPH and acetyl-CoA, which are required for lipid biosynthesis.

Another noteworthy oleaginous organism is microalgae capable of naturally accumulating large amounts of lipids under nutrient starvation conditions and through photosynthesis[106]. Metabolic engineering strategies have also been applied to microalgae in order to improve the lipid accumulation without sacrificing biomass generation[106]. For example, a negative transcriptional regulator of lipid accumulation in *Nannochloropsis gaditana* was newly identified using RNA-Seq analysis under nitrogen starvation conditions. Downregulation of this transcriptional regulator allowed the strain's lipid productivity to double (from 2.5 g m^{-2} d^{-1} to 5.0 g m^{-2} d^{-1}) without negatively affecting the strain's growth under semicontinuous cultivation[107]. In another case, a highly efficient targeted genome editing method based on meganucleases and transcription-activator-like effector nucleases was developed for diatom *Phaeodactylum tricornutum*. The method was used to delete UDP-glucose pyrophosphorylase gene in a *P. tricornutum* strain, which led to a 45-fold increase in lipid content, compared with the control strain[108]. A thorough review of recent studies can be found elsewhere[106] and relevant companies commercializing the use of microalgae for lipid production can be found in Supplementary Table 1.

In addition to use as biofuels, fatty acids themselves are important products in food, nutrition and pharmaceutical industries, and also serve as monomers for various types of polymers such as polyurethane, polyester, polyether, polyolefin, polyamides, epoxies and polyols, which are thoroughly discussed in other review papers[109,110].

Isoprenoids, polyketides and biofuels derived from them. There have been many examples of metabolic engineering studies on the production of isoprenoids and polyketides, which can be used as various functional compounds in food, nutrition, medicine, cosmetics and agricultural industries. The strategies employed for biological and chemical production of these isoprenoid and polyketide compounds are not covered in this paper, and readers can consult many excellent papers cited in a recent review paper[111]. Here, we focus on biofuels and many other industrial chemicals derived from isoprenoids and polyketides[86,111]. Isoprenoids have been widely utilized as therapeutic agents and natural flavouring or fragrance compounds, and various forms of isoprenoids have been biologically produced through mevalonate and/or 1-deoxy-D-xylulose 5-phosphate pathways (Supplementary Data 1d). The simplest isoprenoid compound is isoprene, which is widely used as an alternative for gasoline and its greatest production titre has been reported as 60 g l^{-1} (with a high productivity of 2 g l^{-1} h^{-1}) using a metabolically engineered *E. coli*[112] (Supplementary Data 1d). Pinene, a natural bicyclic monoterpene compound, was also produced by an engineered *E. coli* strain harbouring a heterologous hybrid mevalonate pathway comprising geranyl diphosphate synthase from *Abies grandis* and pinene synthase from *Pinus taeda*[113] (Supplementary Data 1d). Fed-batch fermentation of this engineered *E. coli* strain produced 0.97 g l^{-1} of pinene. Biologically produced pinene can be further subjected to chemical dimerization to generate pinene dimer, a high-energy-density biosynthetic jet fuel[114]. So far, metabolic engineering of *S. cerevisiae* has led to more industrially competitive production titres of isoprenoid-derived biofuels, for example, 130 g l^{-1} of farnesene by fed-batch fermentation using a 200,000 l bioreactor[115] and 5.2 g l^{-1} of bisabolene by fed-batch fermentation using a 2 l bioreactor[116] (Supplementary Data 1d). As a successful industrial application, isoprenoid-based jet fuel through blending with fossil-based jet fuel has been commercially developed for flights (Supplementary Table 1).

Polyketides are a group of secondary metabolites that are naturally produced by a special group of microorganisms such as actinomycetes (for example, *Streptomyces* species) and fungi (for example, *Aspergillus* spp.)[111]. Genes responsible for the biosynthesis of polyketides and other types of secondary metabolites are often clustered in one location of a chromosome, known as a biosynthetic gene cluster. Although polyketides have mainly been used for medical purposes, such as antibiotics, anticancer agents and immunosuppressants, they have also been considered as important precursors of industrial chemicals and biofuels through engineering of polyketide synthases[117,118]. For example, introduction of an iterative type I polyketide synthase with fine-tuned expression of its cognate thioesterase in *E. coli* produced pentadecaheptaene by fed-batch fermentation, which was subsequently subjected to chemical hydrogenation to produce 140 mg l^{-1} of pentadecane[119] (Supplementary Data 1e). In another study, a type I modular polyketide synthase was further engineered to produce short-chain ketones and adipic acid. For short-chain ketones, 3.2 mg l^{-1} of 3-pentanone and 4.1 mg l^{-1} of 2-butanone were produced by adopting varied acyltransferase domains in polyketide synthase heterologously expressed in *E. coli*[120]. Adipic acid was generated in vitro by recombining borrelidin producing polyketide synthase, spinosyn-producing polyketide synthase and thioesterase from 6-deoxyerythronolide B synthase[121]. It will be interesting to see how polyketide biosynthetic machineries are further engineered and polyketide biosynthetic pathways are modularly reconstructed in vivo for the production of diverse chemicals. Readers can consult an excellent review paper on polyketide pathway engineering for the production of biofuels and chemicals[122].

From D-erythrose 4-phosphate in the pentose phosphate pathway. D-Erythrose 4-phosphate in the pentose phosphate pathway is a starting metabolite (together with phosphoenolpyruvate) for the biosynthesis of aromatic amino acids and is converted to chorismate through a series of reactions. Chorismate is a key branch point for the biosynthesis of L-tryptophan, L-phenylalanine, L-tyrosine and other metabolites of industrial value, including benzoic acid[123], styrene[123], catechol[123], *p*-aminobenzoic acid[123], *p*-hydroxybenzoic acid[123], resveratrol[123], toluene[124], phenol[125] and anthranilic acid[126] (Fig. 2 and Supplementary Data 1f), representatives of which are described below.

Aromatic compounds and aromatic polyesters. Anthranilate and prephenate are two immediate downstream metabolites of chorismate in the biosynthetic pathway of aromatic amino acids. Anthranilate leads to the biosynthesis of L-tryptophan and serves as a chemical intermediate for the synthesis of perfumes, dyes and

pharmaceuticals. Anthranilate could be produced to 14 g l^{-1} from glucose using an engineered *E. coli* strain, which has a mutation in the *trpD* gene encoding a variant enzyme having anthranilate synthase activity without anthranilate phosphoribosyl transferase activity, and expresses *aroG*^{fbr} encoding a feedback inhibition resistant variant of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase and *tktA* encoding transketolase[126]. Catechol, another aromatic chemical serving as a precursor to pesticides, flavours and fragrances, can be produced from anthranilate. In a study involving the expression of *Pseudomonas aeruginosa* anthranilate 1,2-dioxygenase, the abovementioned anthranilate-overproducing *E. coli* could produce 4.47 g l^{-1} of catechol from glucose[127].

Prephenate is a precursor metabolite of L-phenylalanine and L-tyrosine, which can be converted to many aromatic chemicals of industrial importance. Phenol recently produced using metabolically engineered *E. coli* is one exemplary chemical that can be derived from prephenate through the L-tyrosine biosynthetic pathway[125] (Fig. 2). In this study, two negative regulators were knocked down and the genes involved in the tyrosine biosynthetic pathway together with the *Pasteurella multocida* tyrosine phenol-lyase gene (*TPL*) were overexpressed for the production of phenol from glucose. Phenol is known to be very toxic to most production hosts, constituting a severe challenge for its efficient microbial production. To overcome this toxicity problem, glycerol tributyrate was used as a phenol extractant for in situ extractive fed-batch fermentation for phenol production. As a proof-of-concept demonstration, this bi-phasic cultivation allowed production of 3.79 g l^{-1} of phenol. Optimal production of L-tyrosine, a direct precursor of phenol, will further improve the production of phenol using *E. coli*[128]. More recently, an alternative phenol biosynthetic pathway was designed where 4-hydroxybenzoate decarboxylase was recruited to convert 4-hydroxybenzoate to phenol and CO_2. The gene encoding this enzyme as well as those encoding 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase and chorismate pyruvate lyase were subsequently integrated in the genome for stable gene expression. Two-phase extractive fermentation with glycerol tributyrate was also applied to the final engineered strain, resulting in production of 9.51 g l^{-1} phenol from glucose[129].

Resveratrol, a natural antioxidant compound used as a food supplement and cosmetic ingredient, has also been successfully produced using microorganisms, which use L-tyrosine or L-phenylalanine as an intermediate precursor. Resveratrol was produced from glucose through L-tyrosine as an intermediate precursor using an engineered *S. cerevisiae* that heterologously expressed genes encoding tyrosine ammonia lyase, 4-coumaryl-CoA ligase and resveratrol synthase. This engineered *S. cerevisiae* produced 415.65 mg l^{-1} of resveratrol from glucose[130]. For resveratrol biosynthesis through L-phenylalanine, the genes encoding phenylalanine ammonia lyase, cinnamic acid hydroxylase, 4-coumaryl-CoA ligase and resveratrol synthase were heterologously expressed in *S. cerevisiae*. Subsequent strain development, which involved optimization of the transfer of electrons to cytochrome P450 monooxygenase, increase of the precursor supply and decrease of the pathway intermediate degradation, led to the production of 800 mg l^{-1} of resveratrol[131]. Bio-based fermentative production of resveratrol by engineered yeast has been commercialized (Supplementary Table 1).

