Chem Soc Rev

REVIEW ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Chem. Soc. Rev.*, 2025, **54**, 8469

α-Ketoglutaric acid as a promising platform chemical for sustainable bio-based industries

Louis-Thibault J. D. Opsommer, †[a] Thomas Schalck,†[b] Sasha Yogiswara,†[c] Kevin J. Verstrepen,*[c] Jan Michiels*[b] and Bert F. Sels *[a]

Received 15th January 2025

DOI: 10.1039/d4cs01125b

rsc.li/chem-soc-rev

The chemical industry is gradually shifting from fossil-derived resources to more sustainable bio-based processes. Natural bio-molecules such as succinic, lactic, and itaconic acid are promising platform chemicals for this green chemistry transition because they can be produced from biomass and converted into various products that are currently produced through fossil-based processes, or they can replace these fossil-based products. One specific bio-molecule, α-ketoglutaric acid (α-KGA), is particularly interesting because it can be directly applied in certain nutrition and healthcare applications, and also serves as a precursor for other commodity and fine chemicals. This review examines the unique chemical properties and application potential of α-KGA and summarises the current state-of-the-art in chemical synthesis and microbial production of α-KGA. Specifically, we discuss how recent advances in precision fermentation, microbial metabolic engineering, and downstream purification are opening new avenues towards sustainable α-KGA production from renewable feedstocks such as sugars, glycerol, fatty acids, alkanes, and alcohols, with titres reaching up to 195 g L^{-1} and productivity up to 1.75 g L^{-1} h^{-1}. Finally, we critically assess the future potential and remaining challenges to implement a cost-competitive industrial bio-based α-KGA chemistry.

[a] *Centre for Sustainable Catalysis and Engineering (CSCE), Department of Microbial and Molecular Systems (M^2S), KU Leuven, Celestijnenlaan 200F, Leuven, 3001, Belgium. E-mail: bert.sels@kuleuven.be*

[b] *VIB-KU Leuven Center for Microbiology & Centre of Microbial and Plant Genetics, Department of Microbial and Molecular Systems (M^2S), KU Leuven, Kasteelpark Arenberg 20, Leuven, 3001, Belgium. E-mail: jan.michiels@kuleuven.be*

[c] *Laboratory for Systems Biology, VIB-KU Leuven Center for Microbiology & Laboratory for Genetics and Genomics, Department of Microbial and Molecular Systems (M^2S), KU Leuven, Gaston Geenslaan 1, Leuven, 3001, Belgium. E-mail: kevin.verstrepen@kuleuven.be*

† Authors contributed equally to this work.

Louis-Thibault J. D. Opsommer

Louis-Thibault Opsommer holds an MSc in Bioscience Engineering (Catalytic Science & Technology) from KU Leuven (2020). Currently, he is pursuing a PhD at the Center for Sustainable Catalysis and Engineering (CSCE) under the guidance of Prof. Bert F. Sels, focusing on the hydrogenation of fermentation products, particularly α-ketoglutaric acid, using heterogeneous catalysis. His main research interests include heterogeneous catalysis, Pd catalysts, kinetic studies, the integration of bio-production and catalytic processes, and the development of polymer materials.

Thomas Schalck

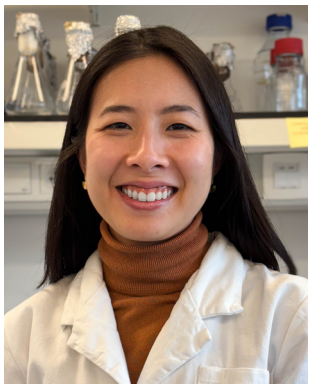
Thomas Schalck obtained his PhD in 2021 from the Centre of Microbial and Plant Genetics under the supervision of Prof. Jan Michiels, focusing on ethanol tolerance mechanisms and development of genome editing tools in E. coli. Currently, as a postdoctoral researcher in the same lab; he is engineering E. coli as a microbial cell factory to convert food and agricultural waste into α-ketoglutarate. His work integrates fermentation technology and analytical chemistry. He is particularly interested in fermentation-enabled valorisation of waste into polymer precursors and co-promotes PhD projects on polyalcohol production from carbohydrate-rich sources using E. coli.

1. Introduction

During the 19th century Industrial Revolution, linear economies emerged that relied heavily on fossil resources to produce energy, fuels, and key chemicals. These relatively cheap and efficient fossil-based industries provided the goods and energy to support a rapidly growing global population. However, the finite reserves of fossil crude oil, coal, and natural gas – along with their severe environmental and climate impacts – necessitate a fundamental shift in industrial processes. The underlying issue is the emission of carbon dioxide (CO_2)[1] and other harmful substances[2,3] (*e.g.*, SO_x, NO_x, and volatile organic compounds) emitted by fossil-based industries, contributing to environmental degradation and public health problems. Therefore, replacing fossil-based feedstocks with renewable biomass that does not increase net atmospheric CO_2 levels is generally considered a logical and crucial shift.

As a result, exploiting biomass to produce value-added products has gained significant attention over the past decade.[4–9] One of the most promising routes is using microorganisms as cell factories to produce chemical compounds or polymer precursors. Bacteria, yeasts, or fungi can be cultivated on sustainable resources, such as plant biomass or waste streams, while producing value-added metabolites that can be used directly or can serve as building blocks for further processing. Developing such processes is complex and interdisciplinary, involving synthetic–catalytic chemistry, biochemistry, and molecular (micro)biology.

Microbes catabolise biomass-derived carbohydrates, fats, and/or proteins, amino acids, and other nitrogen-containing



Sasha Yogiswara

Sasha Yogiswara studied chemical engineering at the University of California, Berkeley, where she discovered an interest in biotechnology. This led her to work at the Joint BioEnergy Institute, focusing on improving plant cell walls for biofuel production. She then pursued a Master's degree in Life Science and Technology at TU Delft, where she studied aroma formation in beer. This experience led to an internship at Nestlé R&D, studying the behaviour of microbial communities in food fermentation. Currently, Sasha is finishing her PhD in Prof. Kevin Verstrepen's lab at KU Leuven, engineering yeast to produce valuable compounds from waste materials.

Kevin J. Verstrepen

Kevin J. Verstrepen is professor in Microbial Genetics and Genomics at KU Leuven, Group Leader in Systems Biology at VIB (Flanders Institute for Biotechnology), director of the VIB-KU Leuven Center for Microbiology, director of the Leuven Institute for Beer Research, guest professor at Tianjin Institute for Industrial Biotechnology, Honorary Professor at Nottingham University, and external advisor for the European Food Safety Authority genetically modified microbes section. He obtained 4 ERC grants (Starting in 2009, Consolidator in 2015, Proof-of-concept in 2020 and Synergy in 2024). He has authored more than 200 scientific publications which have been cited over 20 000 times.

Jan Michiels

Jan Michiels is Full Professor at the KU Leuven Faculty of Bioscience Engineering and vice-director of the Centre of Microbial and Plant Genetics. Since 2017, he has also been a group leader at the Flemish Institute for Biotechnology (VIB). He teaches courses in biochemistry, molecular biology, genetics, and bacterial physiology. His research focuses on molecular aspects of microbe–host interactions and bacterial stress resistance, particularly antibiotic tolerance in pathogens. A second research line targets the discovery of new antibiotics and the development of microbial cell factories through metabolic engineering and synthetic biology. He has published over 170 peer-reviewed journal articles.

Bert F. Sels

Bert F. Sels, currently a full professor at KU Leuven and head of the CSCE research group, earned his PhD in 2000 in the field of heterogeneous oxidation catalysis. His research focuses on heterogeneous catalysis, addressing future challenges in industrial organic and environmental catalysis. A key area of his work is advancing carbon circularity through the use of renewable carbon sources such as biomass, carbon dioxide, and plastic waste. His group has published over 400 peer-reviewed articles and holds more than 40 patents. Professor Sels is the co-chair of the Catalysis Commission of the International Zeolite Association (IZA), a co-founder of the European Research Institute of Catalysis (ERIC), and a member of the European Academy of Sciences and Arts.

compounds into cellular metabolites. Many of these metabolites can be applied directly in food, feed, fuel, pharmaceutical, or chemical sectors. This approach has been indispensable for centuries in the fermentation of ethanol in alcoholic beverages, acetic acid, and lactic acid. Recent advances in biotechnology, including genetic and evolutionary engineering and fermentation process control, have enabled rewiring of microbes' metabolism. As a result, living cells were reprogrammed as microbial cell factories, expediting the production of precise and valuable molecules, including drop-in biofuels (*e.g.*, isoprene)[10] and specific pharmaceuticals (*e.g.*, insulin, vitamins, and vaccines).[11–13] This strategy is called ''precision fermentation,'' hinting that it allows the production of specific, precise molecules with high yields and purity.

Innovative green chemistry methods can further refine fermentation-derived products into more advanced and diverse derivatives.[14,15] For instance, microbial intermediate compounds like succinic or adipic acid have been explored as novel chemical building blocks through specific catalytic processes.[8,16–21] Together, these dual biochemical and chemocatalytic approaches offer sustainable alternatives to traditional fossil-based manufacturing.

Popular guidelines of priority bio-based chemicals include the US Department of Energy's (DOE) 2004 and 2010 Platform Chemical Lists.[22,23] This list contains chemicals that can be produced from biomass with strong commercial potential and environmental benefits. Considering both bio-production and catalytic valorisation, this list has been instrumental for guiding research priorities to advance bio-industries.

α-Ketoglutaric acid (α-KGA) is one such promising molecule that can be produced through precision fermentation for applications in nutrition, pharmaceuticals, and particularly in chemical manufacturing, where it can be catalytically converted into higher-value derivatives. Currently, α-KGA is produced *de novo* using chemical synthesis to meet an annual demand of 100 tonnes in the European Economic Area.[24] However, as α-KGA is a key tricarboxylic acid cycle (TCAc) intermediate in living organisms, many studies have proposed microbial fermentation as a potential source of α-KGA.[25–28]

In this review, we provide a comprehensive overview of sustainable α-KGA production and its potential in various industrial applications. First, we discuss α-KGA's chemical structure and properties. Second, we outline the applications of α-KGA across traditional and emerging fields, ranging from medicine to chemistry. Third, we provide a complete overview of the production strategies of α-KGA through chemical synthesis, biocatalysis, and microbial fermentation. This includes a summary of the strategies for optimising fermentation conditions and strain engineering, as well as the production parameters, reported titres, and productivity. Lastly, we discuss the recovery methods of α-KGA from fermentation broths. In short, this review aims to highlight the immense potential of α-KGA in bio-based industries, even though it has not yet been included in DOE's priority bio-based platform chemical list.[22,29]

2. Structure, properties, and applications of α-KGA

2.1. Structure and properties

The molecular structure of α-KGA, also known as 2-oxopentanedioic acid or 2-oxoglutaric acid, consists of two carboxylic acids and a ketone moiety at the α-position. This conformation is linked to multiple favourable chemical properties and reactivity profiles. First, the position and nature of the functional groups are ideal for cross-linking with other molecules such as dialcohols or diamines, which enables the formation of polymeric networks that could lead to interesting novel materials. Second, the polar groups of α-KGA make it highly soluble in water and non-volatile, enabling high titres in bio-based processes (Table 1). Third, α-KGA exceeds water's boiling point, allowing easy separation from aqueous solutions. Finally, α-KGA does not show any toxic properties.[30]

In aqueous solution, α-KGA can adopt multiple conformations in equilibrium, depending on the environmental pH. This concept was introduced in 1975 by Arthur Cooper and Alfred Redfield with a ^{1}H-NMR study of α-ketoacids[35] and has since been supported by experimental evidence. The main structure at neutral pH is the α-ketone over the α-*geminal*-diol that shifts towards the diol structure when the pH drops below pK_{a1} (*cf.* Table 1). Additionally, the α-ketone may convert into the cyclic lactol structure at an even lower pH due to the nucleophilic attack of the γ-carboxylic acid on the carbonyl group. Such intramolecular reaction is enhanced by the electron-withdrawing effect of the α-carboxylic acid group which is mostly present in acidic solutions. In basic solution, however, deprotonation of β-methylene occurs and results in an enolate conformation (Fig. 1). Besides the acidity, temperature and α-KGA concentration can also influence the equilibrium structure ratio. Eventually, the ratio lactol : keto : diol was typically found to be 16 : 31 : 53 at pH 0.5 and 25 °C, although the values differ among reported studies.[32,35–38] At room temperature and pH 7, α-KGA is mainly in the ketone form with neglectable lactol concentrations (keto : diol 93 : 7).[38]

Understanding the structure of α-KGA under different pH conditions could also clarify its reactivity when exploring new reaction schemes and products (*cf.* Section 2.2).

2.2. Applications

Ketoacids or oxo-carboxylic acids are a broad class of organic acids where the carbonyl group is positioned in the α, β, γ, or δ

Table 1 Properties of α-KGA[31–34]

Property (unit)	α-KGA
CAS	328-50-7
Molecular mass (g mol^{-1})	146.1
Melting point (°C)	115
Boiling point (°C)	345.6
Decomposition point ($T_{10\%}$, °C)	173
Solubility (25 °C, aqueous, unbuffered solution)	1.9 M (278 g L^{-1})
pK_{a1}	2.35
pK_{a2}	4.85

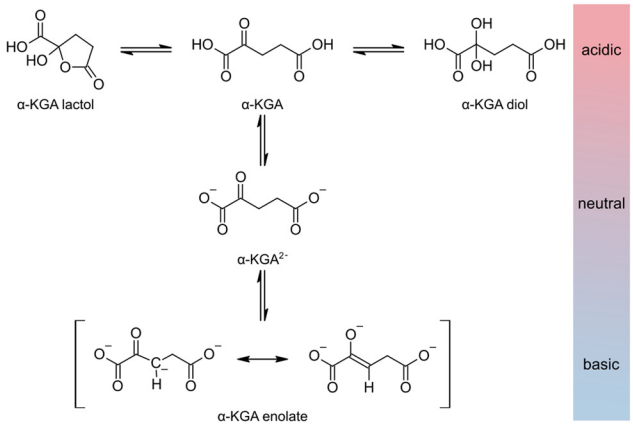


Fig. 1 The equilibrium molecular structures of α-KGA in accordance with the pH of the aqueous solution.[32,35–38]

position relative to the carboxylic acid group. This wide range of keto acids originate from various metabolic pathways, and have applications in various industries, serving as food additives, flavours, feed, medicines, cosmetics, precursors for synthesis, and intermediates in fine chemistry.[39] An extensive overview of the bio-based production and applications of these ketoacids (Fig. 2) has already been published in previous reviews.[40–42]

The α- and γ-ketoacids (and their esters or salts) are relatively stable. The α-ketoacids, such as oxaloacetic acid, pyruvic acid, and α-KGA, often serve as metabolic links between the amino acid, carbohydrate, and fatty acid metabolic pathways in living organisms. In contrast, β-ketoacids are not often found in nature and are less chemically resistant when not esterified (*i.e.*, susceptible to thermal decarboxylation). α-Ketoacids are relatively more stable to thermal decarboxylation (even at 100 °C in diluted HCl solution), although they can undergo catalytic (oxidative) decarboxylation. An example is the enzymatic conversion of α-KGA into succinic acid in living cells.[37,39,43,44] Recent research has also shown the use of α-ketoacids in the synthesis of enolates, Strecker aldehydes, unsaturated carbonyls, pyridoxamines, pyrroles, amides, and furanones.[45–53] Furthermore, the superelectrophilic activation of α-ketoacids upon condensation with weak (aromatic) nucleophiles has also been described for the synthesis of *geminal*-diphenyl compounds (*e.g.*, 1-tetralone derivatives).[54] Lastly,

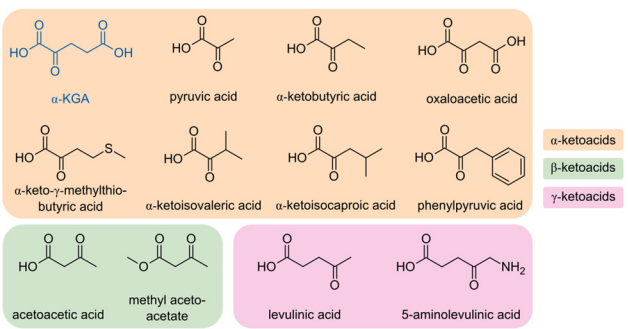


Fig. 2 Overview of relevant ketoacids used in bio-based and chemical industries.[40]

α-ketoacids may also act as green acylating agents in organic chemistry, in which only CO_2 is released as the sole by-product.[55] Because of their structure, α-ketoacids such as α-KGA or α-ketoadipic acid (α-KAA) offer the advantage of a more controlled reactivity compared to oxaloacetic acid, which easily decomposes into pyruvic acid and CO_2 at 25 °C and pH 7, or mesoxalic acid, which readily forms its hydrate form. Thus, working with α-KGA reduces the need for very strict control of reaction conditions such as temperature and pH needed to reduce the decomposition of α-KGA or α-KAA.[37,56]

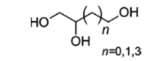
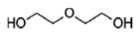
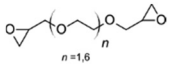
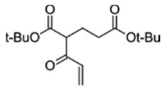
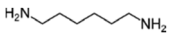
Numerous applications of α-KGA have been described, including the production of building blocks for polymers and the synthesis of more complex chemical compounds used as pharmaceuticals, food supplements or cosmetics. Each of these applications is discussed in more detail in the following sections.