As an extended application, these prephenate-derived aromatic chemicals have been explored for microbial production of novel aromatic polyesters such as poly(3-hydroxybutyrate-co-D-phenyllactate). An engineered *E. coli* strain optimized for D-phenyllactate production also expressing isocaprenoyl-CoA:2-hydroxyisocaproate CoA-transferase and evolved PHA synthase genes were able to produce an aromatic-containing polyester, poly(3-hydroxybutyrate-co-D-phenyllactate), by one-step direct fermentation from glucose[132] (Fig. 2). This study also demonstrated that more diverse aromatic polyesters can be biologically produced by using different aromatic monomers, resembling the strategies described for various lactate-containing polymers above. It will be interesting to see if such aromatic-containing polymers will show material properties suitable for replacing petroleum-derived aromatic polymers (such as PET) of current industrial use in large amounts.

From α-ketoglutarate in the TCA cycle. Several metabolites serve as important precursors in the TCA cycle for the biosynthesis of a wide range of important chemicals. In this section, biological production of amino acids and polyamide monomers derived from α-ketoglutarate is described (Supplementary Data 1g).

Amino acids and derivatives. Several proteinogenic amino acids (that is, L-glutamate, L-proline, L-glutamine and L-arginine) derived from α-ketoglutarate have been subjected to extensive studies for microbial overproduction[12]. Among these amino acids, the production process of monosodium glutamate (MSG) that has evolved from chemical to biological methods is noteworthy[133]. MSG was initially produced by direct chemical synthesis involving acrylonitrile as a starting material and preferential crystallization for the optical resolution of DL-glutamic acid during 1962 to 1973. However, fermentative production of MSG using *C. glutamicum*, first introduced in 1956, has surpassed the chemical process and is now established as the favoured method for its reduced production costs and environmental problems. L-Glutamine is an amino acid that can be produced by an enzymatic conversion of L-glutamate using glutamine synthetase. For the overproduction of L-glutamine, a sulfaguanidine-resistant mutant of *C. glutamicum* was isolated capable of producing more than 40 g l^{-1} of glutamine from 100 g l^{-1} glucose in 48 hours[134].

L-Arginine is a semi-essential amino acid with a wide range of applications as a supplement for food, pharmaceuticals and cosmetics. Biological production of L-arginine had mainly been performed by fermentation of mutant *C. glutamicum* strains obtained through random mutagenesis. Recently, a high-performance L-arginine producer was developed by systems metabolic engineering of *C. glutamicum*[135]. Upon initial implementation of random mutagenesis, systems metabolic engineering strategies were subsequently conducted, which involved removing repressors in the L-arginine biosynthetic pathway, optimizing intracellular levels of NADPH, disrupting precursor exporters and optimizing rate-controlling L-arginine biosynthetic reactions. The resulting rationally designed strain was able to produce 92.5 g l^{-1} of L-arginine with a high yield of 0.40 g per g carbon source (glucose and sucrose) by fed-batch culture in a five-litre fermentor. It is notable that this strain was able to produce 81.2 g l^{-1} of L-arginine with a yield of 0.35 g per g carbon source (glucose and sucrose) in a 1,500-litre demo-plant scale fermentor[135]. This study emphasizes the importance of validating the final strain's production performance at demo-plant scale or at least in pilot-plant scale in addition to the lab-scale experiments because production strains might show different production performances across different cultivation scales. In this regard, the final *C. glutamicum* strain developed in this study might have been subjected to additional around of metabolic engineering (for example, trouble-shooting metabolic engineering) if it showed drastically varied production performances across lab-scale and large-scale fed-batch fermentations (which was not the case in the L-arginine producer).

Non-proteinogenic amino acids and derivatives. C. glutamicum has also been explored for the overproduction of several non-proteinogenic amino acids such as γ-aminobutyric acid[136] and L-ornithine[137] derived from L-glutamate. γ-Aminobutyric acid has a wide range of applications in pharmaceuticals, feeds and polyamide 4 synthesis. By overexpressing the gene encoding glutamate decarboxylase that converts L-glutamate to γ-aminobutyric acid in L-glutamate-overproducing *C. glutamicum*, γ-aminobutyric acid could be produced to

a high titre of 199.29 g l^{-1} from glucose[138]. The microbially produced γ-aminobutyric acid can be chemically converted to butyrolactam (also known as 2-pyrrolidone), a monomer of polyamide 4, through a reaction with Al_2O_3 as a catalyst in toluene[139]. More recently, a single enzymatic step of converting γ-aminobutyric acid to butyrolactam was also demonstrated using *E. coli*[140,141]. L-Ornithine, an important chemical with food and pharmaceutical applications, is largely produced by fermentation using L-arginine- or L-citrulline-auxotrophic *C. glutamicum* mutant strains where mutagenesis plays a crucial role for the strain development[142]. By contrast, rational metabolic engineering of *C. glutamicum* was recently conducted, which led to the high production titre of 51.5 g l^{-1} of L-ornithine with a productivity and yield of 1.29 g l^{-1} h^{-1} and 0.24 g g^{-1} glucose[137]. In this study, competing pathways were removed, arginine biosynthetic gene cluster was overexpressed and the NADPH pool was enriched to enhance the production of L-ornithine. Putrescine, another derivative of L-glutamate and L-ornithine, is a four-carbon diamine monomer of polyamides and has also been subjected to extensive metabolic engineering effort. The first biological production of putrescine was demonstrated using engineered *E. coli*[143]. The engineered *E. coli* strain was constructed by overexpressing putrescine biosynthetic genes (*argABCDE* and *speC*), deleting competing pathways (*speEG*, *puuPA* and *argI*) and deleting a global transcriptional regulator (*rpoS*). Programmable synthetic small RNAs[144], which allow multiplexed regulation of gene expression levels, were further applied to optimize the expression levels of *argF* and *glnA* genes for the enhanced putrescine production. The resulting strain produced 42.3 g l^{-1} of putrescine with productivity of 1.27 g l^{-1} h^{-1} and a yield of 0.26 g g^{-1} glucose[145]. The same strategy of optimizing expression levels of *argF* and *glnA* was also applied to an *E. coli* base strain producing L-proline. The L-proline-producing *E. coli* base strain was first constructed by removing L-proline utilization pathway (*putA*, *proA* and *putP*) and by deleting competing pathway toward L-ornithine (*speC* and *speF*). Upon application of sRNAs targeting the *argF* and *glnA*, the resulting *E. coli* strain produced the highest titre of 33.8 g l^{-1}.

From succinic acid in the TCA cycle. Succinic acid, one of the four-carbon (C_4) dicarboxylic acids in the TCA cycle, is an important industrial chemical product itself as a surfactant, ion chelator and monomer for polymers, and also serves as a platform intermediate chemical for a variety of other industrial chemical derivatives (Supplementary Data 1h).

Succinic, fumaric and malic acids. Succinic acid has been most extensively studied using several different production hosts including *Actinobacillus succinogenes*[146], *Anaerobiospirillum succiniciproducens*[147], *C. glutamicum*[148], *Mannheimia succiniciproducens*[149] (and also *Basfia succiniciproducens*[150], which is very similar to *M. succiniciproducens*), *Pichia kudriavzevii*[151], *S. cerevisiae*[152] and *Y. lipolytica*[153] strains. These microbial strains were metabolically engineered to overproduce succinic acid to high concentrations with high yields and productivities as detailed in a recent review paper[154]. Two strategies, homo-succinic acid production and low-pH yeast fermentation, are emphasized here. *M. succiniciproducens*, one of the native succinic acid-overproducing microorganisms, was metabolically engineered for efficient succinic acid production without any by-products, which is critical for reducing the high costs of succinic acid recovery and purification. As a proof-of-concept demonstration, combination of genome-scale metabolic simulation and consequent target gene engineering together with carbon source utilization engineering allowed production of homo-succinic acid to 69.2 g l^{-1} with a productivity of 2.50 g l^{-1} h^{-1}. This strain's productivity was further increased to 38.6 g l^{-1} h^{-1} using a membrane cell recycle bioreactor system[149]. Production of homo-succinic acid was obviously beneficial for reducing recovery and purification costs, making it unnecessary to employ low-pH fermentation.

Nonetheless, low-pH-tolerant strains were also employed for succinic acid production as in the case of lactic acid production discussed above. *P. kudriavzevii* was considered as a succinic acid production host as it is known to be tolerant to multiple stresses including low pH, high salt concentration and high temperature[151]. Metabolic engineering of *P. kudriavzevii* led to the production of 48.2 g l^{-1} of succinic acid with productivity and yield of 0.97 g l^{-1} h^{-1} and 0.45 g g^{-1}, respectively, by batch fermentation at pH 3.0. However, yeast strains in general appeared to show lower productivity than other succinic acid producers such as *M. succiniciproducens*. Several companies around the world are commercializing biological succinic acid production (Supplementary Table 1).

Succinic acid produced biologically can be further converted chemically to various derivatives, including 1,4-butanediol[155], tetrahydrofuran[156], γ-butyrolactone[157] and *N*-methyl-pyrrolidone[158], and even polymers including polybutylene succinate and polyamides (Supplementary Table 1). 1,4-Butanediol can also be biologically produced using a systematically engineered *E. coli* (see the next section)[159]. During 1,4-butanediol production, γ-butyrolactone was found to be secreted as a by-product, suggesting that γ-butyrolactone can also be biologically produced through applying different metabolic engineering strategies, if needed.

Fumaric and malic acids, two downstream metabolites of succinic acid in the TCA cycle, have been reported at much higher production concentrations when using native overproducing microorganisms than model organisms such as *E. coli* and *S. cerevisiae*. For example, the wild-type *Rhizopus nigricans* strain efficiently produced up to 130 g l^{-1} of fumaric acid with productivity of 0.92 g l^{-1} h^{-1} (ref. [160]). Model organisms such as *E. coli*[161] and *S. cerevisiae*[162] were also used as production hosts for fumaric acid production to possibly overcome disadvantages of the unique morphological characteristics of *Rhizopus* species that hampers oxygen transfer in a large-scale bioreactor. However, these engineered strains appeared to be not as competitive as the *Rhizopus* overproducers. For example, an engineered *E. coli* produced 41.5 g l^{-1} of fumaric acid[163]. In the case of malic acid, the wild-type *Aspergillus flavus* strain produced 113 g l^{-1} of malic acid with productivity and yield on glucose of 0.59 g l^{-1} h^{-1} and 0.94 g g^{-1}, respectively[164]. Adaptive laboratory evolution of another native malic acid producer *Ustilago trichophora* and subsequent medium optimization achieved production of 196 g l^{-1} of malic acid with productivity and yield on glycerol of 0.39 g l^{-1} h^{-1} and 0.82 g g^{-1}, respectively[165]. Model organisms such as *E. coli*[166] and *S. cerevisiae*[167] were also used for malic acid production, but the production performances were inferior to those of the above fungal strains; for example, 59 g l^{-1} of malic acid was produced using an engineered *S. cerevisiae* strain[167]. Fumaric acid can be chemically converted to its ester forms such as methylhydrogenfumarate, dimethylfumarate and ethylhydrogenfumarate, which all have medical applications, especially as a drug[168]. Malic acid can also be chemically and biologically polymerized to polymalic acid, which is also used medically as a drug carrier[169].

Derivatives of C_4 dicarboxylic acids in the TCA cycle. C_4 dicarboxylic acids in the TCA cycle can be further derivatized to various industrial platform chemicals, including 1,4-butanediol[159], 1,2,4-butanetriol[170], levulinic acid[171] and adipic acid[172], using pathways involving additional metabolic enzymes. As briefly mentioned above, *E. coli* was engineered to produce 1,4-butanediol by constructing a novel biosynthetic pathway starting from succinyl-CoA and using 4-hydroxybutyric acid as an intermediate, with the help of computational pathway predictions. From an extensive metabolic engineering campaign to achieve optimal metabolic fluxes toward 1,4-butanediol, the final engineered *E. coli* strain capable of producing over 125 g l^{-1} of 1,4-butanediol was developed[159]. The product can be converted chemically to other important chemicals, including 1,3-butadiene[173], tetrahydrofuran[174], γ-butyrolactone[175] and *N*-methyl-2-pyrrolidone[176].

As another example, adipic acid is a bulk chemical that can also be derived from succinyl-CoA in the TCA cycle. It serves as a monomer of polyamide 6,6, one of the most widely used polymers in everyday life. Adipic acid can be produced through an engineered reverse β-oxidation pathway[177,178] or a reverse adipate-degradation pathway[172], both using succinyl-CoA as a precursor in *E. coli* (Fig. 2). A recently developed engineered *E. coli* strain, BL21(DE3), equipped with the reverse adipate-degradation pathway comprising heterologous β-ketothiolase, 3-hydroxyacyl-CoA dehydrogenase, 3-hydroxyadipyl-CoA dehydrogenase, 5-carboxy-2-pentenoyl-CoA reductase and adipyl-CoA synthetase, all adopted from *Thermobifida fusca*, was able to produce 68.0 g l^{-1} of adipic acid[172]. It should be noted that adipic acid can also be produced through a completely different pathway involving D-erythrose 4-phosphate from the pentose phosphate pathway, 3-deoxy-D-arabino-heptulosonate-7-phosphate, 3-dehydroshikimate and *cis*,*cis*-muconic acid (Supplementary Data 1f). Recently, biological conversion of *cis*,*cis*-muconic acid to adipic acid was demonstrated using metabolically engineered *S. cerevisiae*, thanks to the discovery of novel enoate reductase that can convert *cis*,*cis*-muconic acid to adipic acid[179]; this is comparable to a chemical process that converts *cis*,*cis*-muconic acid to adipic acid via a hydrogenation reaction.

From oxaloacetate in the TCA cycle. Oxaloacetate, another TCA cycle metabolite, can also be converted to various chemicals, including 1-propanol[180], butanol[63], 2-methyl-1-butanol[63], 3-methyl-1-pentanol[181], β-alanine[182], 3-hydroxypropionic acid[183], malonic acid[183], 1,3-diaminopropane[184], cadaverine[185], 5-aminovaleric acid[186], glutaric acid[186] and valerolactam[140,187] (Supplementary Data 1i). All these chemicals can now be biologically produced only using microorganisms (Supplementary Tables 2,3).

Alcohols and their derivatives. 2-Ketobutyrate, which can be biosynthesized from oxaloacetate via L-threonine pathway, serves as a precursor of several alcohols such as 1-propanol[180], butanol[63], 2-methyl-1-butanol[63] and 3-methyl-1-pentanol[181]. All these alcohols could be produced from 2-ketobutyrate by using 2-ketoisovalerate decarboxylase and alcohol dehydrogenase having broad substrate ranges[63,181], which were also used for the production of isobutanol discussed above (Supplementary Data 1c). In particular, metabolic flux toward 2-methyl-1-butanol was further optimized by enhancing its biosynthesis pathway and removing competing pathways, resulting in producing 1.25 g l^{-1} of 2-methyl-1-butanol from flask cultivation of *E. coli*[188]. In another example, a previously developed L-threonine overproducing *E. coli* strain was used to produce 1-propanol[180]. The genes encoding (*R*)-citramalate synthase, feedback-resistant L-threonine dehydratase, acetate kinase A, acetyl-CoA:acetoacetyl-CoA synthase and mutant alcohol dehydrogenase were overexpressed in the L-threonine-overproducing strain, and the resulting strain produced 10.8 g l^{-1} of 1-propanol from glucose (yield of 0.107 g g^{-1}) and 10.3 g l^{-1} of 1-propanol from glycerol (yield of 0.259 g g^{-1})[180]. Subsequent dehydrogenation of this 1-propanol leads to the formation of propylene, one of the most important starting chemicals for a variety of products in the petrochemical industry[189].

Odd-carbon-number monomers for polymer synthesis. Additional downstream derivatives of oxaloacetate are odd-carbon-number monomers that have been used for the synthesis of various polymers. These monomers include 3-hydroxypropionic acid (C_3 hydroxy acid), malonic acid (C_3 dicarboxylic acid), glutaric acid (C_5 dicarboxylic acid), β-alanine (C_3 amino acid), 5-aminovaleric acid (C_5 amino acid), valerolactam (C_5 lactam), 1,3-diaminopropane (C_3 diamine) and cadaverine (C_5 diamine)[9]. Production of β-alanine was first demonstrated using *E. coli* harbouring heterologous L-aspartate-α-decarboxylase[182]. 3-Hydroxypropionic acid[183,190] and malonic acid[183] can be further biosynthesized from β-alanine as a precursor. 3-Hydroxypropionic acid can also be produced using 3-hydroxypropanal or malonyl-CoA as a precursor (Supplementary Data 1b,e). 5-Aminovaleric acid and glutaric acid are also biologically producible now, both using engineered *E. coli* with an L-lysine conversion pathway from *Pseudomonas putida*[191,192]. Recently, an L-lysine-overproducing *C. glutamicum* strain was metabolically engineered for the enhanced production of 5-aminovaleric acid[186,193,194] and glutaric acid[195], resulting in 39.9 g l^{-1} of 5-aminovaleric acid[194] and 90.0 g l^{-1} of glutaric acid[195], respectively, by fed-batch fermentation. Also, fermentative production of valerolactam was recently demonstrated in engineered *E. coli* by using newly discovered enzymes that can convert 5-aminovaleric acid to valerolactam[140,187]. Furthermore, bio-based production of 1,3-diaminopropane (C_3 diamine) was demonstrated in an engineered *E. coli* strain expressing heterologous genes encoding 2-ketoglutarate 4-aminotransferase and L-2,4-diaminobutanoate decarboxylase[184].

For cadaverine, a monomer of polyamides, its bio-based production was demonstrated using several strains including *E. coli*[196] and *C. glutamicum*[197,198] by engineering an L-lysine downstream pathway. The highest production titre of cadaverine achieved was 103.78 g l^{-1} using an engineered *C. glutamicum*[198]. In this study, the *E. coli ldcC* gene encoding L-lysine decarboxylase was integrated into a chromosome of an industrial lysine-overproducing strain of *C. glutamicum*, PKC, in order to convert L-lysine to cadaverine. The *lysE*-encoding L-lysine exporter was also disrupted to concentrate carbon fluxes towards cadaverine. This microbially produced cadaverine was subsequently purified and polymerized with sebacic acid to synthesize bio-based polyamide 5,10 with a high thermal resistance[198]. Bio-based polyamide 5,10 is currently commercially available (Supplementary Table 1).