2.2.1. α-KGA as a building block for polymers. α-KGA consists of one carbonyl and two carboxylic acid groups, making it particularly suited for condensation reactions with other monomers to form polymeric materials with potentially novel properties. As a result, α-KGA-based polymers have a wide range of applications, including tissue engineering and drug delivery systems in medicine and novel plastic materials. An overview is provided in Table 2.

One such innovative biomaterial is poly(triol α-KGA) which is synthesised from the thermal condensation of α-KGA with triols like glycerol, 1,2,4-butanetriol, or 1,2,6-hexanetriol.[57] The abundance of ketone groups in the polymer backbone allows for post-polymerisation modifications to expand the polymer's functionalities and to further modulate its mechanical properties and degradation rates.[57] Owing to these features, this polyketoester holds potential for tissue engineering and drug delivery. It can also serve as a cell scaffolding system, when oxime linkers are integrated into the α-KGA-diethylene glycol copolymer structure.[58] Finally, researchers succeeded in developing a novel type of thermoresponsive polyester that consists of hexa(ethylene glycol) or ethylene glycol-bis(glycidyl ether), two modified di-alcohols. The resulting material is especially suitable for medical applications as it spontaneously degrades over time through self-hydrolysis.[59]

Besides polyesters, α-KGA can also be incorporated into the backbone of polyurethane acrylate materials (PUAs). For example, a bio-based polyurethane was synthesised from hexamethylene diisocyanate and a novel polyol comprising α-KGA and isosorbide. The PUA eventually functioned as a thermally stable, UV-curable coating.[60] Polyacrylic acids (PAAs) are another class of polymers in which α-KGA plays a key role. Di-*tert*-butyl acrylate (diTBA) was fabricated from α-KGA and subsequently polymerised into PAA, which served as a dispersing agent and adsorbent,[61] or as a polymer additive for personal healthcare products and water treatments.[62] More recently, α-KGA was investigated as a commercial building block together with glutaric acid in the melt polymerisation for bio-based nylon 56 and nylon 66 analogue blends. The resulting structures showed interesting thermal properties, including melt temperatures in a range close to that of commercial nylon 66 (250–300 °C).[63]

Table 2 Overview of α-KGA combined with secondary monomers as building blocks for novel polymers. Abbreviations: OEG-BGE, oligo(ethylene glycol)-bis(glycidyl ether); PUAs, polyurethane acrylates; diTBA, di-*tert*-butyl-acrylate; PAA, polyacrylic acid; HMDA, hexamethylenediamine

Monomer	Structure	Material	Applications	Ref.
Tri-alcohols		Polyketoesters	Tissue engineering, drug delivery	57
Diethylene glycol		Polyketoesters-oximes	Cell scaffolds, adhesive elastomers	58
OEG-BGE		Polyesters	Thermoresponsive, degradable materials	59
Isosorbide		Polyol (for PUA synthesis)	Thermally stable, UV-curable coatings	60
diTBA		PAA	Dispersing agents, adsorbents, additives	61 and 62
HMDA		Nylon	Nylon 66 substitution	63

The utility of the carboxylic acid groups on α-KGA to form polyesters and polyamides has been well investigated. Future research could focus on exploring the chemical versatility of the ketone group, which could serve as an interesting moiety to explore degradability of novel materials by means of microorganisms, enzymes, or catalytic hydrolysis.

2.2.2. α-KGA as a precursor or intermediate in the synthesis of chemical building blocks. α-KGA is a precursor molecule for many valuable commodity and fine chemicals. The following paragraphs highlight several synthesis routes currently used for various applications.

Glutamic acid (Glu) is mainly produced from glucose *via* microbial fermentation using the bacteria *Corynebacterium.*[64] Such fermentation processes, however, can have several drawbacks such as high energy and time requirements, low efficiency, and low purity of the product.[65] Although Glu fermentation has been commercially established,[66] researchers have investigated whether chemical transformations from the readily available α-KGA are also feasible (Table 3).

Glu can be made *via* reductive amination from α-KGA, catalyzed by the enzyme Glu dehydrogenase (GDH), with NADH cofactor as the electron donor[67] by using re-engineered cells or their extracted enzymes[68–70] in combination with various nitrogen sources (*cf.* Table 3). The reductive amination can also be performed using inorganic compounds as catalysts, such as TiO_2, FeS, and ZnS.[65,71–73] Glu-analogues can also be synthesized from α-KGA using benzylamine as a reagent and a Rh-based catalyst,[74] or by means of a two-step alkylation-transaminase approach.[75] The transamination of L-Glu can also occur using semi-synthetic enzymes.[76–78] The β-cyclopropane analog of Glu, in turn, was synthesised from α-KGA and used in vitamin K-dependent carboxylase studies.[79] Furthermore, the reductive amination of α-KGA has been frequently related to light-induced NADH-regeneration systems for enzymatic reactions.[80–87]

α-KGA is frequently used to synthesize benzimidazole-type compounds by reacting with *o*-phenylenediamines. These benzimidazoles serve as core intermediates or final products in the synthesis of potential anticancer agents.[88–94] A typical example

Table 3 Overview of various reaction types used for the synthesis of glutamic acid (analogs). Abbreviations: AA, amino acid; PXBr, 5-monobromopyridoxamine dihydrobromide; ALBP, adipocyte lipid binding protein; LHMDS, lithium hexamethyldisilazide

Reaction	Reagent	Product	Catalyst	Ref.
Reductive amination (biocatalytic)	PXBr	Glu	Semi-synthetic enzyme (papain-PX)	71
	$NH_4^+HCOO^-$ $NADH/NAD^+$	D-Glu	*E. coli* (pFADA)	68
	Urea Dextran-NAD^+	L-Glu	Artificial cells Multienzyme system	69
	$NH_4^+HCOO^-$ $NADH/NAD^+$	D-Glu	AA aminotransferase Alanine racemase L-Alanine dehydrogenase Formate dehydrogenase	70
Catalytic	NH_2OH	Glu	TiO_2	65
	NH_4Cl	Glu	FeS	72
	NH_4Cl	Glu	ZnS	73
	Benzylamine	*N*-Benzyl-Glu	Rh complex	74
Alkylation transamination	Cysteine sulfinic acid	(4*R*)-4-Methyl-L-Glu	LHMDS, lipases, transaminases	75
Transamination	Pyridoxamine	L-Glu	ALBP pyridoxamine cofactor (ALBP-PX)	76–78

is bendamustine, a benzimidazole with a glutaric moiety, which has been used to tackle leukemia, multiple myeloma, and non-Hodgkin's lymphoma.[88] 2-alkylamino-1-aminobenzimidazoles in turn react with α-KGA to produce benzimidazolones.[95] Similarly, α-KGA can also react with *o*-phenylenediamines to form quinoxalines, another class of heterocyclic components that have widespread therapeutical applications, such as to treat Chagas disease,[96] HIV,[97,98] cancer,[98–102] and inflammation.[103] Benzoxazinones constitute an alternative category of heterocycles that are formed upon reaction of α-KGA with *o*-aminophenols and display antibacterial[104,105] or fluorescence activity.[106]

α-KGA also acts as a precursor for indoles, a group of compounds with promising therapeutic potential. Members of the indole class target diseases such as leukemia,[107,108] gastroenterological cancer,[109] and bone malignancies,[110] and could serve as antitumour compounds.[110,111] Indoles are also considered important intermediates for the synthesis of β-carbolinium salts, which were tested as antifungal agents[112,113] and novel acetylcholinesterase inhibitors.[114] Similar indole-type structures are involved in the synthesis of quinoline-6-alkanamides, known as melatonin analog drugs for various clinical applications.[115] An overview of these compounds can be found in Fig. 3.

α-KGA can also be used as a building block for novel peptide structures. In this synthesis pathway, oximes are implemented to protect the ketoacid moiety in the so-called annulation reaction. The protected structure is subsequently used in chemoselective peptide synthesis in which a final deprotection step with Zn recovers the ketoacid moiety.[116] These novel peptides could serve as biologically active compounds[117–119] or for drug release strategies.[120] Their novelty lies in preserving the ketoacid or ketoamide moiety along or at the end of the polymer chain. Another strategy to form the amide bond is the decarboxylative acylation of amines, for which *tert*-butyl hydroperoxide is typically used.[121] The structure of α-KGA has a tendency to coordinate with metal centers. As such, it could serve as an ideal bidentate ligand in transition metal complexes (TMCs), based on Ni and Rh,[122,123] that display antibacterial and antitumour properties.[122] Moreover, α-KGA was also used for the synthesis of novel ligands in various TMCs[124–126] with antioxidant characteristics.[124]

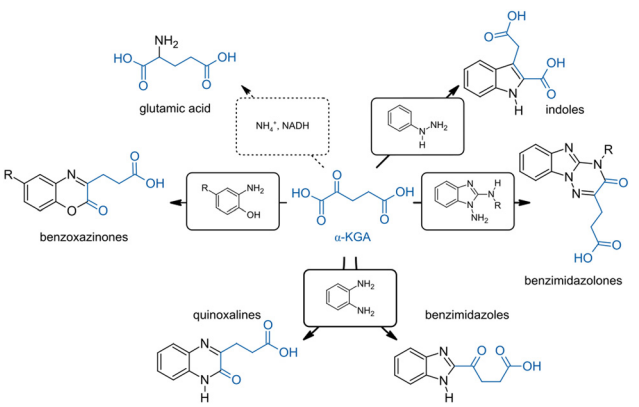


Fig. 3 Synthesis routes from α-KGA. The α-KGA moiety is highlighted in blue, and the reagent for each reaction is provided in a box. The dotted lines represent biochemical reaction pathways, whereas the solid lines indicate synthetic pathways.

The carbonyl group of α-KGA readily reacts with hydrazine ($H_2N–NH_2$) to produce hydrazone, which subsequently converts into the versatile compound 1,4,5,6-tetrahydro-6-oxo-3-pyridazinecarboxylic acid (THOPCA).[127] For instance, THOPCA was transformed into glutamine (Gln) as an alternative for the natural L-isomer using catalytic hydrogenation. In this two-step process, a 5% Pd/C catalyst in water was first used to hydrogenate the imine bond followed by hydrogenolysis of the N–N bond, or *vice versa*.[128] Furthermore, THOPCA was applied as an intermediate for pyridazine-3-carboxamide or pyridazinone synthesis, two promising pharmaceuticals for various diseases.[129–131] Lastly, THOPCA plays a key role in the fabrication of 3,3′-dipyridazinyl disulfide, a molecule that enables the elucidation of active site mechanisms in enzymes.[132]

α-KGA has also been used in combination with various hydrazines ($R_2N–NH_2$) to form novel hydrazones. For instance, isonicotinyl hydrazide can be applied to produce a Schiff base-type ligand, which can be used for organotin complexes in homogeneous catalysis.[133] Similarly, methyl carbazate was employed to form (2-(methoxycarbonyl-hydrazono)-pentanedioic acid), a ligand for Ag, Co, and Zn complexes with antimicrobial and/or anticancer properties.[134,135] Other closely related hydrazine structures are thiosemicarbazides that can react with α-KGA to form thiosemicarbazones. These sulphur-containing compounds are able to form a complex with metals, like Zn,[136] Cu[137] or rare-earth metals,[138] either in an open or in a closed form, to fight leukemia or to develop contrasting compounds for MRI diagnosis. Analogously, cyclic isothiosemicarbazones were prepared from α-KGA as potential novel antibiotics.[139]

Another broad class of antibacterial agents encompasses the canthin-6-one analogs.[140–143] These compounds are made in a multi-step synthesis starting from tryptamine, where α-KGA is employed in the so-called Pictet–Spengler condensation step.[144] β-Carbolines are derived from similar condensation reactions of α-KGA with tryptamine-2-carboxylic acids and may serve as valuable pharmaceutical building blocks.[145,146]

α-KGA as a di-acid precursor can be combined with heterocyclic anilines to yield quinazoline derivatives. Such structures have a broad range of applications in medicine and pharmaceutics as biologically active compounds. Therefore, numerous synthesis procedures for a broad class of quinazolines have been published over the years.[147–150] An overview of some of the earlier-mentioned compounds is given in Fig. 4.

When the decarboxylating enzymes MenD or SucA from *Escherichia coli* or Kgd from *Mycobacterium tuberculosis* are used, α-KGA is an ideal substrate for the biocatalytic two-step decarboxylation-addition reaction with aldehydes to produce *vicinal*-hydroxyketone adducts.[151] The resulting hydroxyketone products, obtained in high enantiomeric excess, are important structures for pharmaceutical compounds.[152–155] When pyruvic acid is used instead of aldehydes, the groups (*i.e.*, ketone and alcohol) switch on the vicinal positions.[156]

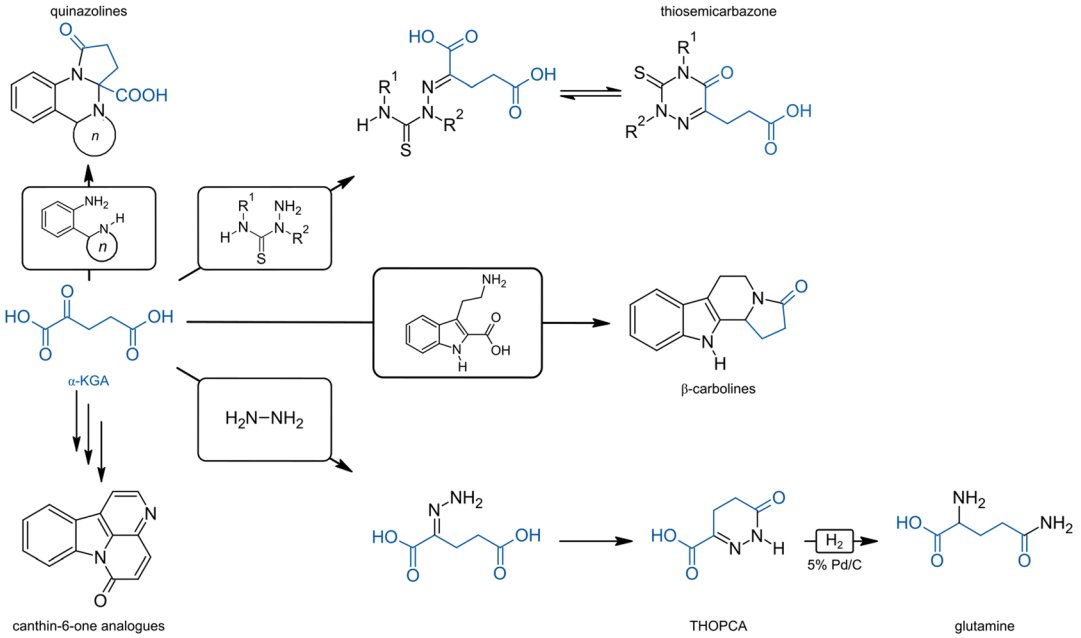


Fig. 4 Synthesis routes from α-KGA (part 2). The α-KGA moiety is highlighted in blue, and the reagent for each reaction is provided in a box.

In the pursuit of a synthetic route to produce industrially relevant α-hydroxy butyrolactones, the inclusion of α-KGA as a potential substrate has been considered. These lactone building blocks are prepared by mixing the α-keto acid with an olefin in the presence of a Lewis acid.[157] When the so-called superacids like trifluoromethanesulfonic acid are used, α-KGA reacts with weak(er) nucleophiles such as benzene to form diphenyl 1-tetralone, owing to the superelectrophilic nature of protonated α-KGA.[54] In addition, α-KGA plays a key role in the synthesis of lactivicin, a lactone-derived antibiotic.[158] Furthermore, homocitric acid lactone can be created from α-KGA in a multistep process, yielding homologs for studying biological nitrogen fixation.[159] α-KGA is also a precursor to produce 2-allyl-5-oxo-tetrahydrofuran-2-carboxylic acids, which can be further converted to form nonanes, which are present in natural products.[160] Finally, chiral 5-oxo-tetrahydrofuran-2-carboxylic acids can be obtained from the hydrogenation reaction of α-KGA using a Pt/alumina catalyst with cinchona alkaloid modifiers. The resulting lactones are in turn used as chiral building blocks or derivatisation agents.[161] An overview of the adducts and lactones is given in Fig. 5.

Lastly, α-KGA is also involved in the synthesis of various other compounds, spanning a wide range of applications, including fluorescent[162,163] and enzyme activity probes,[164] blue pigments,[165] the dihydro-2*H*-pyran-3(4*H*)-one chemical precursor,[166] the vitamin

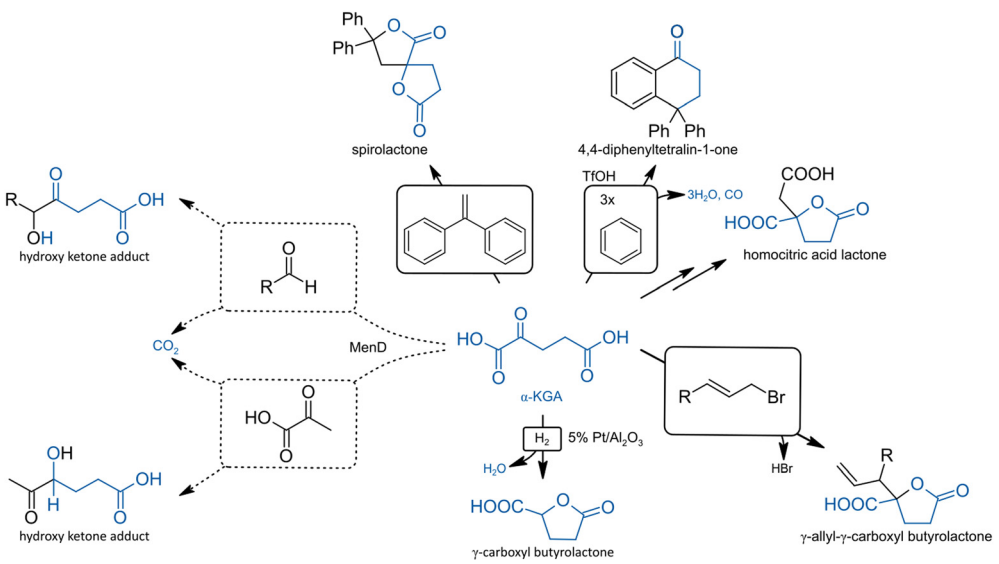


Fig. 5 Synthesis routes from α-KGA (part 3). Abbreviations: TfOH, triflic acid; Pt/Al_2O_3, platinum on γ-alumina catalyst; and MenD, 2-succinyl-5-enol-pyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase.

menaquinone,[167,168] the 2-hydroxy-3-oxoadipate metabolite,[169] thioacetal antidotes,[170] biologically active isoxazolylpyrrolones,[171] enzyme-inhibiting sulfoxide analogs,[172] thiadiazole β-peptides,[173] hydantoin-derivatized pharmaceuticals,[174] and finally, antitumour agents, such as hadacidin analogs,[175] isoxazoleacetic acid,[176] and thiazolidinones.[177]

In this section, we provided extensive insights into the potential of α-KGA as a substrate or intermediate in chemical synthesis. The (m)ethyl esters of α-KGA have also been discussed in the same manner in a recent review paper.[42]

2.2.3. α-KGA in nutrition and healthcare. α-KGA is a non-toxic key metabolite (LD50: 5000 mg kg^{-1} (ref. 178)) in living cells that can be absorbed through the diet or administered to humans as ornithine or calcium α-ketoglutarate salts,[179] or as cell-permeable ester derivatives such as octyl- or di(m)ethyl-α-KGA.[180] While these well-established delivery methods support the administration of the ketoacid to treat various pathologies or to improve general health, the authors wish to highlight that the illustrations presented below are based on pre-clinical studies in model organisms or in a limited number of patients.[181,182]

Treating patients with α-KGA has been demonstrated to improve (muscle) recovery after invasive traumas, such as surgical interventions.[183–185] Studies have shown that administering α-KGA during heart surgeries can prevent muscle degradation and ensure proper blood and oxygen flow to vital organs such as the heart and kidneys. This intervention decreases the likelihood of heart or kidney dysfunction following surgery.[186–189] Although these reports suggest that supplementing α-KGA improves patient recovery and surgical outcomes, this practice is not yet applied in standard clinical procedures due to the lack of large-scale supporting evidence. In addition, a recent study corroborated that (dietary) α-KGA suppresses blood clots, also called thrombosis (Fig. 6, ④f).[190] In this respect, administration of α-KGA to type 2 diabetes (T2D) patients makes sense as they often suffer from thromboinflammation (Fig. 6, ④d and f) that may cause organ damage, pneumonia, asthma, and fibrosis.[191] Besides ameliorating cardiovascular conditions associated with diabetes, α-KGA directly prevents obesity in T2D by improving glucose homeostasis through lowering blood glucose levels, suppressing hepatic gluconeogenesis, and stimulating insulin secretion.[192] Next, α-KGA has been suggested to be effective in treating bone, breast, and skin cancer.[193–195] α-KGA inhibits the proliferation of malicious cancer cells by inducing cell death,[194] attenuating tumour-induced blood vessel growth – as a result of reduced levels of erythropoietin and growth factors (*e.g.*, HIF-1 and VEGF)[196] – and by suppressing tumour cell migration (metastasis) (Fig. 6, ④b).[193] Consequently, combining α-KGA with other cancer treatments significantly improves the efficacy of anticancer drugs, such as 5-fluorouracil, and immunotherapy.[195,196] Similarly, an anti-cancer mixture, composed of B87 and dimethyl-α-KGA, has been proposed to kill tumour cells by shutting down respiration and glycolysis simultaneously.[197] Moreover, α-KGA could alleviate osteopenia, a condition that weakens skeletal bones, by concurrently inhibiting degradation and stimulating mineralisation of bone tissue.[198] Finally, α-KGA can act as an antidote for cyanides, a toxin with detrimental

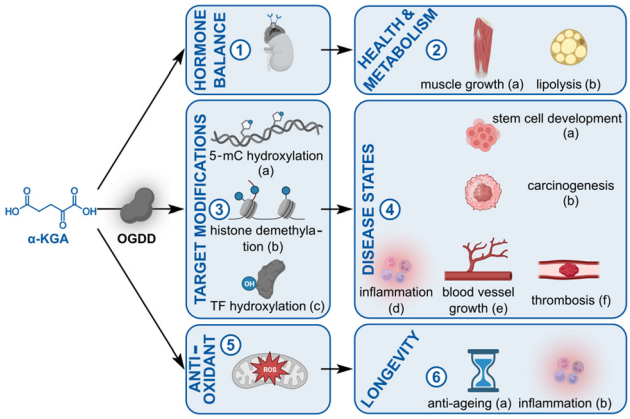


Fig. 6 The application of α-KGA in the healthcare context. α-KGA can bind to chromaffin cells to stimulate the release of epinephrine (1), which, in turn, promotes muscle growth (2a) and lipolysis of adipose tissue (2b). α-KGA drives OGDD-mediated hydroxylation of cytosine base pairs (3a), demethylation of lysine residues at histones (3b), and hydroxylation of key transcription factors (such as Akt and HIF1α) (3c). Since these nucleotide or protein targets of OGDDs are implied in multiple disease states, α-KGA has an indirect, profound impact on stem cell development (4a), carcinogenesis and metastasis (4b), inflammation (4d), the outgrowth and morphology of the vasculature (4e), and blood clot formation (*i.e.*, thrombosis, 4f). Lastly, α-KGA may serve as an antioxidant (5) and, therefore, prolongs the lifespan in (model) organisms (6a) and attenuates inflammation responses (4b). Abbreviations: OGDD, 2-oxoglutarate dependent dioxygenase; OH, hydroxyl; TF, transcription factor; and ROS, reactive oxygen species. The figure is created based on ref. 182, 206, 208, 230, 233 and 240.

effects on the liver, kidneys, and nervous system, by rapidly forming a complex with the cyanide moiety called cyanohydrin.[30,199–202] As such, α-KGA has been shown to mitigate the toxic effects of sodium nitroprusside in fruit flies.[203] Lastly, α-KGA also serves as a biomarker for the diagnosis of hyperinsulinism-hyperammonemia syndrome[204] and Rey's syndrome.[205]

One of the reasons why α-KGA can effectively treat pathologies is because it is strongly connected to the superfamily of 2-oxoglutarate-dependent dioxygenases (2-OGDDs, Fig. 6). These universal enzymes consume α-KGA and oxygen to produce carbon dioxide and succinate while catalysing hydroxylation-initiated oxidation or demethylation of proteins, nucleic acids, or lipids.[206] Particularly, prolyl hydroxylases and histone demethylases, both belonging to 2-OGDDs, are being studied intensively as they are implicated in cancer and diabetes.[207,208] Prolyl hydroxylases (PHDs) are known to hydroxylate proline residues in several regulatory proteins, suppressing their activities. These proteins include Akt, a regulator involved in blood clot formation and inflammation, and HIF1α, a factor promoting blood vessel growth (Fig. 6, ③c).[209] The hydroxyl groups either mask the phosphorylation sites in Akt and increase the affinity of phosphatases for Akt[209,210] or promote proteasomal degradation of HIF1α.[209,211] Since α-KGA drives the hydroxylation reaction, it can be applied as a "PHD booster" to inactivate Akt or HIFα. α-KGA was shown to help prevent thrombosis and inflammation due to reduced platelet aggregation and monocyte activation in COVID-19-infected murine models (Fig. 6, ④d and f).[190] Alternatively, treatment of an oncogenic rat model with octyl-α-

KGA diminished the blood vessel density surrounding the tumour due to decreased HIF1α levels (Fig. 6, ④e).[212,213] HIF1α-targeting strategies have been prioritised in recent years since this regulator is considered a "hallmark" in cancer biology and its activation is linked to cell proliferation, metastasis, and tumour resistance to chemo- and radiotherapy.[214]

Although this anti-angiogenesis strategy improves the efficacy of cancer therapies,[215] patients should be administered α-KGA with caution. Recent reports have indicated that tumour cells, relying heavily on glutamine consumption (*i.e.*, glutaminolysis), exploit the released α-KGA as a messenger that triggers the central cascade pathways (NF-κB[216] and TOR[217]). Subsequent pathway activation promotes cancer cell immortality, medicinally known as anoikis resistance, during circulation in the bloodstream (*i.e.*, metastasis)[218] and accelerates tumour development.[219]

Finally, apart from targeting central regulators, 2-OGDDs are also involved in the demethylation of histones at lysine residues (Fig. 6, ③b)[208] or DNA at cytosine positions (Fig. 6, ③a).[220] Attaching covalent groups, such as methyl moieties, to histones alters the spatial organisation of the chromosome and gene expression patterns, whereas DNA (de)methylation often occurs during cell differentiation and development.[221,222] These epigenetic modifications, at both the histone and DNA level, can cause cancer and neurodevelopmental or autoimmune disorders when they occur aberrantly.[221] Particularly, the nucleotide derivatives methylcytosine (5-mC) and hydroxymethylcytosine (5-hmC) are considered strong determinants of tumour fate and are believed to be associated with heart-related complications in diabetes patients.[223,224] Indeed, the TET (Ten-Eleven Translocation) 2-OGDD superfamily mediates hydroxylation of 5-mC and the resulting increase in 5-hmC inhibits tumour progression (Fig. 6, ④b) or restores cardiac function.[224–227] At the histone level, supplementing α-KGA to mice, suffering from colorectal cancer, drives lysine demethylation by stimulating the Jumonji C-domain containing histone demethylase (JHDM), which belongs to the 2-OGDD superfamily. As a result, this treatment arrested the tumours in a terminally differentiated and non-proliferative state.[228] A similar instance of epigenetic-induced cell reprogramming, linked to α-KGA, has also been observed in pluripotent stem cells. However, in this case, α-KGA seems to exert a divergent effect on stem cell fate as, depending on their state, this ketoacid may either accelerate differentiation or favour pluripotency in stem cells through the activity of the TET and JHDM demethylating enzymes (Fig. 6, ④a).[229,230] Moreover, supplementation of α-KGA attenuates differentiation of immunological T-cells through a complex interplay of altering the epigenetic profile and promoting triacylglyceride synthesis as well as oxidative phosphorylation in mitochondria.[231] The latter provides an excellent illustration showcasing the broad-range impact of the "epigenetic modifier" α-KGA.

Besides being prescribed to treat diseases, α-KGA can also be administered to healthy individuals for its anti-aging benefits,[232] its capacity to induce metabolic effects similar to those attained through intense physical training,[233,234] or its role in procollagen production.[235] The anti-aging benefits were first demonstrated in a mouse model that had improved survival and suppressed morbidity, shown as a reduction in frailty, colour loss, dermatitis, *etc.* (Fig. 6, ⑥a).[236] Moreover, α-KGA promotes longevity in *C. elegans* worms since this molecule binds and inactivates the ATP synthase, resulting in decreased oxygen consumption.[237] Restraining the activity of the ATP synthase also increases autophagy, which prolongs lifespan (Fig. 6, ⑥a and b).[237]

Related to the link between exercise and α-KGA, high intensity and short duration (resistance) training induces α-KGA synthesis in muscles, causing higher blood α-KGA levels.[233] Circulatory α-KGA molecules bind to the $OXGR1^{AG}$ receptor of chromaffin cells in the adrenal gland to induce the release of epinephrine through the NF-κB signaling cascade (Fig. 6, ①).[233] Eventually, elevated epinephrine concentrations enlarge skeletal muscles and promote the breakdown of fat tissue (Fig. 6, ②a and b).[233,238] Therefore, the authors hinted towards the use of α-KGA as an anti-obesity therapeutic.[233]

Finally, α-KGA not only targets receptors and protein complexes but is also involved in mitigating oxidative stress. The antioxidising properties of α-KGA result from its direct scavenging function towards toxic reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), its role in the cellular synthesis of glutathione, a well-known antioxidant, or its ability to trigger and modulate ROS-dedicated stress response pathways (Fig. 6, ⑤). The H_2O_2-scavenging function of α-KGA is a result of a spontaneous conversion of α-KGA into succinate in the presence of H_2O_2, thereby releasing the harmless products H_2O and CO_2.[239,240] Moreover, α-KGA serves as an indirect precursor for glutathione synthesis, as this tripeptide is produced from glutamic acid—the transamination product of α-KGA—along with cysteine and glycine.[241] Alternatively, α-KGA may also act as a signaling molecule that engages the constitutive-androstane-receptor (CAR) pathway and as a result, promotes the expression of ROS detoxifying enzymes, including superoxide dismutases.[242] In hospital settings, administering α-KGA as an antioxidising agent to patients showed improved recovery after lung surgery and reduced myocardial injury in pressure-overloaded heart.[243,244] Furthermore, in combination with 5-hydroxymethylfurfural (5-HMF), α-KGA has also been patented as an antioxidant agent for humans and animals.[245]

Next to its antioxidant activity, α-KGA supplementation improves nitrogen metabolism and has a positive effect on the intestinal microbiota of pigs. Therefore, α-KGA could be potentially applied as a growth-promoting factor for enhancing pork production in livestock farming.[246]

3. Chemical synthesis routes for the production of α-KGA

α-KGA can be produced from a condensation reaction of diethyl succinate (DES) with diethyl oxalate (DEO), followed by hydrolysis of the obtained triethyl oxalyl succinate (Fig. 7). The first precursor, DES, is obtained by the oxidation of butane into

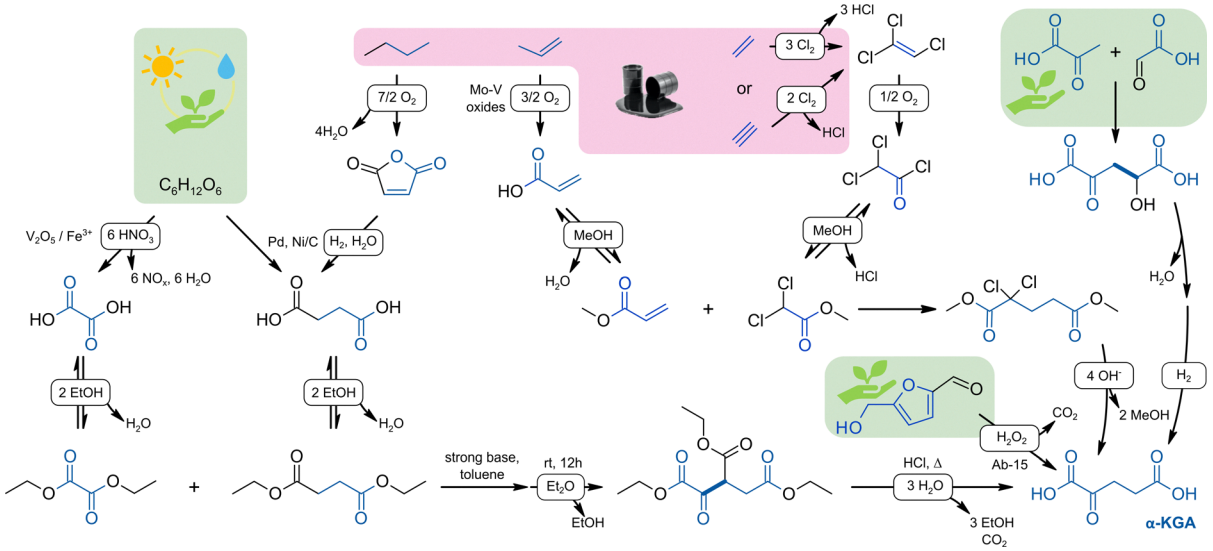


Fig. 7 Industrial production of α-KGA. Compounds highlighted in green represent biomass-derived reagents and those in red are petroleum-derived reagents. Abbreviations: MeOH, methanol; EtOH, ethanol; Et_2O, diethyl ether; rt, room temperature; Δ, heat, and Ab-15, Amberlyst-15.

maleic anhydride, then the ring-opening hydrogenation of maleic anhydride to succinic acid using a Ni- or Pd-catalyst, followed by an esterification of succinic acid into DES.[247,248] Alternatively, succinic acid can also be obtained from fermentation using glucose as a substrate.[249] The second α-KGA precursor, DEO, is typically generated from the reaction of glucose with nitric acid using V–Fe catalysts to yield oxalic acid, which is then esterified into DEO. Other routes towards oxalic acid include the propylene and ethylene glycol oxidation.[250–252] The two-step condensation process of DES with DEO, despite its overall yield of 63–76%,[39,253,254] is far from ideal as it involves dangerous chemicals, such as sodium or potassium ethoxides, toluene, and diethyl ether. Other compounds involved are considered as acute toxic according to ECHA (including maleic anhydride and oxalic acid with LD_{50} values of 1090 and 375 mg kg^{-1} for oral uptake, respectively).[255,256] Moreover, the reaction chemistry leads to environmental issues due to the partial use of fossil resources and the emission of nitric oxides during synthesis.