Future challenges

Industrial biotechnology is a highly multidisciplinary field as can be seen from the bio-based chemicals map and relevant discussions above. In order to firmly establish industrial biotechnology platforms in chemical production, several challenges need to be overcome. First, metabolic and biochemical engineers need to more actively interact with synthetic chemists, chemical engineers and (bio)process engineers, especially those from industrial partners[199], to develop an optimal biosynthetic and/or chemical route for a target chemical. In particular, a systematic method of comparing biological reactions versus chemical reactions for the conversion of given chemicals needs to be developed with respect to raw materials costs, direct fixed capital costs, operation costs, and technical feasibility, which can be collectively called as technoeconomic analysis. Active interactions among experts with complementary research backgrounds and knowledge will help implement more creative and efficient (bio)synthetic pathways and, ultimately, an optimal process.

Second, more powerful computational methods and tools need to be developed in order to further optimize the process for target chemical production. Obviously, development of the (bio)processes for the production of chemicals and materials solely by experiments requires too much time, money and effort. For example, a framework based on a deep neural network was recently developed to design synthesis schemes for small organic molecules[200]. Such a framework can be applied with sufficient modification to more systematically design (bio)synthetic pathways of a variety of industrial chemicals. Novel computational methods and tools can be effectively combined with those that have been widely used in systems metabolic engineering, including genome-scale metabolic modelling[201], retrobiosynthesis[25] and protein-structure modelling[202] to bring about a breakthrough in strain development campaigns.

Third, the discussions in this paper have so far focused on biosynthetic pathways starting from fermentable sugars that mainly come from lignocellulosic biomass. The scope of carbon sources

needs to be further extended to reflect pathways that utilize a more diverse range of carbon sources, including food wastes (including fatty acids), methane, CO_2 and syngas[203]. Much effort is being exerted worldwide to develop microbial cell factories and chemical processes for utilizing such raw materials in the production of bio-based chemicals and materials.

Fourth, use of freshwater in fermentation needs to be smartly managed. Most microorganisms are cultured in a fermentation medium based on freshwater. Considering the forthcoming freshwater shortage, it is our responsibility to consider use and reuse strategies for the freshwater needed for fermentation. As an alternative, use of much more abundant seawater as a fermentation medium basis can be considered. As discussed in a recent article, the use of halophilic microorganisms can be a good alternative to use seawater instead of freshwater[204]. However, freshwater-based fermentations of microorganisms will likely prevail for the foreseeable future to achieve the titres, yields and productivities of chemicals and materials of interest. More studies are needed to decipher the range of chemicals and materials that can be efficiently produced using halophilic microorganisms.

Fifth, sufficient dialogue among all stakeholders is needed to successfully establish the bio-based economy. Government, industry, scientists, engineers and the public alike need to appreciate the importance of moving toward achieving sustainable society and at the same time participate in designing the circular economy system. Considering the whole value chain from the raw material (for example, biomass) to bio-based products, a proper profit-sharing model also needs to be established. Most importantly, all the stakeholders need to communicate transparently to avoid unnecessary pullbacks as we have seen from the genetically modified organisms case.

The bio-based chemicals map accompanying this paper covers all the achievements made through biological, chemical or combined biological and chemical methods. At the same time, the bio-based chemicals map provides open opportunities for producing a more diverse range of industrial chemicals by creatively designing new methods based on the strategies presented. Although the number of processes for bio-based production of chemicals that can compete or replace the current petrochemical processes is limited, this situation is likely to change as the strategies reviewed in this paper further advance and evolve to make bio-based chemical production more efficient. With growing concerns on environmental problems and climate change and increasing regulatory rules preventing the use of fossil-derived chemicals and materials, it is evident that bio-based chemicals production will be essential for the future of the chemical industry. This also aligns well with at least four of the United Nation's 17 Sustainable Development Goals, "affordable and clean energy", "sustainable cities and communities", "responsible consumption and production" and "climate change", through which bio-based chemicals production contributes to the sustainability of our world.

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References

1. *A European Strategy for Plastics in a Circular Economy* (European Commission, 2018).
2. The future of plastic. *Nat. Commun.* **9**, 2157 (2018).
3. Yang, D., Cho, J. S., Choi, K. R., Kim, H. U. & Lee, S. Y. Systems metabolic engineering as an enabling technology in accomplishing sustainable development goals. *Microb. Biotechnol.* **10**, 1254–1258 (2017).
4. Lee, J. W. et al. Systems metabolic engineering of microorganisms for natural and non-natural chemicals. *Nat. Chem. Biol.* **8**, 536–546 (2012).
5. Chubukov, V., Mukhopadhyay, A., Petzold, C. J., Keasling, J. D. & Martin, H. G. Synthetic and systems biology for microbial production of commodity chemicals. *NPJ Syst. Biol. Appl.* **2**, 16009 (2016).
6. Clomburg, J. M., Crumbley, A. M. & Gonzalez, R. Industrial biomanufacturing: the future of chemical production. *Science* **355**, aag0804 (2017).
7. Lee, S. Y. & Kim, H. U. Systems strategies for developing industrial microbial strains. *Nat. Biotechnol.* **33**, 1061–1072 (2015).
8. Lee, J. W., Kim, T. Y., Jang, Y. S., Choi, S. & Lee, S. Y. Systems metabolic engineering for chemicals and materials. *Trends Biotechnol.* **29**, 370–378 (2011).
9. Jang, Y. S. et al. Bio-based production of C2–C6 platform chemicals. *Biotechnol. Bioeng.* **109**, 2437–2459 (2012).
10. Sarria, S., Kruyer, N. S. & Peralta-Yahya, P. Microbial synthesis of medium-chain chemicals from renewables. *Nat. Biotechnol.* **35**, 1158–1166 (2017).
11. Pfleger, B. F., Gossing, M. & Nielsen, J. Metabolic engineering strategies for microbial synthesis of oleochemicals. *Metab. Eng.* **29**, 1–11 (2015).
12. Becker, J. & Wittmann, C. Advanced biotechnology: metabolically engineered cells for the bio-based production of chemicals and fuels, materials, and health-care products. *Angew. Chem. Int. Ed. Engl.* **54**, 3328–3350 (2015).
13. Rudroff, F. et al. Opportunities and challenges for combining chemo- and biocatalysis. *Nat. Catal.* **1**, 12–22 (2018).
14. Zhang, M. M., Wang, Y., Ang, E. L. & Zhao, H. Engineering microbial hosts for production of bacterial natural products. *Nat. Prod. Rep.* **33**, 963–987 (2016).
15. Krivoruchko, A., Zhang, Y., Siewers, V., Chen, Y. & Nielsen, J. Microbial acetyl-CoA metabolism and metabolic engineering. *Metab. Eng.* **28**, 28–42 (2015).
16. Long, M. R., Ong, W. K. & Reed, J. L. Computational methods in metabolic engineering for strain design. *Curr. Opin. Biotechnol.* **34**, 135–141 (2015).
17. Copeland, W. B. et al. Computational tools for metabolic engineering. *Metab. Eng.* **14**, 270–280 (2012).
18. King, Z. A., Lloyd, C. J., Feist, A. M. & Palsson, B. O. Next-generation genome-scale models for metabolic engineering. *Curr. Opin. Biotechnol.* **35**, 23–29 (2015).
19. Chae, T. U., Choi, S. Y., Kim, J. W., Ko, Y. S. & Lee, S. Y. Recent advances in systems metabolic engineering tools and strategies. *Curr. Opin. Biotechnol.* **47**, 67–82 (2017).
20. Choi, K. R. & Lee, S. Y. CRISPR technologies for bacterial systems: current achievements and future directions. *Biotechnol. Adv.* **34**, 1180–1209 (2016).
21. Jensen, M. K. & Keasling, J. D. Recent applications of synthetic biology tools for yeast metabolic engineering. *FEMS Yeast Res.* **15**, 1–10 (2015).
22. Smanski, M. J. et al. Synthetic biology to access and expand nature's chemical diversity. *Nat. Rev. Microbiol.* **14**, 135–149 (2016).
23. Caspi, R. et al. The MetaCyc database of metabolic pathways and enzymes. *Nucleic Acids Res.* **46**, 633–639 (2018).
24. Hadadi, N., Hafner, J., Shajkofci, A., Zisaki, A. & Hatzimanikatis, V. ATLAS of Biochemistry: A repository of all possible biochemical reactions for synthetic biology and metabolic engineering studies. *ACS Synth. Biol.* **5**, 1155–1166 (2016).
25. Hadadi, N. & Hatzimanikatis, V. Design of computational retrobiosynthesis tools for the design of *de novo* synthetic pathways. *Curr. Opin. Chem. Biol.* **28**, 99–104 (2015).
26. Kumar, A., Wang, L., Ng, C. Y. & Maranas, C. D. Pathway design using *de novo* steps through uncharted biochemical spaces. *Nat. Commun.* **9**, 184 (2018).
27. Shin, J. H., Kim, H. U., Kim, D. I. & Lee, S. Y. Production of bulk chemicals via novel metabolic pathways in microorganisms. *Biotechnol. Adv.* **31**, 925–935 (2013).
28. Feher, T. et al. Validation of RetroPath, a computer-aided design tool for metabolic pathway engineering. *Biotechnol. J.* **9**, 1446–1457 (2014).
29. Kan, S. B., Lewis, R. D., Chen, K. & Arnold, F. H. Directed evolution of cytochrome c for carbon-silicon bond formation: Bringing silicon to life. *Science* **354**, 1048–1051 (2016).
30. Kan, S. B. J., Huang, X., Gumulya, Y., Chen, K. & Arnold, F. H. Genetically programmed chiral organoborane synthesis. *Nature* **552**, 132–136 (2017).
31. Choi, S., Song, C. W., Shin, J. H. & Lee, S. Y. Biorefineries for the production of top building block chemicals and their derivatives. *Metab. Eng.* **28**, 223–239 (2015).
32. Pereira, B. et al. Efficient utilization of pentoses for bioproduction of the renewable two-carbon compounds ethylene glycol and glycolate. *Metab. Eng.* **34**, 80–87 (2016).
33. Chae, T. U., Choi, S. Y., Ryu, J. Y. & Lee, S. Y. Production of ethylene glycol from xylose by metabolically engineered *Escherichia coli*. *AIChE J.* (2018).
34. Sousa, A. F. et al. Biobased polyesters and other polymers from 2,5-furandicarboxylic acid: a tribute to furan excellency. *Polym. Chem.* **6**, 5961–5983 (2015).
35. Luo, Z. W., Kim, W. J. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for efficient production of 2-pyrone-4,6-dicarboxylic acid from glucose. *ACS Synth. Biol.* **7**, 2296–2307 (2018).
36. Masuno, M. N. et al. Methods of producing *para*-xylene and terephthalic acid. US patent 2013/0245316 A1 (2013).
37. Luo, Z. W. & Lee, S. Y. Biotransformation of *p*-xylene into terephthalic acid by engineered *Escherichia coli*. *Nat. Commun.* **8**, 15689 (2017).

38. Tomas, R. A., Bordado, J. C. & Gomes, J. F. *p*-Xylene oxidation to terephthalic acid: a literature review oriented toward process optimization and development. *Chem. Rev.* **113**, 7421–7469 (2013).
39. Sangeetha, V. H., Deka, H., Varghese, T. O. & Nayak, S. K. State of the art and future prospectives of poly(lactic acid) based blends and composites. *Polym. Compos.* **39**, 81–101 (2018).
40. Sauer, M., Porro, D., Mattanovich, D. & Branduardi, P. 16 years research on lactic acid production with yeast - ready for the market? *Biotechnol. Genet. Eng. Rev.* **27**, 229–256 (2010).
41. Jung, Y. K., Kim, T. Y., Park, S. J. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of polylactic acid and its copolymers. *Biotechnol. Bioeng.* **105**, 161–171 (2010).
42. Paddon, C. J. et al. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* **496**, 528–532 (2013).
43. Burk, M. J., Burgard, A. P., Osterhout, R. E. & Pharkya, P. Microorganisms and methods for the biosynthesis of adipate, hexamethylenediamine and 6-aminocaproic acid. US patent 2010/0317069 A1 (2010).
44. Cordova, L. T. & Alper, H. S. Central metabolic nodes for diverse biochemical production. *Curr. Opin. Chem. Biol.* **35**, 37–42 (2016).
45. Sánchez-Riera, F., Cameron, D. C. & Cooney, C. L. Influence of environmental factors in the production of R(−)-**1**, 2-propanediol by *Clostridium thermosaccharolyticum*. *Biotechnol. Lett.* **9**, 449–454 (1987).
46. Siebert, D. & Wendisch, V. F. Metabolic pathway engineering for production of 1,2-propanediol and 1-propanol by *Corynebacterium glutamicum*. *Biotechnol. Biofuels* **8**, 91 (2015).
47. Nakamura, C. E. & Whited, G. M. Metabolic engineering for the microbial production of 1,3-propanediol. *Curr. Opin. Biotechnol.* **14**, 454–459 (2003).
48. Chen, Z. et al. Metabolic engineering of *Corynebacterium glutamicum* for the production of 3-hydroxypropionic acid from glucose and xylose. *Metab. Eng.* **39**, 151–158 (2017).
49. Li, Y., Wang, X., Ge, X. & Tian, P. High production of 3-hydroxypropionic acid in *Klebsiella pneumoniae* by systematic optimization of glycerol metabolism. *Sci. Rep.* **6**, 26932 (2016).
50. Chu, H. S. et al. Direct fermentation route for the production of acrylic acid. *Metab. Eng.* **32**, 23–29 (2015).
51. Matsubara, M. et al. Fermentative production of 1-propanol from D-glucose, L-rhamnose and glycerol using recombinant *Escherichia coli*. *J. Biosci. Bioeng.* **122**, 421–426 (2016).
52. Yang, P. et al. A new strategy for production of 5-aminolevulinic acid in recombinant *Corynebacterium glutamicum* with high yield. *Appl. Environ. Microbiol.* **82**, 2709–2717 (2016).
53. Chu, H. S. et al. Metabolic engineering of 3-hydroxypropionic acid biosynthesis in *Escherichia coli*. *Biotechnol. Bioeng.* **112**, 356–364 (2015).
54. Karp, E. M. et al. Renewable acrylonitrile production. *Science* **358**, 1307–1310 (2017).
55. Craciun, L. et al. Preparation of acrylic acid derivatives from α- or β-hydroxy carboxylic acids. US patent US7538247B2 (2009).
56. Corma, A., Iborra, S. & Velty, A. Chemical routes for the transformation of biomass into chemicals. *Chem. Rev.* **107**, 2411–2502 (2007).
57. Dishisha, T., Pyo, S. H. & Hatti-Kaul, R. Bio-based 3-hydroxypropionic- and acrylic acid production from biodiesel glycerol via integrated microbial and chemical catalysis. *Microb. Cell Fact.* **14**, 200 (2015).
58. Guo, Z. et al. Dehydration of lactic acid to acrylic acid over lanthanum phosphate catalysts: the role of Lewis acid sites. *Phys. Chem. Chem. Phys.* **18**, 23746–23754 (2016).
59. Meng, Y., Xue, Y., Yu, B., Gao, C. & Ma, Y. Efficient production of L-lactic acid with high optical purity by alkaliphilic *Bacillus* sp. WL-S20. *Bioresour. Technol.* **116**, 334–339 (2012).
60. Thomas, K. C. & Ingledew, W. M. Production of 21% (v/v) ethanol by fermentation of very high gravity (VHG) wheat mashes. *J. Ind. Microbiol. Biotechnol.* **10**, 61–68 (1992).
61. Ma, C. et al. Enhanced 2,3-butanediol production by *Klebsiella pneumoniae* SDM. *Appl. Microbiol. Biotechnol.* **82**, 49–57 (2009).
62. Kim, J. W. et al. Enhanced production of 2,3-butanediol by engineered *Saccharomyces cerevisiae* through fine-tuning of pyruvate decarboxylase and NADH oxidase activities. *Biotechnol. Biofuels* **9**, 265 (2016).
63. Atsumi, S., Hanai, T. & Liao, J. C. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature* **451**, 86–89 (2008).
64. Abdel-Rahman, M. A., Tashiro, Y. & Sonomoto, K. Recent advances in lactic acid production by microbial fermentation processes. *Biotechnol. Adv.* **31**, 877–902 (2013).
65. Kwon, S., Yoo, I. K., Lee, W. G., Chang, H. N. & Chang, Y. K. High-rate continuous production of lactic acid by *Lactobacillus rhamnosus* in a two-stage membrane cell-recycle bioreactor. *Biotechnol. Bioeng.* **73**, 25–34 (2001).
66. Carlos Serrano-Ruiz, J. & Dumesic, J. A. Catalytic upgrading of lactic acid to fuels and chemicals by dehydration/hydrogenation and C–C coupling reactions. *Green Chem.* **11**, 1101–1104 (2009).
67. Carlson, T. L. & Peters, J., E. M. Low PH lactic acid fermentation. US patent US6475759B1 (2002).
68. Choi, S. Y. et al. One-step fermentative production of poly(lactate-*co*-glycolate) from carbohydrates in *Escherichia coli*. *Nat. Biotechnol.* **34**, 435–440 (2016).
69. Choi, S. Y. et al. Engineering the xylose-catabolizing Dahms pathway for production of poly(D-lactateco-glycolate) and poly(D-lactate-*co*-glycolate-*co*-D-2-hydroxybutyrate) in. *Escherichia coli*. *Microb. Biotechnol.* **10**, 1353–1364 (2017).
70. Shi, D. J., Wang, C. L. & Wang, K. M. Genome shuffling to improve thermotolerance, ethanol tolerance and ethanol productivity of *Saccharomyces cerevisiae*. *J. Ind. Microbiol. Biotechnol.* **36**, 139–147 (2009).
71. Alper, H., Moxley, J., Nevoigt, E., Fink, G. R. & Stephanopoulos, G. Engineering yeast transcription machinery for improved ethanol tolerance and production. *Science* **314**, 1565–1568 (2006).
72. Fan, D., Dai, D. J. & Wu, H. S. Ethylene formation by catalytic dehydration of ethanol with industrial considerations. *Materials* **6**, 101–115 (2012).
73. Song, D. Kinetic model development for dehydration of 2,3-butanediol to 1,3-butadiene and methyl ethyl ketone over an amorphous calcium phosphate catalyst. *Ind. Eng. Chem. Res.* **55**, 11664–11671 (2016).
74. Vecchini, N., Galeotti, A. & Pisano, A. Process for the production of 1,3 butadiene from 1,3 butanediol. US patent 20170313633 A1 (2014).
75. Vecchini, N., Galeotti, A. & Pisano, A. Process for the production of 1,3-butandiene from 1,4-butanediol via tetrahydrofuran. WO patent 2016092517 (2016).
76. Kataoka, N., Vangnai, A. S., Tajima, T., Nakashimada, Y. & Kato, J. Improvement of (R)-1,3-butanediol production by engineered *Escherichia coli*. *J. Biosci. Bioeng.* **115**, 475–480 (2013).
77. Burgard, A. P., Burk, M. J. & Pharkya, P. Methods and organisms for converting synthesis gas or other gaseous carbon sources and methanol to 1,3-butanediol. US patent 9284581 B2 (2009).
78. Baez, A., Cho, K. M. & Liao, J. C. High-flux isobutanol production using engineered *Escherichia coli*: a bioreactor study with *in situ* product removal. *Appl. Microbiol. Biotechnol.* **90**, 1681–1690 (2011).
79. van Leeuwen, B. N., van der Wulp, A. M., Duijnstee, I., van Maris, A. J. & Straathof, A. J. Fermentative production of isobutene. *Appl. Microbiol. Biotechnol.* **93**, 1377–1387 (2012).
80. Peters, M. W., Taylor, J. D., Jenni, M., Manzer, L. E. & Henton, D. E. Integrated process to selectively convert renewable isobutanol to *p*-xylene. US patent 2011/0087000 A1 (2011).
81. Moon, H. G. et al. One hundred years of clostridial butanol fermentation. *FEMS Microbiol. Lett.* **363**, fnw001 (2016).
82. Patakova, P. et al. Comparative analysis of high butanol tolerance and production in clostridia. *Biotechnol. Adv.* **36**, 721–738 (2018).
83. Jimenez-Bonilla, P. & Wang, Y. *In situ* biobutanol recovery from clostridial fermentations: a critical review. *Crit. Rev. Biotechnol.* **38**, 469–482 (2018).
84. Lee, J. et al. Metabolic engineering of *Clostridium acetobutylicum* ATCC 824 for isopropanol-butanol-ethanol fermentation. *Appl. Environ. Microbiol.* **78**, 1416–1423 (2012).
85. Anbarasan, P. et al. Integration of chemical catalysis with extractive fermentation to produce fuels. *Nature* **491**, 235–239 (2012).
86. Beller, H. R., Lee, T. S. & Katz, L. Natural products as biofuels and bio-based chemicals: fatty acids and isoprenoids. *Nat. Prod. Rep.* **32**, 1508–1526 (2015).
87. Peralta-Yahya, P. P., Zhang, F., del Cardayre, S. B. & Keasling, J. D. Microbial engineering for the production of advanced biofuels. *Nature* **488**, 320–328 (2012).
88. Fairley, P. Introduction: Next generation biofuels. *Nature* **474**, S2–5 (2011).
89. Xu, P. et al. Modular optimization of multi-gene pathways for fatty acids production in *E. coli*. *Nat. Commun.* **4**, 1409 (2013).
90. Gajewski, J., Pavlovic, R., Fischer, M., Boles, E. & Grininger, M. Engineering fungal *de novo* fatty acid synthesis for short chain fatty acid production. *Nat. Commun.* **8**, 14650 (2017).
91. Liao, J. C., Mi, L., Pontrelli, S. & Luo, S. Fuelling the future: microbial engineering for the production of sustainable biofuels. *Nat. Rev. Microbiol.* **14**, 288–304 (2016).
92. Dellomonaco, C., Clomburg, J. M., Miller, E. N. & Gonzalez, R. Engineered reversal of the beta-oxidation cycle for the synthesis of fuels and chemicals. *Nature* **476**, 355–359 (2011).
93. Sheppard, M. J., Kunjapur, A. M. & Prather, K. L. J. Modular and selective biosynthesis of gasoline-range alkanes. *Metab. Eng.* **33**, 28–40 (2016).
94. Choi, Y. J. & Lee, S. Y. Microbial production of short-chain alkanes. *Nature* **502**, 571–574 (2013).
95. Cheon, S., Kim, H. M., Gustavsson, M. & Lee, S. Y. Recent trends in metabolic engineering of microorganisms for the production of advanced biofuels. *Curr. Opin. Chem. Biol.* **35**, 10–21 (2016).
96. Cao, Y. X. et al. Heterologous biosynthesis and manipulation of alkanes in *Escherichia coli*. *Metab. Eng.* **38**, 19–28 (2016).
97. d'Espaux, L. et al. Engineering high-level production of fatty alcohols by *Saccharomyces cerevisiae* from lignocellulosic feedstocks. *Metab. Eng.* **42**, 115–125 (2017).