Anther chemical route to synthesise α-KGA or its salts is *via* the intermediate adduct dimethyl 2,2-dichloroglutarate (DMDG). The reaction between methyl dichloroacetate (MDA) and methyl acrylate (MA) yields DMDG, which is subsequently transformed into an aqueous α-KGA solution using a hydroxide medium.[257] The precursor MDA is derived from alcoholysis of trichloroethylene-originated dichloroacetyl chloride.[258,259] The precursor MA is derived from the liquid-phase methyl esterification of acrylic acid that arises from the vapor-phase catalytic oxidation of propylene.[260] Again, these compounds are considered toxic.[258–260] Under mild temperatures, α-KGA can be produced through the transamination reaction between glutamate and glyoxylate, with Cu, Ni, Co, or V metal salts as a catalyst, and with glycine as a by-product.[261]

Another possible chemical pathway is the oxidation of aqueous 5-hydroxymethyl furfural (5-HMF) to α-KGA on an Amberlyst®-15 catalyst with H_2O_2. However, only a yield of 31% was achieved at 75 °C after 24 h, with formic acid and succinic acid as primary by-products.[262]

More recently, a heterogeneously catalysed route towards α-KGA has been developed *via* an aldol condensation between pyruvic acid and glyoxylic acid. This reaction resulted in 2-hydroxy-4-oxoglutaric acid, which formed α-KGA upon dehydration and hydrogenation using a Pd/TiO_2 catalyst. The overall yield of this reaction was 85%, with a volumetric productivity of 50 g L^{-1} h^{-1}, and could therefore offer a valuable green alternative to the classic industrial production routes.[263] Moreover, pyruvic acid can be obtained from consecutive dehydration-decarboxylation chemistry from tartaric acid, or can be sourced from microbial fermentation.[39,264] Similarly, glyoxylic acid can be obtained from the oxidation of glyoxal, derived from the oxidation of ethylene glycol,[265] or can be produced through enzymatic oxidation of bio-based glycolic acid.[266,267]

4. Cell-free biocatalytic pathways towards α-KGA

Apart from chemical synthesis, α-KGA can also be produced through *in vitro* metabolic pathways comprising a few enzymatic steps that are applied in a cell-free system. This approach is considered safe and sustainable since generally high selectivity and yield of the desired end-product are achieved without the burden of toxic catalysts or reagents. Additionally, this strategy requires only mild reaction temperatures (4–60 °C).[268] The enzyme's reactivity and selectivity are also tuneable through protein engineering.[269] However, producing α-KGA using this strategy may require isolation of enzymes from the microbial cells that produce them, therefore potentially increasing production costs.[270]

Different enzymes can be applied to produce α-KGA in a cell-free biocatalytic system. Glutamic acid (Glu) or its cyclic version, 2-pyrrolidone-5-carboxylic acid, can be transformed into α-KGA with the oxygen-dependent *Streptomyces ghanaensis* L-glutamate oxidase (L-GOX) (Fig. 8, ①),[271] an engineered variant of the *Proteus mirabilis* L-amino acid deaminase (L-AAD)[272] (Fig. 8, ②) or the NAD^+-dependent *Clostridium symbiosum* glutamate dehydrogenase (L-GDH) (Fig. 8, ③).[273] Often, the primary Glu-converting enzymes are combined with either a NADH oxidase or a catalase to regenerate NAD^+ or to detoxify the released hydrogen peroxide, respectively.[271,273] Applying immobilised L-GOX in combination with catalase in a 1 L reactor yielded the highest α-KGA production metrics in a cell-free system, reaching a titre of 71 g L^{-1} and a spatiotemporal yield of 14.2 g L^{-1} h^{-1}.[271]

More elaborate enzyme cascades have been designed to use other sugar(-derived) substrates for the production of α-KGA. For example, D-glucuronic acid can be converted into α-KGA in a four-step, redox-balanced pathway with a yield of 92% and with H_2O and CO_2 as primary side-products (Fig. 9A).[274] Recently, a similar approach has been proposed using D-xylose or L-arabinose as feedstocks while simultaneously co-producing green hydrogen gas (Fig. 9B).[275,276] This setup enabled the production of 41.6 g L^{-1} α-KGA, corresponding to a theoretical yield of at least 99%, with a spatiotemporal yield of 4.6 g L^{-1} h^{-1}.

Producing α-KGA using cell-free biocatalysis seems to be a promising strategy, primarily due to the high yields and product purity. The substrate for biocatalytic production of α-KGA, mainly glutamic acid, has been industrially produced on a large scale (over 2 million tons per year) using the bacterium *Corynebacterium glutamicum*.[277] Substrate availability is thus not an issue.

Currently, cell-free biocatalysis for α-KGA production primarily relies on purified enzymes, contributing to higher overall process costs. Although not yet applied to α-KGA synthesis, the use of crude cell extracts could offer a more cost-effective alternative. A second approach is whole-cell biocatalysis, where intact living cells serve as catalysts, which do not require enzyme purification as well. However, this may compromise product purity due to metabolic side reactions within the host organism. The specific advantages and limitations of whole-cell biocatalysis for α-KGA production are discussed in Section 5.2.

5. Producing α-KGA using microbial cell factories

5.1. The biochemistry of α-KGA in microorganisms

(Micro)organisms typically synthesise α-KGA as part of aerobic fatty acid and carbohydrate catabolism. Specifically, hexose or pentose sugars are first metabolised through the Emden Meyerhof pathway (glycolysis) or pentose phosphate pathway to yield phosphoenolpyruvate or pyruvate. These central C_3 intermediates are converted into either oxaloacetate or acetyl-CoA through a carboxylation or

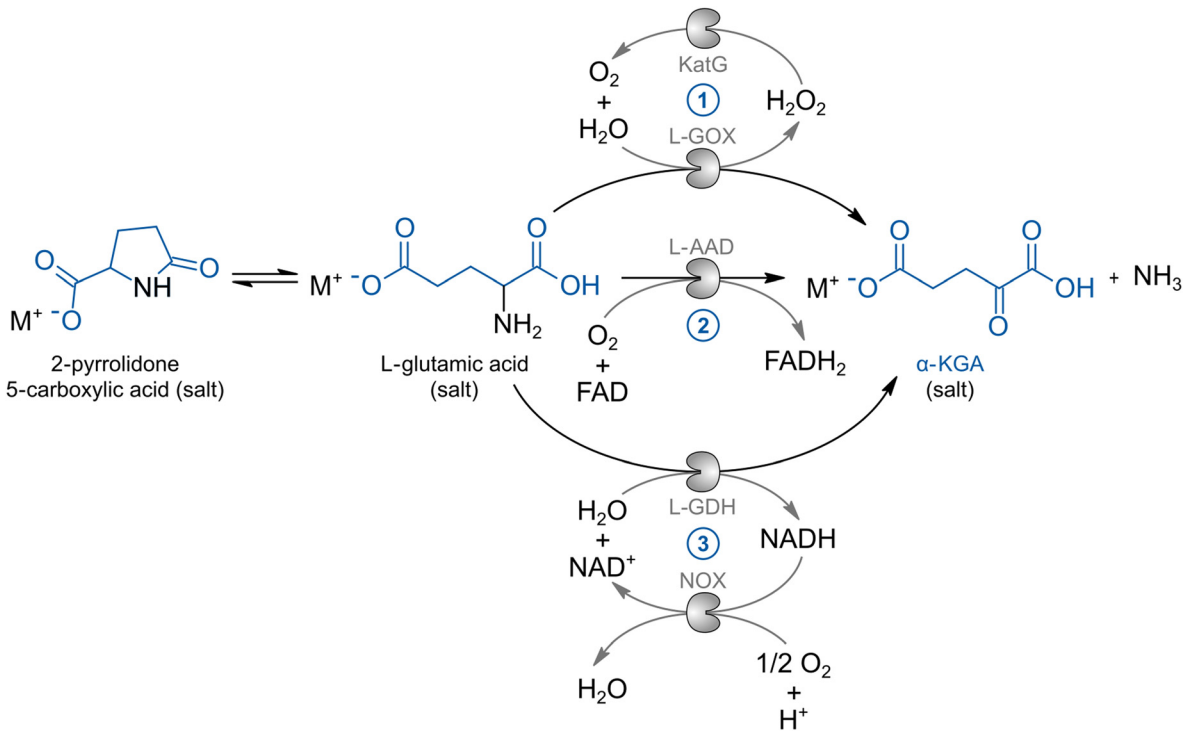


Fig. 8 *In vitro* biocatalytic conversion of L-glutamic and 2-pyrrolidone 5-carboxylic acid (or their corresponding salts) into α-KGA (salt). Three enzyme-based strategies can be distinguished using L-glutamate oxidase (L-GOX) ①, L-amino acid deaminase (L-AAD) ②, and L-glutamate dehydrogenase (L-GDH) ③. Furthermore, the NADH oxidase (NOX) is responsible for NAD^+ regeneration. M^+ represents either a proton, in the case of an acid, or an alkali metal cation (*e.g.*, Na^+), in the case of a salt. The figure is based on ref. 273, 338 and 614.

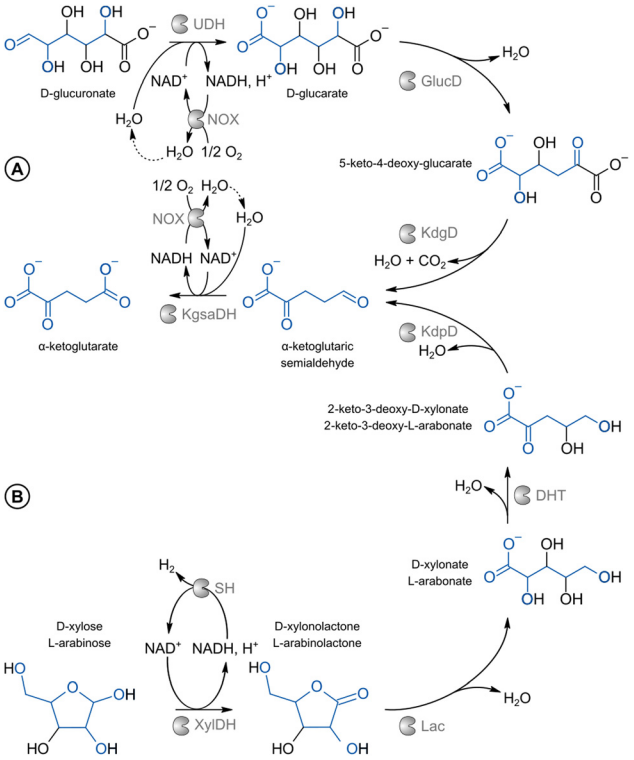


Fig. 9 *In vitro* enzyme cascades for the production of α-KGA from the hexose D-glucuronate (A) or pentoses D-xylose or L-arabinose (B). Enzyme abbreviations: UDH, uronate dehydrogenase; GlucD, glucarate dehydratase; KdgD; 5-keto-4-deoxyglucarate dehydratase; KgsaDH; α-ketoglutaric semialdehyde dehydrogenase; XylDH, xylose dehydrogenase; Lac, lactonase; DHT, dehydratase; NOX, NADH oxidase; SH, soluble hydrogenase; and KdpD, 2-keto-3-deoxy-D-xylonate or arabonate dehydratase. The figure is based on ref. 274–276.

decarboxylation reaction, respectively. The resulting products serve as key precursors for the tricarboxylic acid cycle (TCAc; also known as the Krebs cycle) within the cytoplasm of bacteria or the mitochondria of yeast (Fig. 10 and Table 4).[278–280] Fatty acids are first degraded through the β-oxidation pathway into acetyl-CoA, which then enters the TCAc (Fig. 10 and Table 4).[281–285] When the TCA cycle operates in its oxidative mode ("clockwise" orientation in Fig. 10), acetyl-CoA combines with oxaloacetate to form citrate. Citrate then undergoes a series of (de)hydration, decarboxylation, acetyltransferase, and oxidation reactions, sequentially generating (iso)citrate (C_6), α-KGA (C_5), and C_4 intermediates. This oxidative TCAc (oTCAc) produces reducing equivalents (NADH, $FADH_2$), which are crucial for multiple cellular reactions and also fuel the respiratory chain to yield energy in the form of ATP.[278,286] Under anaerobic conditions, the TCAc can also function in reductive mode to convert oxaloacetate, originating from (phosphoenol)pyruvate carboxylation, into succinic acid (Fig. 10).[287–289] Combined with the oTCAc, the reductive cycle prevents accumulation of reduced coenzymes (NADH, $FADH_2$) that cannot be readily re-oxidised in the absence of oxygen or other electron acceptors, while still ensuring the formation of intermediary TCA products that are essential for the cell's anabolism. In most model microorganisms, including *Escherichia coli* and *Saccharomyces cerevisiae*, succinic acid is the end point of the reductive TCAc (rTCAc) because the reaction catalysed by α-KGA dehydrogenase (KGDH, step 10 in Fig. 10) is irreversible in the clockwise orientation. However, in (thermophilic) green sulphur (proteo)bacteria, a fully functional reverse TCA cycle (rTCAc) enables CO_2 fixation into TCA cycle intermediates, catalysed by the alternative enzymes 2-oxoglutarate synthase and aconitate hydratase.[290–292]

Apart from being an indispensable intermediate of TCA cycles, α-KGA serves as the main precursor for the *de novo* synthesis of glutamate, which, in turn, plays a key role as a nitrogen donor in transamination reactions.[293–295] Furthermore, *Pseudomonas fluorescens* exploits α-KGA as an antioxidant and possesses a dedicated pathway to "recycle" α-KGA from succinate based on the spontaneous reaction between α-KGA and reactive oxygen species.[296–298]

While the TCAc is the most universal pathway leading to α-KGA biosynthesis, several other pathways can also contribute to α-KGA formation, including oxidative glutamate deamination, the Weimberg pathway, and galacturonic acid degradation. Firstly, oxidative deamination of glutamate may proceed in a cofactor-dependent or -independent way. The former reaction is performed by glutamate dehydrogenase, which also converts α-KGA into glutamate in the opposite direction, and requires $NAD(P)^+$.[299] As discussed previously, L-amino acid deaminase (L-AAD)[300,301] or L-glutamate oxidase (L-GOX)[302] solely requires oxygen and releases hydrogen peroxide while deaminating glutamate. Finally, the xylose oxidative (Weimberg) pathway is a thermodynamically more favourable route that converts xylose into α-KGA in only five consecutive steps (Fig. 14).[303] This atypical sugar metabolism can be found in hyperthermophilic Archaea from the *Sulfolobus* genus,[304] the bacterial *Pseudomonas*[303,305,306] or *Caulobacter crescentus*[307] species, and the filamentous fungus *Myceliophthora thermophila*.[308]

5.2. L-Glutamic acid to α-KGA *via* whole-cell biocatalysis

Whole-cell biocatalysis (or biotransformation) leverages living microbial cells as natural biocatalysts to convert precursor molecules into α-KGA. In this approach, the entire microbial cell is utilised rather than purified or extracted enzymes as employed in the cell-free biocatalytic approach discussed in the previous chapter (Fig. 8). These cells contain the desired enzymes and biochemical pathways to catalyse a small number of key reactions without the need to disrupt the cells or purify individual enzymes, thereby reducing costs.[327]

Specifically for α-KGA production, whole-cell biocatalysis has been explored to deaminate the substrate L-glutamic acid or its cyclic derivative, 2-pyrrolidone-5 carboxylic acid. In this case, the L-amino acid oxidase (L-AAO) or L-AAD from *Proteus* species[328,329] and L-GOX from *Streptomyces*[321,330–336] are heterologously expressed in a microbial host, such as *B. subtilis* or *E. coli* (Table 5). Importantly, when using oxidases (either L-AAO or L-GOX) as a catalyst, the reaction generates hydrogen peroxide, a toxic by-product for the microbial host cell. Hence, a catalase is often co-expressed alongside L-GOX to convert the released hydrogen peroxide into water and oxygen.[323,337] Protein engineering can be applied to enhance the enzyme activity,