98. Rodriguez, G. M., Tashiro, Y. & Atsumi, S. Expanding ester biosynthesis in *Escherichia coli*. *Nat. Chem. Biol.* **10**, 259–265 (2014).
99. Qiao, K., Wasylenko, T. M., Zhou, K., Xu, P. & Stephanopoulos, G. Lipid production in *Yarrowia lipolytica* is maximized by engineering cytosolic redox metabolism. *Nat. Biotechnol.* **35**, 173–177 (2017).
100. Xu, P., Qiao, K., Ahn, W. S. & Stephanopoulos, G. Engineering *Yarrowia lipolytica* as a platform for synthesis of drop-in transportation fuels and oleochemicals. *Proc. Natl Acad. Sci. USA* **113**, 10848–10853 (2016).
101. Zhang, F., Carothers, J. M. & Keasling, J. D. Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids. *Nat. Biotechnol.* **30**, 354–359 (2012).
102. Zhou, Y. J., Buijs, N. A., Siewers, V. & Nielsen, J. Fatty acid-derived biofuels and chemicals production in *Saccharomyces cerevisiae*. *Front. Bioeng. Biotechnol.* **2**, 32 (2014).
103. Zhou, Y. J. et al. Production of fatty acid-derived oleochemicals and biofuels by synthetic yeast cell factories. *Nat. Commun.* **7**, 11709 (2016).
104. Kurosawa, K., Boccazzi, P., de Almeida, N. M. & Sinskey, A. J. High-cell-density batch fermentation of *Rhodococcus opacus* PD630 using a high glucose concentration for triacylglycerol production. *J. Biotechnol.* **147**, 212–218 (2010).
105. Li, Y., Zhao, Z. & Bai, F. High-density cultivation of oleaginous yeast *Rhodosporidium toruloides* Y4 in fed-batch culture. *Enzyme Microb. Technol.* **41**, 312–317 (2007).
106. Levering, J., Broddrick, J. & Zengler, K. Engineering of oleaginous organisms for lipid production. *Curr. Opin. Biotechnol.* **36**, 32–39 (2015).
107. Ajjawi, I. et al. Lipid production in *Nannochloropsis gaditana* is doubled by decreasing expression of a single transcriptional regulator. *Nat. Biotechnol.* **35**, 647–652 (2017).
108. Daboussi, F. et al. Genome engineering empowers the diatom *Phaeodactylum tricornutum* for biotechnology. *Nat. Commun.* **5**, 3831 (2014).
109. Miao, S., Wang, P., Su, Z. & Zhang, S. Vegetable-oil-based polymers as future polymeric biomaterials. *Acta Biomater.* **10**, 1692–1704 (2014).
110. Zhu, Y., Romain, C. & Williams, C. K. Sustainable polymers from renewable resources. *Nature* **540**, 354–362 (2016).
111. Park, S. Y., Yang, D., Ha, S. H. & Lee, S. Y. Metabolic engineering of microorganisms for the production of natural compounds. *Adv. Biosys.* **2**, 1700190 (2018).
112. Whited, G. M. et al. Development of a gas-phase bioprocess for isoprene-monomer production using metabolic pathway engineering. *Ind. Biotechnol.* **6**, 152–163 (2010).
113. Yang, J. et al. Metabolic engineering of *Escherichia coli* for the biosynthesis of alpha-pinene. *Biotechnol. Biofuels* **6**, 60 (2013).
114. Sarria, S., Wong, B., Garcia Martin, H., Keasling, J. D. & Peralta-Yahya, P. Microbial synthesis of pinene. *ACS Synth. Biol.* **3**, 466–475 (2014).
115. Meadows, A. L. et al. Rewriting yeast central carbon metabolism for industrial isoprenoid production. *Nature* **537**, 694–697 (2016).
116. Ozaydin, B., Burd, H., Lee, T. S. & Keasling, J. D. Carotenoid-based phenotypic screen of the yeast deletion collection reveals new genes with roles in isoprenoid production. *Metab. Eng.* **15**, 174–183 (2013).
117. Kim, H. U., Charusanti, P., Lee, S. Y. & Weber, T. Metabolic engineering with systems biology tools to optimize production of prokaryotic secondary metabolites. *Nat. Prod. Rep.* **33**, 933–941 (2016).
118. Curran, S. C. et al. Probing the flexibility of an iterative modular polyketide synthase with non-native substrates *in vitro*. *ACS Chem. Biol.* **13**, 2261–2268 (2018).
119. Liu, Q. et al. Engineering an iterative polyketide pathway in *Escherichia coli* results in single-form alkene and alkane overproduction. *Metab. Eng.* **28**, 82–90 (2015).
120. Yuzawa, S. et al. Comprehensive *in vitro* analysis of acyltransferase domain exchanges in modular polyketide synthases and its application for short-chain ketone production. *ACS Synth. Biol.* **6**, 139–147 (2017).
121. Hagen, A. et al. Engineering a polyketide synthase for *in vitro* production of adipic acid. *ACS Synth. Biol.* **5**, 21–27 (2016).
122. Yuzawa, S., Keasling, J. D. & Katz, L. Insights into polyketide biosynthesis gained from repurposing antibiotic-producing polyketide synthases to produce fuels and chemicals. *J. Antibiot.* **69**, 494–499 (2016).
123. Averesch, N. J. H. & Kromer, J. O. Metabolic engineering of the shikimate pathway for production of aromatics and derived compounds-Present and future strain construction strategies. *Front. Bioeng. Biotechnol.* **6**, 32 (2018).
124. Fischer-Romero, C., Tindall, B. J. & Juttner, F. *Tolumonas auensis* gen. nov., sp. nov., a toluene-producing bacterium from anoxic sediments of a freshwater lake. *Int. J. Syst. Bacteriol.* **46**, 183–188 (1996).
125. Kim, B., Park, H., Na, D. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of phenol from glucose. *Biotechnol. J.* **9**, 621–629 (2014).
126. Balderas-Hernandez, V. E. et al. Metabolic engineering for improving anthranilate synthesis from glucose in *Escherichia coli*. *Microb. Cell Fact.* **8**, 19 (2009).
127. Balderas-Hernandez, V. E. et al. Catechol biosynthesis from glucose in *Escherichia coli* anthranilate-overproducer strains by heterologous expression of anthranilate 1,2-dioxygenase from *Pseudomonas aeruginosa* PAO1. *Microb. Cell Fact.* **13**, 136 (2014).