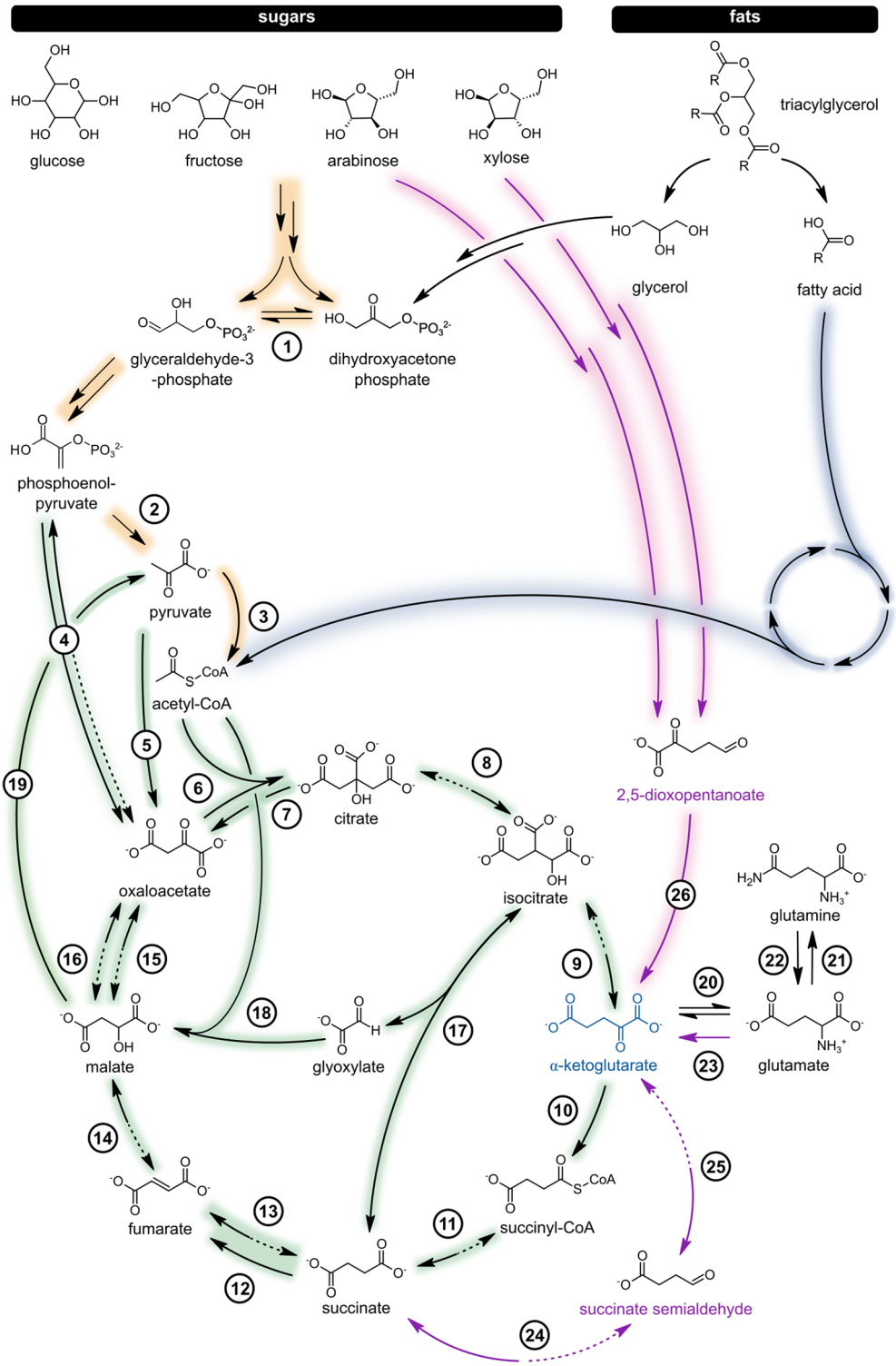


Fig. 10 The microbial metabolism of sugars and fats towards α-KGA. Yellow shading refers to the glycolysis and pentose phosphate pathways, green to the TCAc, blue to the fatty acid (β-oxidation) degradation pathway, and fuchsia to the Weimberg pathway. The purple arrows indicate heterologous enzymes that cannot be natively found in *E. coli*, *C. glutamicum*, and *Y. lipolytica*. The solid lines highlight the oxidative reactions in the TCAc and Weimberg pathway, whereas the dotted lines represent reductive reactions. The numbers and the associated reaction chemistry are explained in more detail in Table 4. The figure is created based on ref. 296, 410, 615 and 616.

resulting in a higher conversion efficiency. For instance, L-GOX from *Streptomyces mobaraensis* was subjected to two rounds of site-saturation mutagenesis, which increased the enzyme activity 1.9-fold due to two combinatorial amino acid substitutions

Table 4 Supplementary table to Fig. 10, describing the reactions and the genes involved in the microbial metabolism more extensively. Gene names are provided in italics and species names are included in the gene accession IDs: ECK, *Escherichia coli* (*Ec*) K12-MG1655; cg, *Corynebacterium glutamicum* (*Cg*) ATCC 13032; YALI0, *Yarrowia lipolytica* CLIB1222; PFLU, *Pseudomonas fluorescens* SBW25; CCNA, *Caulobacter crescentus/vibrioides* NA1000; and Bxe, (*Para*)*Burkholderia xenovorans* LB400. Gene information and reactions retrieved from **https://www.ecocyc.org**[309] for *E. coli*, from **https://www.pseudomonas.com**[310] for *P. fluorescens*, and from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database[311] for *C. glutamicum, Y. lipolytica, C. crescentus, P. fluorescens, and B. xenovorans*. Additional information resources are provided in the Ref column. In the case of heterologous expression, the host organism is indicated after ⟾. Gene names that are found between square brackets encode for peptides that constitute a single polypeptide enzyme complex. Colours of the outlines correspond to the shaded backgrounds behind the arrows in Fig. 10. Abbreviations: (M)Q, (mena)quinone; (M)QH_2, (mena)quinol; OPO_3^{3-}, orthophosphate; CoA, coenzyme A; and §, regulator (inhibitor). (*) *C. glutamicum* possesses a single complex that acts as a hybrid pyruvate- and α-ketoglutarate dehydrogenase complex[312]

Reaction number	Ref.	Enzyme name	Gene name Gene accession ID	Reaction
1		triose phosphate isomerase	*tpiA* (ECK3911) *tpiA* (cg1789) *YALI0F05214g*	glyceraldehyde-3-phosphate ↔ dihydroxyacetone phosphate
2		pyruvate kinase	*pykF* (ECK1672) or *pykA* (ECK1855) *pyk* (cg2291) *PYK1 (YALI0F09185g)*	phosphoenolpyruvate + H^+ + ADP → pyruvate + ATP
		phosphoenolpyruvate synthetase	*ppsA* (ECK1700)	phosphoenolpyruvate + $2H^+$ + OPO_3^{3-} + AMP ↔ pyruvate + ATP + H_2O
	313	Enzyme I (EI) of sugar phosphotransferase system (PTS)	*ptsI* (ECK2411) *ptsI* (cg2117)	phosphoenolpyruvate + $2H^+$ + OPO_3^{3-} + AMP ↔ pyruvate + ATP + H_2O
3	314 315	pyruvate dehydrogenase	[*aceE, aceF, lpd*] (ECK0113, ECK0114, ECK0115) [*aceE, aceF, lpd, odhA, odhI*§] (cg2466, cg2421, cg0441, cg1280, cg1630§) (*) [*PDA1, PDB1, LAT1, LPD1*] (*YALI0F20702g, YALI0E27005g, YALI0D23683g, YALI0D20768g*)	pyruvate + CoA-SH + NAD^+ → acetyl-CoA + CO_2 + NADH
4	316	phosphoenolpyruvate carboxylase phosphoenolpyruvate carboxykinase	*ppc* (ECK3947) *ppc* (cg1787) *pck* (ECK3390) *pck* (cg3169) *YALI1C24367g*	phosphoenolpyruvate + HCO_3^- → oxaloacetate + OPO_3^{3-} phosphoenolpyruvate + CO_2 + ADP ↔ oxaloacetate + ATP
5		pyruvate carboxylase	*pyc* (cg0791) *PYC1 (YALI0C24101g)*	pyruvate + CO_2 + ATP → oxaloacetate + ADP + OPO_3^{3-}
6		citrate synthase	*gltA* (ECK0709) *gltA* (cg0949) *CIT1 (YALI0E00638g), CIT2 (YALI0E02684g)*	oxaloacetate + acetyl-CoA + H_2O → citrate + CoA-SH + H^+
7		citrate lyase	[*citD, citE, citF*] (ECK0610, ECK0609, ECK0608) *citE* (cg0985) [*ACL1, ACL2*] (*YALI0D24431g, YALI0E34793)*	citrate → oxaloacetate + acetate citrate + ATP + CoA-SH → oxaloacetate + acetyl-CoA + ADP
8		aconitase	*acnA* (ECK1271) *acn* (cg1737) *ACO1 (YALI0D09361g), ACO2 (YALI0E14949g)*	citrate + H_2O ↔ isocitrate
9		isocitrate dehydrogenase	*icd* (ECK1122) *icd* (cg0766) *IDP2 (YALI0F04095g)* *IDH2 (YALI0D06303g), IDH1 (YALI0E05137g)*	isocitrate + $NADP^+$ ↔ α-ketoglutarate + CO_2 + NADPH isocitrate + NAD^+ ↔ α-ketoglutarate + CO_2 + NADH
10	314	α-ketoglutarate dehydrogenase	[*sucA, sucB, lpd*] (ECK0714, ECK0715, ECK0115) [[*aceE, aceF, lpd, odhA, odhI*§] (cg2466, cg2421, cg0441, cg1280, cg1630§) (*) [*KGD1, KGD2, LPD1*] (*YALI0E33517g, YALI0E16929g, YALI0D20768g)*	α-ketoglutarate + CoA-SH + NAD^+ → succinyl-CoA + CO_2 + NADH

Table 4 (*continued*)

Reaction number	Ref.	Enzyme name	Gene name Gene accession ID	Reaction
11		succinyl-CoA synthetase	[*sucC, sucD*] (ECK0716, ECK0717) [*sucC, sucD*], *actA* (cg2837, cg2836, cg2840) [*SCS1, SCS2*] (*YALI0E24013g, YALI0D04741g*)	succinyl-CoA + ADP + OPO_3^{3-} ↔ succinate + CoA-SH + ATP succinyl-CoA + GDP + OPO_3^{3-} ↔ succinate + CoA-SH + GTP
12	317 318	succinate dehydrogenase (succinate:quinone oxidoreductase)	[*sdhA, sdhB, sdhC, sdhD*] (ECK0712, ECK0713, ECK0710, ECK0711) [*sdhA, sdhB, sdhCD*] (cg0446, cg0447, cg0445) [*SDH1, SDH2, SDH3, SDH4, SDH5*] (YALI0D11374g, YALI0D23397g, YALI0E29667g, YALI0A14784g, YALI0F11957g)	succinate + Q → fumarate + QH_2 succinate + FAD → fumarate + $FADH_2$
13		fumarate reductase	[*frdA, frdB, frdC, frdD*] (ECK4150, ECK4149, ECK4148, ECK4147)	succinate + MQ ↔ fumarate + MQH_2
14		fumarase	*fumA* (ECK1607), *fumB* (ECK4115), *fumC* (ECK1606), *fumD* (ECK1671), or *fumE* (ECK2925) *fum* (cg1145) *FUM1 (YALI0C06776g)*	fumarate + H_2O ↔ malate
15	319	malate dehydrogenase	*maeA* (ECK1473) *mdh* (cg2613) *mdh2* (cg0763) *maeB* (ECK2458) *IDH1 (YALI0D16753g), IDH2 (YALI0E14190g)*	malate + NAD^+ ↔ oxaloacetate + NADH malate + $NADP^+$ ↔ oxaloacetate + NADPH malate + $NAD(P)^+$ ↔ oxaloacetate + NAD(P)H
16	319	malate:quinone oxidoreductase	*mqo* (ECK2202) *mqo* (cg2192)	malate + Q ↔ oxaloacetate + QH_2
17		isocitrate lyase	*aceA* (ECK4007) *aceA* (cg2560) *ICL1* (*YALI0C16885g*)	isocitrate ↔ glyoxylate + succinate
18		malate synthase	*aceB* (ECK4006) or *glcB* (ECK2970) *aceB* (cg2559) *MLS1 (YALI0E15708g), YALI0D19140g*	acetyl-CoA + glyoxylate + H_2O → malate + CoA-SH + H^+
19		malate dehydrogenase	*maeA* (ECK1473) *maeB* (ECK2458)	malate + NAD^+ → pyruvate + NADH malate + $NADP^+$ → pyruvate + NADPH
20	320	glutamate dehydrogenase glutamate synthetase	*gdhA* (ECK1759) *gdh* (cg2280) *GDH1 (YALI0F17820g)* *GDH2 (YALI0E09603g)* [*gltB, gltD*] (ECK3202, ECK3203)	α-ketoglutarate + NADPH + H^+ + NH_4^+ ↔ glutamate + $NADP^+$ + H_2O α-ketoglutarate + NADH + H^+ + NH_4^+ ↔ glutamate + NAD^+ + H_2O
21		glutamine synthetase	*glnA* (ECK3863) *glnA* (cg2429) *glnA2* (cg2447) *GLN1 (YALI0F00506g)*	glutamate + ATP + NH_4^+ → glutamine + OPO_3^{3-} + ADP + H^+
22		glutamate synthase (glutamine 2-oxoglutarate aminotransferase)	[*gltB, gltD*] (ECK3202, ECK3203) [*gltB, gltD*] (cg0229, cg0230) *GLT1 (YALI0B19998g)*	glutamine + α-ketoglutarate + NADPH + H^+ → 2 glutamate + $NADP^+$ glutamine + α-ketoglutarate + NADH + H^+ → 2 glutamate + NAD^+
23 (→)		L-glutamate oxidase	*Lgox* (*Streptomyces* sp.) ⟾ *Ec*	glutamate + O_2 + H_2O → α-ketoglutarate + NH_4^+ + H_2O_2
24 (→)		succinate semialdehyde dehydrogenase	*gabD* (PFLU0180) ⟾ *Ec*	succinate + NADPH + $2H^+$ ↔ succinate semialdehyde + $NADP^+$ + H_2O
25 (→)		α-ketoglutarate decarboxylase	*alkB* (PFLU2976) ⟾ *Ec*	succinate semialdehyde + CO_2 ↔ α-ketoglutarate + H^+
26 (→)		α-ketoglutarate semialdehyde dehydrogenase	*xylA* (CCNA00865) ⟾ *Ec* Bxe_C1357 ⟾ *Ec*	2,5-dioxopentanoate + H_2O + NAD^+ ↔ α-ketoglutarate + $2H^+$ + NADH

Table 5 Performance indicators of α-KGA production *via* whole-cell biocatalysis. Symbols: ⟹, introduction and heterologous expression; *, mutant; Δ, gene deletion/knock-out; ↑, increase; and ⊗, inhibition. Strain designation: *Ec*, *E. coli*; *Bs*, *Bacillus subtilis*; *Sg*, *Streptomyces ghanaensis*; *Sm*, *Streptomyces mobaraensis*; *Sp*, *Streptomyces platensis*; *Sl*, *Streptomyces lividans*; *Sv*, *Streptomyces viridosporus*; and *Pm*, *Proteus mirabilis*. Abbreviations: L-Glu, L-glutamic acid

Species	Strain	Strategy	Substrate [g/L]	Production period [h]	Titre [g/L]	Yield [g/g]	Productivity [g/L/h]	Ref.
Ec	BL21	⟹ *Lgox* from *Sm* Expression catalase (*katE*)↑	L-Glu [100]	12	77.40	0.98	6.5	321
		⟹ mutagenised *Lgox** $S_{280}T$, $H_{533}L$ from *Sm* Expression catalase (*katE*)↑	Monosodium glutamate [270]	12	181.90	0.75	N/A	322
		9:1 double-strain cultivation ⟹ *Lgox* from *Sp* (strain 1) ⟹ Catalase (*cat*) from *Sl* (strain 2)	L-Glu [100]	6	95.40	0.95	15.9	323
		1:1 double-strain cultivation, immobilised on zinc-imidazole framework ⟹ *Lgox* from *Sv* (strain 1) ⟹ Catalase *katG* from *Ec* (strain 2)	L-Glu [40]	12	38.40	0.97	3.2	324
Bs	168	⟹ *aad* from *Pm* Cell-immobilisation	L-Glu [15]	24	4.65	0.31	0.2	325
		⟹ mutagenised *aad** $F_{110}I$ $A_{255}T$ $E_{349}D$ $R_{228}C$ $T_{249}S$ $I_{352}A$ from *Pm* Δ*sucA*: ⊗ α-KGA degradation		24	12.21	0.81	N/A	326

($S_{280}T$ and $H_{533}L$). When the engineered L-GOX mutant was introduced together with the KatE catalase in *E. coli*, this whole-cell biocatalysis system was able to reach an average spatio-temporal yield of 15.2 g L^{-1} h^{-1} α-KGA over a 12 h period and a molar conversion efficiency of 86.3% from L-glutamic acid.[337]

Due to the different enzyme activities of L-GOX and catalase, and to reduce the burden of expressing multiple heterologous enzymes simultaneously, fine-tuning their expression ratio can be crucial. One study adjusted the co-expression of L-GOX from *Streptomyces ghanaensis* and the native KatG catalase in *E. coli* using promoter and ribosome binding site engineering to obtain an LGOX : KatG activity ratio of 2 : 1185. This resulted in a spatiotemporal yield of 13.25 g L^{-1} h^{-1} with a conversion efficiency of 96% over an 8 h period.[338] Another solution exploited a double-strain setup in which one of the *E. coli* strains expressed the enzyme L-GOX from *Streptomyces platensis* and the other the enzyme catalase from *Streptomyces lividans*. Through fine-tuning the inoculum ratios of the two *E. coli* strains, α-KGA productivity was increased by 97% compared to the single-strain system. Moreover, a scale-up strategy brought the spatiotemporal yield to 15.9 g L^{-1} h^{-1}, the highest reported value to date.[339]

To further improve productivity and reduce operational costs of whole-cell α-KGA synthesis, cell immobilisation has been explored.[324,325,340] Here, microbial cells are attached to a solid support (*i.e.*, alginate beads or metal–organic frameworks) within the reaction vessels, allowing higher cell densities and easier cell recycling and separation from the final product. However, conversion efficiencies are often lower in immobilised compared to planktonic cells, and enzymatic activity decreases by 20–30% after each cycle.[325,340] Hence, it is not entirely clear whether cell immobilisation is truly a viable strategy.