128. Kim, B., Binkley, R., Kim, H. U. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the enhanced production of L-tyrosine. *Biotechnol. Bioeng.* **115**, 2554–2564 (2018).
129. Miao, L., Li, Q., Diao, A., Zhang, X. & Ma, Y. Construction of a novel phenol synthetic pathway in *Escherichia coli* through 4-hydroxybenzoate decarboxylation. *Appl. Microbiol. Biotechnol.* **99**, 5163–5173 (2015).
130. Li, M. et al. *De novo* production of resveratrol from glucose or ethanol by engineered *Saccharomyces cerevisiae*. *Metab. Eng.* **32**, 1–11 (2015).
131. Li, M., Schneider, K., Kristensen, M., Borodina, I. & Nielsen, J. Engineering yeast for high-level production of stilbenoid antioxidants. *Sci. Rep.* **6**, 36827 (2016).
132. Yang, J. E. et al. One-step fermentative production of aromatic polyesters from glucose by metabolically engineered *Escherichia coli* strains. *Nat. Commun.* **9**, 79 (2018).
133. Sano, C. History of glutamate production. *Am. J. Clin. Nutr.* **90**, 728S–732S (2009).
134. Shimizu, H. & Hirasawa, T. in *Amino Acid Biosynthesis: Pathways, Regulation and Metabolic Engineering* (ed. Wendisch, V. F.) 1–38 (Springer, Heidelberg, 2007).
135. Park, S. H. et al. Metabolic engineering of *Corynebacterium glutamicum* for L-arginine production. *Nat. Commun.* **5**, 4618 (2014).
136. Cho, J. S. et al. CRISPR/Cas9-coupled recombineering for metabolic engineering of *Corynebacterium glutamicum*. *Metab. Eng.* **42**, 157–167 (2017).
137. Kim, S. Y., Lee, J. & Lee, S. Y. Metabolic engineering of *Corynebacterium glutamicum* for the production of L-ornithine. *Biotechnol. Bioeng.* **112**, 416–421 (2015).
138. Zelder, O. et al. Improved process for the production of gamma-aminobutyric acid (GABA). WO patent 2015/092599 A1 (2015).
139. Park, S. J. et al. Synthesis of nylon 4 from gamma-aminobutyrate (GABA) produced by recombinant *Escherichia coli*. *Bioprocess Biosyst. Eng.* **36**, 885–892 (2013).
140. Chae, T. U., Ko, Y. S., Hwang, K. S. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of four-, five- and six-carbon lactams. *Metab. Eng.* **41**, 82–91 (2017).
141. Zhang, J. et al. Metabolic engineering of *Escherichia coli* for the biosynthesis of 2-pyrrolidone. *Metab. Eng. Commun.* **3**, 1–7 (2016).
142. Kinoshita, S., Nakayama, K. & Udaka, S. The fermentative production of L-ornithine preliminary report. *J. Gen. Appl. Microbiol.* **3**, 276–277 (1957).
143. Qian, Z. G., Xia, X. X. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of putrescine: a four carbon diamine. *Biotechnol. Bioeng.* **104**, 651–662 (2009).
144. Na, D. et al. Metabolic engineering of *Escherichia coli* using synthetic small regulatory RNAs. *Nat. Biotechnol.* **31**, 170–174 (2013).
145. Noh, M., Yoo, S. M., Kim, W. J. & Lee, S. Y. Gene expression knockdown by modulating synthetic small RNA expression in *Escherichia coli*. *Cell Syst.* **5**, 418–426 (2017). e414.
146. Guettler, M. V., Jain, M. K. & Rumler, D. Method for making succinic acid, bacterial variants for use in the process, and methods for obtaining variants. US patent 5573931 A (1996).
147. Lee, P. C., Lee, W. G., Lee, S. Y. & Chang, H. N. Succinic acid production with reduced by-product formation in the fermentation of *Anaerobiospirillum succiniciproducens* using glycerol as a carbon source. *Biotechnol. Bioeng.* **72**, 41–48 (2001).
148. Okino, S. et al. An efficient succinic acid production process in a metabolically engineered *Corynebacterium glutamicum* strain. *Appl. Microbiol. Biotechnol.* **81**, 459–464 (2008).
149. Lee, J. W. et al. Homo-succinic acid production by metabolically engineered *Mannheimia succiniciproducens*. *Metab. Eng.* **38**, 409–417 (2016).
150. Lange, A. et al. Bio-based succinate from sucrose: High-resolution ^{13}C metabolic flux analysis and metabolic engineering of the rumen bacterium *Basfia succiniciproducens*. *Metab. Eng.* **44**, 198–212 (2017).
151. Rush, B. J. & Fosmer, A. M. Methods for succinate production. US patent application US20140363862A1 (2014).
152. Raab, A. M., Gebhardt, G., Bolotina, N., Weuster-Botz, D. & Lang, C. Metabolic engineering of *Saccharomyces cerevisiae* for the biotechnological production of succinic acid. *Metab. Eng.* **12**, 518–525 (2010).
153. Gao, C. et al. Robust succinic acid production from crude glycerol using engineered *Yarrowia lipolytica*. *Biotechnol. Biofuels* **9**, 179 (2016).
154. Ahn, J. H., Jang, Y. S. & Lee, S. Y. Production of succinic acid by metabolically engineered microorganisms. *Curr. Opin. Biotechnol.* **42**, 54–66 (2016).
155. Hong, U. G. et al. Hydrogenation of succinic acid to 1,4-butanediol over rhenium catalyst supported on copper-containing mesoporous carbon. *J. Nanosci. Nanotechnol.* **13**, 7448–7453 (2013).
156. Hong, U. G. et al. Hydrogenation of succinic acid to tetrahydrofuran (THF) over rhenium catalyst supported on H_2SO_4-treated mesoporous carbon. *Appl. Catal. A Gen.* **415**, 141–148 (2012).
157. Hong, U. G., Hwang, S., Seo, J. G., Lee, J. & Song, I. K. Hydrogenation of succinic acid to γ-butyrolactone (GBL) over palladium catalyst supported on alumina xerogel: Effect of acid density of the catalyst. *J. Ind. Eng. Chem.* **17**, 316–320 (2011).