Whole-cell α-KGA production has gained considerable interest, achieving higher titres and productivity than cell-free systems (see Section 4). Advances in metabolic engineering and process scale-up have contributed to improved yields. In addition, whole-cell biocatalysis can make costly enzyme purification redundant and even allows reusing cells across multiple reaction cycles, potentially further improving cost-efficiency.[327]

5.3. Native α-KGA-producing microbial candidates for industrial settings

Over the years, several candidate microbial producers have been discovered that naturally produce high levels of α-KGA. In the bacterial kingdom, *Escherichia coli*,[341] *Pseudomonas fluorescens*,[342–344] *Corynebacterium glutamicum*,[345,346] *Micrococcus paraffinolyticus*, *Arthrobacter paraffineus* or *hydrocarboglutamicus*[347,348] have been reported to natively secrete the ketoacid of interest. In the case of yeasts, natural α-KGA production occurs in *Candida glabrata*,[349–352] *Yarrowia lipolytica*,[353] and several *Pichia* species.[354,355] Although the above-mentioned microbial species all show potential as microbial α-KGA cell factories, most research thus far has focused on *E. coli*, *C. glutamicum*, or *Y. lipolytica* for metabolic or process engineering to enhance α-KGA biosynthesis.

5.3.1. *Escherichia coli* and *Corynebacterium glutamicum*. The Gram-negative bacterium *E. coli* is arguably the most-studied bacterial species.[356] The genome of this ϒ-proteobacterium has been fully sequenced[357] and the function of many genes (*ca.* 93%) has been annotated.[358–361] Moreover, the availability of an extensive molecular toolbox, including high-throughput and precise gene- or genome-editing methods,[360,362,363] has enabled researchers to redesign *E. coli* into a versatile fuel (*e.g.*, alcohols, isoprenoid/terpenes, and fatty acid ethyl esters) or chemical (*e.g.*, lactic, succinic, and diols/triols) bio-factory for precision fermentation.[364–367]

Since its discovery in the mid-1950s in Japan, *C. glutamicum* has also received considerable interest as a potential chassis strain for producing various value-added compounds.[368–370]

Originally, this non-spore-forming Gram-positive soil species was appreciated as an excellent glutamate producer but later on it was also exploited for industrial-scale lysine production.[370–372] Today, the list of *C. glutamicum*-derived products has gradually extended towards other proteogenic (*e.g.*, L-methionine and L-isoleucine) and non-proteogenic (*e.g.*, ϒ-aminobutyric acid (GABA) and ectoine) amino acids, including their less common D-isoforms.[373,374] Furthermore, *C. glutamicum* has been successfully applied for the synthesis of TCAc-derived acids including itaconic,[375] glutaric,[376] ornithine,[377] and 5-aminolevulinic acid[378] as well as for the production of aromatic amino acid-derived compounds such as muconic acid.[379,380] While *E. coli* is well known for its broad carbon utilisation spectrum, *C. glutamicum* has a narrow substrate range, which is an important limitation. Specifically, *C. glutamicum* lacks the key pathways and enzymes for xylose, arabinose, lactose, galactose, and glycerol consumption.[345] This limitation can, however, be overcome by introducing the corresponding carbon catabolism pathways from *E. coli* to enable fermentation using less expensive carbon substrates (see further).[381]

5.3.2. Yarrowia lipolytica. *Y. lipolytica* is an oleaginous yeast that is most studied for the production of lipids, biofuels, and various chemicals derived from fatty acids. More recently, *Y. lipolytica* cells have also been engineered to synthesise non-oleochemical compounds such as organic acids, polyketides, lactones, aromatic alcohols, polyalcohols, and terpenes.[382,383] This yeast is particularly suitable for the production of α-KGA because of its ability to utilise a wide range of carbon substrates, including glucose, glycerol, ethanol, alkanes, and lipids, and excrete high amounts of organic acids.[384,385] Moreover, its obligate aerobic metabolism shuttles most carbons through the TCAc which, consequently, limits substrate losses associated with the fermentative pathway of ethanol, commonly-found in *S. cerevisiae*.[386] Finally, *Y. lipolytica* belongs to the GRAS ("generally recognised as safe") microorganisms and exhibits high tolerance to low pH conditions, making it an interesting cell factory chassis for industrial precision fermentation applications.[385,387]

Although the microorganisms discussed above already produce α-KGA to some extent, achieving economically sustainable production rates requires process optimisation, *i.e.*, creating the ideal production environment, and strain improvement through metabolic engineering. Hence, the next sections elaborate on these two aspects. Although the focus is primarily on α-KGA (Table 6), publications on α-KGA-derivatives (including glutamic, glutaric, mesaconic, ϒ-aminobutyric acid, arginine, ornithine, and 1,4-butanediol) (Table 7) and succinic/malic acid (Table 8) are also covered. These citations often mention similar or overlapping optimisation approaches to those commonly pursued for α-KGA and can therefore serve as a valuable source of inspiration.

5.4. Optimising fermentation conditions

This section discusses the efforts invested in identifying fermentation conditions, such as nutrients, additives, temperature, aeration, and pH, that can be regulated and controlled within bioproduction units and positively influence α-KGA production titres. Profound knowledge of the optimal conditions for a given bioproduction process is key to achieving economic sustainability in fermentation activities.

5.4.1. Minimising KGDH activity by changing medium composition. One way to enhance α-KGA production in microbes is by decreasing α-KGA breakdown through inhibition of the α-ketoglutarate dehydrogenase (KGDH) complex, which converts α-KGA into succinyl-CoA. In both *Y. lipolytica* and *C. glutamicum*, the activity of this key enzyme complex can be modulated by adjusting the concentration of cofactors and other compounds in the growth medium.

In the case of *Y. lipolytica*, this yeast species takes up thiamine from the growth broth as it is an essential cofactor for the KGDH complex.[388] Hence, limiting thiamine levels is the key strategy to reduce the KGDH complex activity without harming cell growth (Fig. 11, ①).[388–391] Indeed, several studies have demonstrated that thiamine concentrations between 0.15 and 4 μg L^{-1} are optimal for α-KGA production.[388,390,391] However, when thiamine is too low, α-KGA production drops because the pyruvate dehydrogenase complex (PDH) is also thiamine-dependent. As a result, low PDH activity causes accumulation of pyruvate as a major by-product.[390,392–394] Generally, the optimal thiamine concentration depends on the strain, carbon substrate, and culture conditions. However, the common trend is that low thiamine enhances α-KGA but limits biomass production, and *vice versa*. As such, the initial thiamine concentration is typically set higher than the abovementioned optimal concentrations to support biomass growth before transitioning into α-KGA production under thiamine-limited conditions.

In *C. glutamicum*, biotin limitation, supplementation of Tween 40 and 60 detergents and copper,[395] and sublethal penicillin doses are known to trigger α-KGA and glutamate production (Fig. 11, ①).[396] Regarding exposure to Tween and penicillin, this observation can be partially attributed to an increase in pyruvate carboxylase activity, an enzyme that is involved in the synthesis of oxaloacetate, an α-KGA precursor.[397] Moreover, Tween and penicillin might also affect the flux from α-KGA to succinyl-CoA through KGDH, thereby promoting α-KGA accumulation.

Alternatively, medium supplements can also modulate the activity of other key enzymes. Indeed, exposure to surfactants destabilises DtsR, a critical component of the Acyl-CoA carboxylase complex. Instead, depriving cells of biotin also attenuates the activity of the complex because this vitamin is essential for the enzyme's function. Both strategies compromise the flux of acetyl-CoA towards fatty acids and consequently promote α-KGA accumulation as inhibition of fatty acid synthesis indirectly reduces KGDH activity.[396,398] Practically, adjusting the biotin concentration in the medium to about 7 μg L^{-1} proved most efficient in ensuring normal growth of *C. glutamicum* while stimulating α-KGA production (25% more α-KGA than with 9 μg L^{-1} biotin).[399] Penicillin's mode-of-action is more transcription-oriented since this antibiotic decreases *odhA* expression, encoding the catalytic subunit of KGDH, and increases the transcript levels of its corresponding repressor, *odhI*.[400] Another strategy to disrupt KGDH activity is by specifically targeting the complex using the dehydrogenase inhibitor methotrexate (also called Rheumatrex), a medicine to cure

Table 6 Overview of the fermentation condition optimisation (FCO) and metabolic engineering (ME) strategies to improve α-KGA production. The blue numbers refer to the engineering strategies that are graphically explained in Fig. 11, in the case of FCO, or Fig. 12, for ME. Abbreviations: F, flask; B, batch bioreactor; FB, fed-batch bioreactor; ALE, adaptive laboratory evolution; CSL, corn steep liquor; ⟾, introduction of non-native genes; ↕, expression optimisation; ↑, increase; ↓, decrease; ⊗, disruption/deactivation. E following a numerical value indicates that the substrate concentration was maintained around that value, once the initial substrate concentration i was consumed. Strain designation: *Ec*, *E. coli*; *Cg*, *C. glutamicum*; *Cc*, *C. crescentus*; *Tg*, *T. glabrata*; *Yl*, *Y. lipolytica*; *Pk*, *Pichia kudriavzevii*; *Km*, *Kluyveromyces marxianus*; *Sc*, *Saccharomyces cerevisiae*, and *Af*, *Aspergillus flavus*. Enzyme abbreviations: KGDH, α-ketoglutarate dehydrogenase (complex)

Species	Strain	Strategy	Substrate [g/L]	Fermentation setup	Production period [h]	Titre [g/L]	Yield [g/g]	Productivity [g/L/h]	Ref.
Ec	W3110Δ4-$P_{(H)}CAI_{(H)}A$	**ME:** ②**b:** ⟾ pyruvate carboxylase (*pyc*) from *Af* ③**a**: ↕ expression of citrate synthase (*gltA*), aconitase (*acnA*), and isocitrate dehydrogenase (*icd*) ③**b,d**: Δ fumarate reductase (*frdBC*) and fumarase (*fumABC*). ⑤: *ΔldhA, pflB, poxB, ackA-pta*: ⊗ byproduct formation of lactate formate, and acetate	glucose [50 + 50]	FB [5L]	60	32.2	0.32	0.54	410
Cg	JH107	**ME:** ④**a:** *ΔsucA*: ⊗ KGDH ④**b**: *ΔgdhA*, *gltB*: ⊗ amination of α-KGA into glutamate **FCO:** ③: Ammonium ↓	glucose + molasses [total Sugar: 10-80E]	FB	120	51.1	N/A	N/A	406
Cg	GKG-047	Random mutagenesis **FCO:** ①: Methotrexate treatment ②: Biotin supplementation optimisation ③: Ammonium ↓(NH_4OH ▶ NaOH) **ME:** ④**b**: *Δgdh1, 2*: ⊗ amination of α-KGA into glutamate	glucose [40i ▶ 10E]	FB [5L]	32	45.6	0.42	1.52	399
			Glucose [100] + CSL [2]	FB [6|10 L]		47.2	N/A	N/A	401
Cg	N/A	⟾ Weimberg pathway (*xylABCD*) from *Cc*, combined with ALE.	xylose [67.5-132.1]	B [1L]	65	6.7-11.5	0.05-0.09	0.005-0.008	411
Tg	CCTCC M202019	**FCO:** ① [thiamine] ↓ (0.04 mg/L) ② [glucose] ↓	glucose [100]	B [4L in 7L reactor]	64	30.0	0.3	0.47	349
Tg	CCTCC M202019	**FCO:** ① [thiamine] ↓ (0.02 mg/L) ④ Buffered ($CaCO_3$)	glucose [100]	B [4L in 7L reactor]	64	43.7	0.44	0.68	350
Tg	CCTCC M202019	**FCO:** ① Methotrexate treatment	glucose [30i ▶ 100E]	N/A	40	23.2	0.59	N/A	402
Yl	AJ5004	**FCO:** ① [thiamine] ↓, [metals] ↓ (Cu, Zn, and Fe)	*n*-alkanes (C_{14}-C_{16}) [80]	B [0.5L]	72	46.0	0.59	0.64	412
Yl	D 1805	UV-mutagenesis	*n*-alkanes (C_{13}-C_{18}) [2.5 ml/h E]	FB	240	185.0	N/A	0.77	387
Yl	H355	**FCO:** ① [thiamine] ↓	*n*-alkanes (C_{12}-C_{18})	B	150	195.0	0.94	1.30	413
Yl	VKM Y-2373	**FCO:** constant low ethanol (≤2.5 g/L) ① [thiamine] ↓ ③ [nitrogen] ↑ ⑤ O_2 ↓	ethanol [2.5i, 2.5E]	FB [6L]	144	49.0	0.42	0.57	388
Yl	VKM Y-2412	**FCO:** ① [thiamine] ↓ (0.15 μg/L) ⑤ two regime aeration and pH control	biodiesel [80]	B [5L]	192	80.4	1.01	0.42	390

Table 6 (*continued*)

Species	Strain	Strategy	Substrate [g/L]	Fermentation setup	Production period [h]	Titre [g/L]	Yield [g/g]	Productivity [g/L/h]	Ref.
Yl	A-8	**FCO:** ① [thiamine] ↓ (3 μg/L) alternating glycerol-rapeseed oil substrate feeding	glycerol [40i, 0-40E]	FB [2L]	260	58.2	0.58	0.22	394
		Increase of selectivity from 58% to 99% towards α-KGA than pyruvate at the end of the cycles ⑤ two regime pH control	rapeseed oil [0-40E]						
Yl	CBS146773	**FCO:** ① [thiamine] ↓ and sorbitan monolaurate supplementation ⑤ pH and aeration control	glycerol [20i, 10-45E]	FB [2L]	144	82.4	0.59	0.57	393
Yl			rapeseed oil [unknown E]						
Yl		**ME:** Expression of glycerol kinase ↑ ③a: expression methyl citrate synthase ↑ ⑥: mitochondrial organic acid transporter ↑	glycerol [20i unknown E]	FB [2L]	168	53.1	0.53	0.35	404
		FCO: ③: [nitrogen] ↑ [phosphorus] ↓	rapeseed oil [unknown E]						
Yl	WSH-Z06	**ME**②a: ⟾ ACS1 of *Sc*: [acetyl-CoA] ↑, [pyruvate] ↓ **FCO**⑤: pH and aeration control	glycerol [100]	B [1.5L]	144	52.6	0.53	0.37	414
Yl		**ME**②a: ⟾ expression of Acyl-ATP citrate lyase (ACL1) of *Mus musculus*: [acetyl-CoA] ↑, [pyruvate] ↓ **FCO**⑤: pH and aeration control				56.5	0.57	0.39	
Yl	WSH-Z06	**ME:** ②a: Expression of pyruvate dehydrogenase E1α subunit (PDA1)	glycerol [100]	B [1.5L]	144	43.3	0.43	0.30	315
Yl	WSH-Z06	**ME:** ④a: Expression mutant KGD2 $D_{423}E$ ↑: 40% [α-KGA] ↑	glycerol [100]	B [1.5L]	144	50.4	0.50	0.35	26
Yl	WSH-Z06	**ME:** ②b: ⟾ *Sc* PYC1	glycerol [100]	B [1.5L]	144	53.6	0.54	0.37	392
		②b: ⟾ *Rhizopus oryzae* PYC2				62.5	0.63	0.43	
Yl	H355	**ME:** ②b: Expression of pyruvate carboxylase (PYC) ↑ ③a: Expression of $NADP^+$-dependent isocitrate dehydrogenase (IDP1)	glycerol [150i unknown E]	FB [0.6L]	117	186	0.36	1.75	415
Yl	WSH-Z06	**ME:** ⑥: Extra copy of *YALI0B19470g* carboxylate transporter ↑	glycerol [100]	B [0.05]	144	46.7	0.47	0.32	416
Yl	WSH-Z06	Random mutagenesis	glycerol [100]	B [1.5]	168	31.5	0.31	0.19	417
Pk	TFL108	High temperature: 45°C Optimising $MgSO_4$ concentration	glycerin [100i + 50 extra]	B [5]	48	83.0	0.55	1.73	355

rheumatoid arthritis and an anticancer agent. Indeed, treatment with this pharmaceutical improved α-KGA titres in both *C. glutamicum*[401] and the yeast *T. glabrata*.[402]

Finally, besides their influence on enzyme activity and transcription, biotin limitation and Tween 40 or penicillin treatment may have an even higher impact on glutamate synthesis than on α-KGA production. It has been demonstrated that the induced fatty acid synthesis defect elicits membrane tension, which in turn triggers the mechanosensitive and major glutamate-exporting MscCG channel in *C. glutamicum*.[403]

5.4.2. Adjusting the concentration of nitrogen, phosphate, and micronutrients. The nitrogen content within the fermentation

Table 7 Overview of the fermentation condition optimisation (FCO) and metabolic engineering (ME) strategies to improve the parameters of α-KGA-derived products. Abbreviations and symbols are the same as in Table 6. Additional abbreviations: CSL, corn steep liquor; RBS, ribosome binding site; PTS, phosphotransferase system; i, initial; E, at equilibrium. Strain designation: *Ec*, *Escherichia coli*; *Cg*, *Corynebacterium glutamicum*; *Bs*, *Bacillus subtilis*, *Bm*, *Bacillus methanolicus*; *Bst*, *Bacillus stearothermophilus*; *Cc*, *Caulobacter crescentus*; *Bmu*, *Burkholderia multivorans*, *Xc*, *Xanthomonas campestris*, and *Bx*, *Burkholderia xenovorans*. Product abbreviations: GABA, ϒ-aminobutyric acid; BDO, 1,4-butanediol. Enzyme or gene abbreviations: GAD, glutamic acid decarboxylase; GDH; glutamate dehydrogenase; and KGDH, α-ketoglutarate dehydrogenase (complex). Metabolite abbreviations: PEP, phosphoenolpyruvate. Note: gray fonts indicate engineering strategies that were pursued for the intended product but are not relevant for α-KGA production