158. Werpy, T., Frye, J., J. G., Wang, Y. & Zacher, A. H. Methods of making pyrrolidones. US patent US 6706893 B2 (2004).
159. Burgard, A., Burk, M. J., Osterhout, R., Van Dien, S. & Yim, H. Development of a commercial scale process for production of 1,4-butanediol from sugar. *Curr. Opin. Biotechnol.* **42**, 118–125 (2016).
160. Ling, L. B. & Ng, T. K. Fermentation process for carboxylic acids. US patent 4877731 A (1989).
161. Song, C. W., Kim, D. I., Choi, S., Jang, J. W. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of fumaric acid. *Biotechnol. Bioeng.* **110**, 2025–2034 (2013).
162. Xu, G., Liu, L. & Chen, J. Reconstruction of cytosolic fumaric acid biosynthetic pathways in *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **11**, 24 (2012).
163. Li, N. et al. Engineering *Escherichia coli* for fumaric acid production from glycerol. *Bioresour. Technol.* **174**, 81–87 (2014).
164. Battat, E., Peleg, Y., Bercovitz, A., Rokem, J. S. & Goldberg, I. Optimization of L-malic acid production by *Aspergillus flavus* in a stirred fermentor. *Biotechnol. Bioeng.* **37**, 1108–1116 (1991).
165. Zambanini, T. et al. Efficient malic acid production from glycerol with *Ustilago trichophora* TZ1. *Biotechnol. Biofuels* **9**, 67 (2016).
166. Zhang, X., Wang, X., Shanmugam, K. T. & Ingram, L. O. L-Malate production by metabolically engineered *Escherichia coli*. *Appl. Environ. Microbiol.* **77**, 427–434 (2011).
167. Zelle, R. M. et al. Malic acid production by *Saccharomyces cerevisiae*: engineering of pyruvate carboxylation, oxaloacetate reduction, and malate export. *Appl. Environ. Microbiol.* **74**, 2766–2777 (2008).
168. Moharregh-Khiabani, D., Linker, R. A., Gold, R. & Stangel, M. Fumaric acid and its esters: an emerging treatment for multiple sclerosis. *Curr. Neuropharmacol.* **7**, 60–64 (2009).
169. Vert, M. Chemical routes to poly(beta-malic acid) and potential applications of this water-soluble bioresorbable poly(beta-hydroxy alkanoate). *Polym. Degradation Stab.* **59**, 169–175 (1998).
170. Li, X., Cai, Z., Li, Y. & Zhang, Y. Design and construction of a non-natural malate to 1,2,4-butanetriol pathway creates possibility to produce 1,2,4-butanetriol from glucose. *Sci. Rep.* **4**, 5541 (2014).
171. Cheong, S., Clomburg, J. M. & Gonzalez, R. Energy- and carbon-efficient synthesis of functionalized small molecules in bacteria using non-decarboxylative Claisen condensation reactions. *Nat. Biotechnol.* **34**, 556–561 (2016).
172. Zhao, M. et al. Metabolic engineering of *Escherichia coli* for producing adipic acid through the reverse adipate-degradation pathway. *Metab. Eng.* **47**, 254–262 (2018).
173. Sato, S., Takahashi, R., Sodesawa, T. & Yamamoto, N. Dehydration of 1,4-butanediol into 3-buten-1-ol catalyzed by ceria. *Catal. Commun.* **5**, 397–400 (2004).
174. Hunter, S. E., Ehrenberger, C. E. & Savage, P. E. Kinetics and mechanism of tetrahydrofuran synthesis via 1,4-butanediol dehydration in high-temperature water. *J. Org. Chem.* **71**, 6229–6239 (2006).
175. Zhao, J. & Hartwig, J. F. Acceptorless, neat, ruthenium-catalyzed dehydrogenative cyclization of diols to lactones. *Organometallics* **24**, 2441–2446 (2005).
176. Subba Rao, Y. V., Kulkarni, S. J., Subrahmanyam, M. & Ramo Rao, A. V. Modified ZSM-5 catalysts for the synthesis of five- and six-membered heterocyclic compounds. *J. Org. Chem.* **59**, 3998–4000 (1994).
177. Clomburg, J. M. et al. Integrated engineering of beta-oxidation reversal and omega-oxidation pathways for the synthesis of medium chain omega-functionalized carboxylic acids. *Metab. Eng.* **28**, 202–212 (2015).
178. Yu, J. L., Xia, X. X., Zhong, J. J. & Qian, Z. G. Direct biosynthesis of adipic acid from a synthetic pathway in recombinant *Escherichia coli*. *Biotechnol. Bioeng.* **111**, 2580–2586 (2014).
179. Raj, K. et al. Biocatalytic production of adipic acid from glucose using engineered *Saccharomyces cerevisiae*. *Metab. Eng. Commun.* **6**, 28–32 (2018).
180. Choi, Y. J., Park, J. H., Kim, T. Y. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of 1-propanol. *Metab. Eng.* **14**, 477–486 (2012).
181. Zhang, K., Sawaya, M. R., Eisenberg, D. S. & Liao, J. C. Expanding metabolism for biosynthesis of nonnatural alcohols. *Proc. Natl Acad. Sci. USA* **105**, 20653–20658 (2008).
182. Song, C. W., Lee, J., Ko, Y. S. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of 3-aminopropionic acid. *Metab. Eng.* **30**, 121–129 (2015).
183. Song, C. W., Kim, J. W., Cho, I. J. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of 3-hydroxypropionic acid and malonic acid through beta-alanine route. *ACS Synth. Biol.* **5**, 1256–1263 (2016).
184. Chae, T. U., Kim, W. J., Choi, S., Park, S. J. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of 1,3-diaminopropane, a three carbon diamine. *Sci. Rep.* **5**, 13040 (2015).
185. Mimitsuka, T., Sawai, H., Hatsu, M. & Yamada, K. Metabolic engineering of *Corynebacterium glutamicum* for cadaverine fermentation. *Biosci. Biotechnol. Biochem.* **71**, 2130–2135 (2007).
186. Shin, J. H. et al. Metabolic engineering of *Corynebacterium glutamicum* for enhanced production of 5-aminovaleric acid. *Microb. Cell Fact.* **15**, 174 (2016).
187. Zhang, J. et al. Application of an acyl-CoA ligase from *Streptomyces* aizunensis for lactam biosynthesis. *ACS Synth. Biol.* **6**, 884–890 (2017).
188. Cann, A. F. & Liao, J. C. Production of 2-methyl-1-butanol in engineered *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **81**, 89–98 (2008).
189. Lepore, A. W. et al. Catalytic dehydration of biomass derived 1-propanol to propene over M-ZSM-5 (M = H, V, Cu, or Zn). *Ind. Eng. Chem. Res.* **56**, 4302–4308 (2017).
190. Borodina, I. et al. Establishing a synthetic pathway for high-level production of 3-hydroxypropionic acid in *Saccharomyces cerevisiae* via beta-alanine. *Metab. Eng.* **27**, 57–64 (2015).
191. Park, S. J. et al. Metabolic engineering of *Escherichia coli* for the production of 5-aminovalerate and glutarate as C5 platform chemicals. *Metab. Eng.* **16**, 42–47 (2013).
192. Adkins, J., Jordan, J. & Nielsen, D. R. Engineering *Escherichia coli* for renewable production of the 5-carbon polyamide building-blocks 5-aminovalerate and glutarate. *Biotechnol. Bioeng.* **110**, 1726–1734 (2013).
193. Rohles, C. M., Giesselmann, G., Kohlstedt, M., Wittmann, C. & Becker, J. Systems metabolic engineering of *Corynebacterium glutamicum* for the production of the carbon-5 platform chemicals 5-aminovalerate and glutarate. *Microb. Cell Fact.* **15**, 154 (2016).
194. Joo, J. C. et al. Production of 5-aminovaleric acid in recombinant *Corynebacterium glutamicum* strains from a *Miscanthus hydrolysate* solution prepared by a newly developed Miscanthus hydrolysis process. *Bioresour. Technol.* **245**, 1692–1700 (2017).
195. Rohles, C. M. et al. A bio-based route to the carbon-5 chemical glutaric acid and to bionylon-6,5 using metabolically engineered *Corynebacterium glutamicum*. *Green Chem.* **20**, 4662–4674 (2018).
196. Qian, Z. G., Xia, X. X. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of cadaverine: a five carbon diamine. *Biotechnol. Bioeng.* **108**, 93–103 (2011).
197. Buschke, N. et al. Systems metabolic engineering of xylose-utilizing *Corynebacterium glutamicum* for production of 1,5-diaminopentane. *Biotechnol. J.* **8**, 557–570 (2013).
198. Kim, H. T. et al. Metabolic engineering of *Corynebacterium glutamicum* for the high-level production of cadaverine that can be used for the synthesis of biopolyamide 510. *ACS Sustain. Chem. Eng.* **6**, 5296–5305 (2018).
199. Pronk, J. T. et al. How to set up collaborations between academia and industrial biotech companies. *Nat. Biotechnol.* **33**, 237–240 (2015).
200. Segler, M. H. S., Preuss, M. & Waller, M. P. Planning chemical syntheses with deep neural networks and symbolic AI. *Nature* **555**, 604–610 (2018).
201. Kim, W. J., Kim, H. U. & Lee, S. Y. Current state and applications of microbial genome-scale metabolic models. *Curr. Opin. Syst. Biol.* **2**, 9–17 (2017).
202. Chen, Z., Wilmanns, M. & Zeng, A. P. Structural synthetic biotechnology: from molecular structure to predictable design for industrial strain development. *Trends Biotechnol.* **28**, 534–542 (2010).
203. Durre, P. & Eikmanns, B. J. C1-carbon sources for chemical and fuel production by microbial gas fermentation. *Curr. Opin. Biotechnol.* **35**, 63–72 (2015).
204. de Lorenzo, V. et al. The power of synthetic biology for bioproduction, remediation and pollution control: the UN's Sustainable Development Goals will inevitably require the application of molecular biology and biotechnology on a global scale. *EMBO Rep.* **19**, e45658 (2018).

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Author contributions

S.Y.L. conceived the project and designed the study. All authors analysed literature, compiled data, planned the content and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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