Species	Strain	Strategy	Product	Substrate [g/L]	Fermentation setup	Production period [h]	Titre [g/L]	Yield [g/g]	Productivity [g/L/h]	Ref.
Cg	AJ110214/ pgdh	**ME** (Expressing GDH ⬆) ④a: *ΔodhA*: ⊗ KGDH **FCO** ①: Treating with penicillin ②: [biotin] ⬇ and treating with Tween40.	glutamate	glucose [65]	B [1 L]	12	~20	~0.57	N/A	[436]
Cg	MX-14	**ME** ⟹ methanol consumption pathway: methanol dehydrogenase from *Bm* and 3-hexulose-6-phosphate synthase from *Bst* Engineering xylose consumption pathway (⟹ *xylA* from *Ec*) **FCO** ① penicillin G supplementation Improving methanol tolerance using ALE.	glutamate	xylose [4] methanol [15]	F [N/A]	144	0.23	N/A	N/A	[437,438]
Cg	GADΔpknG	**ME** (GAD (*gadB*) expression ⬆) ④a: *ΔpknG*: lock KGDH in an inactive state.	GABA	glucose [100]	F [200 mL]	168	31.16	0.72	0.26	[439]
Cg	SNW201	**ME** (GAD expression (*gadB1,2*)⬆) ④a: *ΔodhA*: ⊗ KGDH	GABA	glucose [100i ▶ >20E]	FB [3 L]	72	29.50	0.21	N/A	[440]
Ec	JWZ08/pW Zt7-g3/ pWZt7-xyl	**ME** (Expression GAD ⬆: *gadB*) (*ΔpuuE*, *gabP*, and *gabT* aminotransferases: ⊗ GABA-consumption) ⟹ Weimberg pathway (*xylABCDX*) from *Cc* *ΔxylAB*: ⊗ native xylose catabolism ④a: *ΔsucA*: ⊗ KGDH	GABA	xylose [20]	F [250 mL]	72	3.95	0.20	N/A	[441]
Cg	G7-1	**ME** (⟹ and expression ↕ of glycerol catabolism pathway) (⊗ GABA degradation pathway)	GABA	glucose [25-30i ▶ 0-20E]	FB [7.5 L]	72	45.60	0.40	N/A	[442]

Table 7 (*continued*)

Species	Strain	Strategy	Product	Substrate [g/L]	Fermentation setup	Production period [h]	Titre [g/L]	Yield [g/g]	Productivity [g/L/h]	Ref.
		③a: Growth phase-dependent ⬆ of citrate synthase (*gltA*) ④a: Growth phase-dependent ⬇ of KGDH (*odhA*)								
Ec	JW14	ME (⊗ Dahms pathway) (Expression glutarate biosynthesis pathway⬆) ⟾ Weimberg pathway (*xylABCDX*) from *Cc* *ΔxylAB*: ⊗ native xylose catabolism	glutarate	xylose [10]	F [125 mL]	60	0.60	N/A	N/A	[443]
Ec	EM38	ME ①: *ΔptsG*: ⊗ PTS transport and expression permease (*galP*) and PEP synthase (*ppsA*) ⬆ to [PEP]⬆ ②b: Expression PEP carboxylase (*ppc*) ⬆: [oxaloacetate]⬆ ③a: Expression oTCAc genes (*gltA*, *acnA*, and *icd*)⬆ ③d: *Δfum*: ⊗ oTCAc-downstream pathway ④a: *ΔsucA*: ⊗ KGDH complex	mesaconate	glucose [50]	F [125 mL]	75	23.10	0.46	0.36	[444]
Ec	17	ME (⟾ pentose importer *araE* from *Bx*) (Expression mesaconate biosynthesis pathway ⬆) ⟾ Weimberg operon from *Cc* and *Bx* ④a: *ΔsucA*: ⊗ KGDH complex	mesaconate	glucose [20], xylose [10], arabinose [10]	F [125 mL]	48	14.75	0.74	N/A	[445]
Ccr	Cc6	ME (Expression Arg biosynthesis pathway⬆) (⊗ Asp and Pro side production) ①: Glucose transport engineering ②b: *pyc* expression ⬆: [oxaloacetic acid] ⬆. ③a: Expression oTCAc genes (*gltA* and *icd*)⬆. ③d: Flux through PPP ⬆: [NADPH] ⬆ ④a: Weaken RBS of *odhA*: KGDH level⬇	arginine	Glucose/ Corn steep liquor [80i ▶ 20-50E]	FB [1000 L]	80	78.40	0.38	0.98	[446]
Cg	AR6	(Tolerance to Arg⬆) ME (⬆Arg-specific biosynthesis pathway and Δ of its repressors *argR* and *farR*) ③d: PGI activity ⬇ and expression of PPP genes (*zwf, tal, tkt, pgl*, and *opcA*)⬆: Flux through PPP ⬆: [NADPH]⬆	arginine	1:1 glucose: sucrose [~110i ▶ 15-70E]	FB [1500 L]	96	81.20	0.35	0.91	[447]

Table 7 (*continued*)

Species	Strain	Strategy	Product	Substrate [g/L]	Fermentation setup	Production period [h]	Titre [g/L]	Yield [g/g]	Productivity [g/L/h]	Ref.
Cg	YW06 (pSY223)	**ME** (*ΔproB, ΔargF*: ⊗ Pro and Arg synthesis) (*ΔargR* regulator: expression *arg* operon ⬆) ③**d**: start codon engineering of PPP genes *pgi* and *zwf*: [NADPH] ⬆	ornithine	glucose [80i ▶ 15-80E]	FB [6.6 L]	40	51.50	0.24	1.29	[448]
Cg	Sorn11	**ME** (*ΔargF*: ⊗ ornithine-citrulline conversion) (*ΔargR*: ⊗ feedback inhibition of the *arg* biosynthesis cluster) (⊗ Pro and Glu transport) (*lysE* expression ⬆: ornithine export ⬆) ④**a**: Weaken RBS of *odhA*: KGDH activity ⬇	ornithine	glucose [100]	F [250 mL]	72	18.40	N/A	N/A	[449]
Cg	SO16	**ME** (*lysE* expression ⬆: ornithine export ⬆) (*Δncgl1221*: ⊗ export of glutamate) (Expression *arg*, and *gdh* ⬆ and *ΔargR* repressor) (*ΔargF*: ⊗ ornithine degradation) (*ΔproB*: ⊗ proline synthesis) ③**d**: Expression of G6P isomerase *pgi* ⬇ and PPP genes (*zwf, tkt, tal pgl,* and *opcA*) ⬆: [NADPH]⬆ ④**a**: Weaken RBS of *odhA*: KGDH activity ⬇ ⑤**:** Expression of acetate synthesis pathway (*ackA, pta*, and *cat*) ⬇	ornithine	glucose [100]	F [250 mL]	72	32.30	0.40	N/A	[450]
Cg	SO26 (glucose) or SO29 (xylose)	**ME** (*Δncgl1221, mscCG2* exporters: glutamate export ⬇) (*gdh* expression ⬆) (⟾ utilisation pathway *xylAB* of *Xc*) ①**:** *ΔiolR*, expression *iolT* ⬆: ⊗ PTS-mediated sugar uptake ③**a**: Overexpression of oTCAc enzymes: citrate synthase (*glt*), aconitase (*acn*), and isocitrate dehydrogenase (*icdh*) ⬆	ornithine	glucose [60i ▶ 30-60E] or xylose [100]	FB [15 L] or F [250 mL]	60 or 72	43.60 or 18.90	0.34 or 0.40	N/A	[451]
Ec	BDO03 (xylose) or BDO05 (arabinose) or BDO07 (galacturonic acid)	**ME** (⟾ 2-ketoacid decarboxylases and alcohol dehydrogenases to convert 2,5-dioxopentanoate into BDO) ⟾ Weimberg pathway from *Cc* and *Bmu*	BDO	xylose [42.1] arabinose [70.5] galacturonic acid [50.5]	FB [1.3 L]	36 72 90	9.21 15.60 16.50	N/A	N/A	[452]

Table 8 Overview of the fermentation condition optimisation (FCO) and metabolic engineering (ME) strategies for improving the production of the closely related succinic and malic acid products. Abbreviations and symbols are the same as in Tables 6 and 7; *, mutation. Strain designation: *Ec*, *Escherichia coli*; *Cg*, *Corynebacterium glutamicum*; *Bm*, *Bacillus methanolicus*; *Cc*, *Caulobacter crescentus*; *As*, *Actinobacillus succinogenes*; *Ll*, *Lactococcus lactis*; *Cb*, *Candida boidinii*; and *Xc*, *Xanthomonas campestris*. Metabolite abbreviations: F6P, fructose-6-phosphate; G3P, glyceraldehyde-3-phosphate; PYR, pyruvate; and PEP, phosphoenolpyruvate. Gray refers to engineering strategies that are not relevant for α-KGA

Species	Strain	Strategy	Product	Substrate [g/L]	Fermentation setup	Production period [h]	Titre [g/L]	Yield [g/g]	Productivity [g/L/h]	Ref.
Ec	KJ134	**ME:** ②b: *ΔmaeA*: ⊗ back flux from oxaloacetate to pyruvate: [oxaloacete]↑ ⑤: *ΔackA, ldhA, adhE, focA-pflB, mgsA, tcdDE, pta*, and *poxB*: [mixed organic acids] and [ethanol] ↓.	succinic acid	glucose [100]	F [350 mL]	96	71.56	1.00	0.84	472
Ec	KJ073	**ME:** ①: *ptsI**: ⊗ PTS transport and compensation with ↑ expression of *galP* permease and *glk* glucokinase ②b: *pck** promoter mutation: expression of PEP carboxykinase↑: [oxaloacetate]↑ ⑤: *ΔpflB, ldhA*: [ethanol], [lactate], and [acetate]↓.	succinic acid	glucose [100]	F [350 mL]	N/A	78.88	0.79	0.90	473
Ec	XZ721	① *ΔptsI*: ⊗ PTS transport ②b: *pck** promoter mutation: expression of PEP carboxykinase↑: [oxaloacetate]↑ ⑤: *ΔpflB*: [formate], [ethanol] ↓	succinic acid	glycerol [50]	F [300 mL]	144	12.75	1.02	N/A	474
Ec	XZ658	**(Based on KJ073)** (*ΔfumABC*: ⊗ conversion of malate to fumarate) ②b: *ΔmaeA*: ⊗ back flux from oxaloacetate to pyruvate: [oxaloacete]↑ ⑤: *ΔpykF* (pyruvate kinase): [lactate]↓	malic acid	glucose [50]	B[1.2 L]	144	33.92	1.06	0.47	435
Ec	WSA163	**ME** (Expression of C_4-dicarboxylate exporters *dcuCB* ↑) ①: ⊗ PTS and compensation with ↑ expression of *galP* permease and *glk* glucokinase ②b: ⟾ PEP carboxykinase from *As*	succinic acid	glucose [10]	F [50 mL]	60	9.53	1.01	N/A	468

Table 8 (*continued*)

Species	Strain	Strategy	Product	Substrate [g/L]	Fermentation setup	Production period [h]	Titre [g/L]	Yield [g/g]	Productivity [g/L/h]	Ref.
		and PYR carboxylase from *Cg*: [oxaloacetate]↑ ③d: Expression of feedback-deregulated *zwf* and *gnd* genes ↑ + expression of lactonase (*pgl*), transaldolase (*talB*), and transketolase (*tktA*) ↑: carbon flux PPP↑ + expression of transhydrogenase (*sthA*)↑: [NADH]↑ ⑤: *ΔackA-pta, ldhA*, and *pflB*: [acetate], [lactate], and [formate]↓								
Ec	Suc-P02	ME: ③d: ↕ Expression of PPP genes through RBS engineering + expression of transhydrogenase (*sthA*)↑: [NADH] ↑	succinic acid	glucose [50]	F [250 mL]	N/A	45.11	1.06	N/A	475
Ec	Suc460	ME: ③d: ⟹ methanol dehydrogenase (*mdh*) from *Bm*: [NADH]↑ + RuMP ⟹ RuMP pathway from *Bm*: assimilation of formaldehyde into F6P	succinic acid	glucose [100] methanol [6.4]	F [1.5L]	N/A	26.72	0.89	N/A	476
Ec	G5	ME: ②b: ⟹ pyruvate carboxylase (*pyc*) from *Ll*: [oxaloacetate]↑ ③d: ⟹ methanol and formate dehydrogenase from *Bm* and *Cb* resp.: oxidation of methanol or formate into CO_2: [NADH] ↑ + assimilation of formaldehyde into F6P (biofilm-based cell-immobilised fermentation)	succinic acid	glucose [50i ▶ 10-50E], methanol [6.4], and sodium formate [2i ▶ 1.5 E)	FB [2L]	60	65.44	0.98	N/A	477
Cg	NC-3b-3	ME ①: *ΔptsG*: ⊗ PTS transport and compensation with ↑ expression of *iolT1, 2* transporters and *glk*, *ppgk* kinases; *ΔiolR* regulator.	succinic acid	glucose [66.12i ▶ 0-50E]	FB [3 L]	48	90.81	0.96	N/A	461
Cg	BOL-3/pAN6-gap	ME: ①: Expression G3P dehydrogenase (*gapA*)↑: glucose consumption ↑.	succinic acid	glucose [40] sodium formate [13.60-16.32]	FB [1.4 L] (Anaerobic)	72	134	1.09	2.48	478

Table 8 (*continued*)

Species	Strain	Strategy	Product	Substrate [g/L]	Fermentation setup	Production period [h]	Titre [g/L]	Yield [g/g]	Productivity [g/L/h]	Ref.
		②b: ↑Expressing *pyc* $P_{458}S$: [oxaloacetate]↑ ③d: ⟹ fumarate dehydrogenase (*fdh*) from *Mv*: [NADH]↑ ⑤: *Δcat, pqo, ldhA*, and *pta-ack*: [acetate] and [lactate]↓								
Cg	BL-1/pAN6-pyc$P_{458}S$ ppc	**ME:** ②b: ↑Expressing *pyc* $P_{458}S$ and native PEP carboxylase (*ppc*): [oxaloacetate]↑ ③b: *ΔsdhCAB*: ⊗ succinate dehydrogenase complex ⑤: *Δcat, pqo*, and *pta-ack*: [acetate]↓ **FCO:** ③: nitrogen in medium↓	succinic acid	glucose [40]	B [1.4L] (Aerobic)	120	10.63	0.29	N/A	479
Cg	CGS5	**ME** ⟹ *xylA* from *Xc*, expression native *xylB*, transaldolase (*tal*), and transketolase (*tkt*)↑ ⟹ pentose transporter (*araE*) from *Bs*. ②b: Expression of pyruvate carboxylase (*pyc*)↑ ③a: Expression of citrate synthase (*gltA*)↑ ⑤: *Δcat, pqo*, and *pta-ack*: [acetate]↓	succinic acid	glucose/xylose [81.3/40.3] hydrolysate from corn stalk	F [25 mL]	24	100.20 98.60	0.82 0.98	4.35 4.29	480
Cg	S071/pGEX4-NCgl0275	**ME** ⑤: *ΔldhA, pta-ackA, poxB*, and acetate coenzyme A transferase (*actA*): [lactate] and [acetate]↓ (End-product inhibition ↓ by ↑ expression of *NCgl0275* regulator)	succinic acid	glucose [70-100]	FB [1 L]	160	152.20	1.10	1.11	460

medium affects α-KGA productivity in both *Y. lipolytica* and *C. glutamicum* (Fig. 11, ②). In *Y. lipolytica*, a carbon-to-nitrogen ratio of 17.4 : 1 to 29 : 1 is shown to promote α-KGA production, regardless of the carbon substrate used.[388,390,404] Below or above this level, α-KGA production decreases, and this effect is even more pronounced under extremely limiting nitrogen conditions. These results contradict the consensus that nitrogen deficiency would stop the amination of α-KGA to glutamate, allowing more α-KGA accumulation. However, in *Y. lipolytica*, nitrogen limitation triggers lipid production and the storage response, diverting carbon catabolites away from the TCAc, causing a reduction in α-KGA.[405]

Similarly, limiting the nitrogen supply to *C. glutamicum* improves α-KGA productivity, because nitrogen starvation prevents the α-KGA end-product from being transformed into glutamate. In practice, a pH-neutralising, ammonium-rich base such as ammonium hydroxide is added to the fermentation broth during the growth stage and exchanged for an ammonium-free base such as sodium hydroxide during the stationary phase to switch the cells into production mode.[399,406]

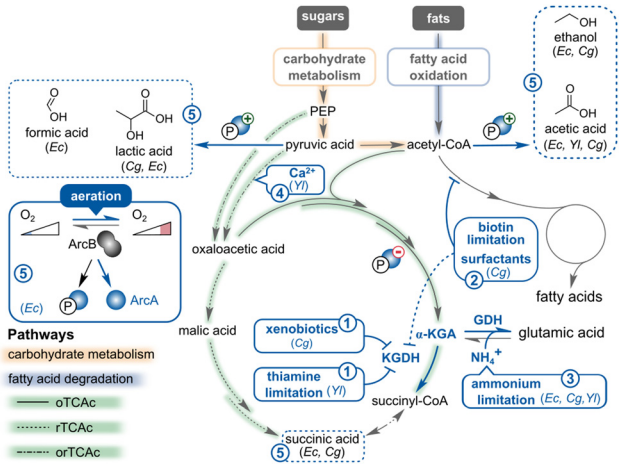


Fig. 11 The impact of optimizing fermentation conditions on the α-KGA-oriented metabolism in *E. coli* (*Ec*), *C. glutamicum* (*Cg*), and *Y. lipolytica* (*Yl*). ① The activity of the α-ketoglutarate dehydrogenase (KGDH) complex can be attenuated in *C. glutamicum* by treating with xenobiotics (such as penicillin and methotrexate) or in *Y. lipolytica* by reducing the thiamine concentration in the medium. ② Limiting biotin levels decreases the activity of the acyl-CoA carboxylase complex in *C. glutamicum*, which indirectly represses the KGDH complex as well. ③ Constraining nitrogen levels prevents the glutamate dehydrogenase (GDH)-mediated amination reaction and therefore blocks the transformation of α-KGA into glutamic acid. ④ Supplementing metal ions (such as calcium) stimulates pyruvate carboxylase in *Y. lipolytica* which promotes the conversion of pyruvic acid into oxaloacetic acid. ⑤ Improving oxygen supply (*i.e.*, aeration) shuts down anaerobic pathways and avoids accumulation of organic acids and alcohols. In *E. coli*, oxygen interrupts ArcA phosphorylation which diminishes the activity of the anaerobic pathways and relieves the repression of the oTCAc. The figure is created based on ref. 396, 403, 431 and 615.

Adjusting the ammonium concentration results in a 24% increase in α-KGA, while the glutamate titre drops by *ca.* 80%.[406] Apart from reducing the ammonium content, the conversion of α-KGA into glutamate can be selectively blocked by adding methionine sulfoximine, an inhibitor of the ammonium assimilating glutamine synthetase (GS)–glutamine 2-oxoglutarate amidotransferase (GOGAT) pathway.[407,408] These enzymes convert α-KGA biochemically into glutamate, and consecutively into glutamine.[409]

Optimising the level of micronutrients such as calcium or metal ions can also improve α-KGA production in *Y. lipolytica*.[390,414] Elevated calcium ion concentrations (*e.g.*, from using calcium carbonate as a buffering agent in the fermentation broth) lower pyruvate by-product levels, as calcium activates pyruvate carboxylase which converts pyruvate into oxaloacetate (Fig. 11, ③).[350,414] Higher concentrations of other metal ions, including zinc, iron, copper, or manganese, were shown to be favourable, but the mechanism remains to be elucidated in *Y. lipolytica*.[390] In the baker's yeast *S. cerevisiae*, the role of metal ions in its metabolism has been extensively investigated,[418,419] providing a foundational framework for the research on metal utilisation in *Y. lipolytica*.

5.4.3. Controlling pH and aeration conditions. Low medium pH (*ca.* 3–4) is crucial for enhancing α-KGA production as well as repressing pyruvate accumulation.[185,391,420] However, a pH of 5–5.5 is most optimal for cell growth, highlighting the importance of strict pH control to balance growth and α-KGA synthesis. Hence, the acidity is initially maintained around 5 to support microbial growth and subsequently lowered as soon as thiamine is depleted and growth stops.[421–423]

Similar to pH levels, oxygen levels in the broth also increase α-KGA accumulation by influencing the cells' metabolism. Dissolved oxygen (DO) alters the activity of aerobic and anaerobic pathways, which in turn affects growth kinetics and the formation of metabolites (Fig. 11, ④). Since *Y. lipolytica* is an obligate aerobic yeast, sufficient aeration of the culture medium is required to ensure cell growth.[26] Additionally, α-KGA production *via* the oTCAc necessitates oxygen as the electron acceptor because the cycle is intertwined with the electron transport chain, which recycles cofactors (*i.e.*, NADH) and generates ATP. Indeed, multiple studies showed that a 50–60% DO saturation is necessary for α-KGA production regardless of the carbon substrate.[390,420,424]

In bacterial cells, low DO levels are less appropriate for α-KGA production as oxygen limitation triggers the secretion of organic acid by-products. Insufficient oxygen availability predominantly promotes lactate accumulation followed by succinate and acetate in *C. glutamicum*[425–428] due to expression induction of *ldhA*, *gapA*, *tpi*, *pgk*, *ppc*, and *mdh*.[429] Similarly, reduced DO concentrations trigger the production of acetate, formate, succinate, and a little ethanol in *E. coli*[430] because the redox state sensor, ArcAB, induces fermentative pathways and represses the expression of oxidative TCAc enzymes simultaneously.[431] Hence, ensuring appropriate oxygen availability throughout the entire fermentation vessel is essential to prevent accumulation of undesired acid by-products (Fig. 11, ④).[425–427]

5.4.4. Fine-tuning bioreactor setups. Implementing a fed-batch process for *Y. lipolytica* cultures increases α-KGA titres 1.2–1.6 fold compared to standard batch setups.[385,388,422] The superior production parameter of fed-batch processes is attributed to a constant feeding regime that improves cell growth and stimulates switching towards α-KGA production. However, the α-KGA-to-pyruvate ratio remains the same as in batch mode phase, at around 2.5.[422] To favour the production of α-KGA over pyruvate, an alternating substrate feeding pattern has been suggested in which glycerol or rapeseed oil is supplemented every 24 h, resulting in an α-KGA-to-pyruvate ratio of 20 : 1 with a minimal pyruvate concentration of 2.9 g L^{-1}.[394] In the case of a nitrogen-feeding fed-batch setup, a 20% increase in α-KGA production is obtained when nitrogen levels are maintained above 1 g L^{-1}.[388] The results, however, contradict the nitrogen limitation (high C : N ratio) rule-of-thumb for high α-KGA production that was previously discussed. Apparently, restricting thiamine and maintaining a constant feeding rate of ammonium sulphate can force cells to convert the nitrogen source into amino acids to alleviate 'ammonium stress'. In turn, the activation of enzymes like glutamate dehydrogenase (GDH) impels an increased flow towards α-KGA, which serves as the primary substrate of GDH.[388]

5.5. Metabolic strain engineering

Apart from optimising production culture conditions, the fluxes within microbial cells can also be re-routed through

genetic engineering to maximise α-KGA yields. Regardless of the selected α-KGA-producing route (oTCA, rTCA, Glu-based oxidation, and Weimberg pathway), most strain engineering strategies pursued so far can be categorised into (i) replenishing the key precursor pools for α-KGA, (ii) maximally directing key precursors (such as acetyl-CoA and (phosphoenol)pyruvate) into the α-KGA-biosynthesis cascade, and (iii) diminishing accumulation of side-products (Fig. 12). These approaches are expected to enhance α-KGA production, if they do not cause any growth defects. Next to improving the efficiency of α-KGA biosynthesis, the industrial potential of the cell factories can be further enhanced by stimulating the α-KGA efflux (to aid product recovery), and by expanding the microbe's substrate utilisation range.

5.5.1. Replenishing the (phosphoenol)pyruvate precursor pool. Both pyruvate and phosphoenolpyruvate (PEP) serve as key intermediates in microbial anaplerotic metabolisms. These C_3-metabolites typically originate from the glycolysis and the pentose phosphate pathway and can be further converted into oxaloacetate by carboxylases or carboxykinases.[432,433] Oxaloacetate may react with acetyl-CoA to form citrate to furnish the oTCA branch.[434] Alternatively, oxaloacetate can also be routed towards malate as part of the anaerobic rTCA branch.[435]

Since PEP is also the direct precursor for pyruvate, high PEP levels enhance the flux throughout the TCAc in bacteria and therefore stimulate α-KGA production. Cellular processes that do not contribute to α-KGA synthesis and drain the PEP-pool should thus be blocked. The sugar phosphotransferase system (PTS) is the dominant PEP-consuming process in bacteria and constitutes a mechanism in which sugars such as glucose or trehalose are simultaneously imported and phosphorylated.[453–455] Hence, inactivating this sugar transport system is a well-established strategy in the case of rTCAc-based succinic acid production to ensure that PEP is routed toward the desired product instead of pyruvate (Fig. 12, ①). Therefore, the glucose-specific permease, encoded by *ptsG*, is often deleted in *E. coli*[444,456–458] and *C. glutamicum*.[459–461] Similarly, disrupting the EI subunit (encoded by *ptsI*)[462,463] and the histidine protein (encoded by *ptsH*)[464] also abolishes PTS-mediated glucose import, but this strategy is less frequently applied. In the specific case of α-KGA production, the α-keto acid renders the native glucose PTS inactive through inhibition of the PTS enzyme I (E1) subunit.[465,466] To compensate for the deliberate loss or α-KGA-mediated inactivation of the glucose transport system, the galactose permease (*galP*)-glucokinase (*glk*)[463,467,468] or the recently discovered ExuT transporter[469] are often recruited in *E. coli* to rescue glucose uptake. Combined with a mutant version of phosphoenolpyruvate carboxykinase (*pck**), disrupting the PTS in *E. coli* can improve the succinic acid production titres up to 4-fold.[462] In *C. glutamicum*, a similar approach has been pursued in which the myo-inositol transporter (*iolT1*), linked to (polyphosphate) glucokinases (*ppgk* or *glk*), serves as the substitute for the inactivated PTS.[461] This strategy increases the succinic acid titres in this species by *ca.* 12%.[461]

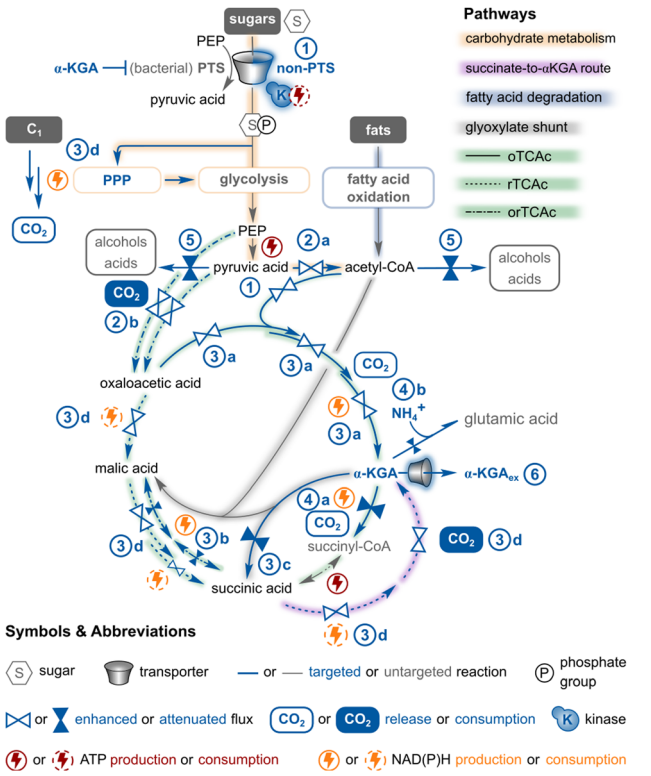


Fig. 12 Metabolic engineering strategies to improve α-KGA production in the bacteria *E. coli* and *C. glutamicum* and the yeast *Y. lipolytica*. Encircled alphanumeric codes represent the different approaches that have been pursued to enhance α-KGA titres in the aforementioned species: ① improved replenishment of the (phosphoenol)pyruvate pool and α-KGA is also shown to inhibit the PTS system and reduce malate and acetate formation. ② maximising conversion of (phosphoenol)pyruvate into acetyl-CoA and oxaloacetate, ③ improving the flux through the oxidative and reductive TCAc branches, ④ suppressing degradation of α-KGA, ⑤ preventing other by-product accumulation, and ⑥ enhancing product export. orTCAc refers to pathways that are shared among the oTCAc and rTCAc. Each strategy is more elaborately discussed in the main text. Abbreviation: $\alpha\text{-KGA}_{ex}$, exported α-KGA towards the growth broth. The figure is based on ref. 27, 309, 311, 410, 468, 477, 479 and 615.

5.5.2. Maximising conversion of (phosphoenol)pyruvate into oxaloacetate and acetyl-CoA. Before being routed toward the TCAc, PEP needs to be carboxylated into oxaloacetate. This C_4 intermediate is sufficient to drive the rTCAc, but to fuel the oTCAc, acetyl-CoA is also required. Acetyl-CoA is produced from the PEP-derivative, pyruvate, through the pyruvate dehydrogenase (PDH) complex. Because this enzyme plays a crucial role in shuttling the intermediates from lower glycolysis towards the oTCAc, optimising the corresponding enzymatic reaction is key for improving the yield of α-KGA and other TCAc products. In *Y. lipolytica*, the prime focus is to compensate for the diminished pyruvate dehydrogenase activity when thiamine levels are deliberately kept low. As discussed before, thiamine-limitation stimulates α-KGA production but also affects the PDH activity as it requires thiamine as a cofactor. Therefore, overexpressing the enzymes involved in acetyl-CoA synthesis is a common strategy to replenish acetyl-CoA, even under suboptimal low thiamine conditions (Fig. 12, ②a). One strategy is to express the heterologous ATP-citrate lyase (*ACL1*) from *Mus musculus* in *Y. lipolytica* to release more acetyl-CoA from citrate in the cytosol.[414]

Alternatively, the native *PDA1* gene, encoding the E1 α-subunit of the PDH complex,[315] and the acetyl-CoA synthetase (*ACS1*) from *S. cerevisiae* can be induced to provide more acetyl-CoA for α-KGA production.[414] When the three genes are separately overexpressed in *Y. lipolytica*, the improvements in α-KGA titres are comparable, at around 20–30% increase (Table 6), indicating that there might be a pathway bottleneck elsewhere.

As is the case in yeast, enhancing the activity of the PDH complex in *E. coli* promotes the conversion of pyruvate to acetyl-CoA (Fig. 12, ②a). Hereto, the *aceE* and *aceF* genes, encoding the E1 and E2 subunits of PDH, respectively, can be overexpressed.[410] Furthermore, introducing the $E_{354}K$ substitution into the NAD^+-interacting lipoamide dehydrogenase, LpdA, renders the PDH less sensitive to NADH feedback inhibition. This increases PDH activity by 80%, contributing to an increment of *ca.* 12% in succinic acid titres.[470] Alternatively, induction of the entire *aceEF-lpd* operon, encoding all subunits of the PDH complex, can be achieved by eliminating the dual transcription regulator PdhR.[471]

Apart from acetyl-CoA, oxaloacetate, which originates from the carboxylation of pyruvate, is the other prime precursor for initiating the TCAc (Fig. 12, ②b). In *Y. lipolytica*, this reaction can be accelerated by expressing heterologous pyruvate carboxylases from either *S. cerevisiae* or *Rhizopus oryzae*.[392,481,482] Surprisingly, overexpressing the native pyruvate carboxylase (*PYC1*) in *Y. lipolytica* does not improve α-KGA levels but instead improves the levels of other TCAc intermediates. This may be due to the imbalance of pyruvate carboxylase activity and the unchanged activities of downstream enzymes.[483] However, when *PYC1* is overexpressed together with the $NADP^+$-dependent isocitrate dehydrogenase (IDP1), α-KGA titres increase by 19% and pyruvate accumulation is neglectable. This approach, combined with limited thiamine and fed-batch cultivation, yielded the highest reported α-KGA titre (186 g L^{-1}) in *Y. lipolytica* when glycerol was used as the feedstock (Table 6).[415]

In bacteria, oxaloacetate is uniquely derived from PEP, as in *E. coli*, or also from pyruvate, as in *C. glutamicum*. The key enzymes involved in these reaction pathways are called (phosphoenol)pyruvate carboxylases and phosphoenolpyruvate carboxykinases (Fig. 12, ②b).[432,433] Natively, PEP acts as the direct precursor for oxaloacetate in *E. coli* and therefore overexpressing the endogenous phosphoenolpyruvate carboxylase gene (*ppc*) or a heterologous copy from the cyanobacterium *Anabaena* sp. 7120 increases the production of α-KGA or its derived products such as mesaconate and succinic acid.[444,457] Additionally, base substitutions in the upstream region of phosphoenolpyruvate carboxykinase (*pck*) enhance gene expression levels and, consequently, stimulate succinic acid productivity in *E. coli* by 30–600% (in terms of spatiotemporal yield).[462,463] In *C. glutamicum*, oxaloacetate can originate from either pyruvate or PEP. Therefore, enhancing the expression of the native phosphoenolpyruvate or pyruvate carboxylase genes, called *ppc* or *pyc* respectively, results in higher titres of succinic acid[460,478–480] and the α-KGA-mediated hydroxylation product hydroxy-isoleucine.[484] Regarding *pyc*, the $P_{458}S$ mutant is often exploited to further improve the conversion into oxaloacetate as this substitution renders the pyruvate carboxylase insensitive for feedback inhibition.[478] Combined with overexpressing *ppc*, the *pyc* mutant improved the succinate production titres in *C. glutamicum* by 24% under aerobic conditions.[479]

5.5.3. Improving the carbon flux through the oxidative and reductive TCAc branches. Oxaloacetate can be directed towards α-KGA through either the oxidative or reductive TCAc. Following the oxidative mode, oxaloacetate sequentially undergoes condensation, hydration, and oxidative decarboxylation, leading to α-KGA.[280] In *E. coli*, each of these conversions can be stimulated by optimising the expression of the corresponding enzymes: citrate synthase (*gltA*), aconitase (*acnA* or *acnB*), and isocitrate dehydrogenase (*icd*), respectively (Fig. 12, ③a). At the same time, the fumarases (*fumABC*) and fumarate reductases (*frdBC*) are often knocked-out to improve the α-KGA titre even further, *i.e.*, by *ca.* 47% (Fig. 12, ③b).[410] This strategy directs the carbon flux through isocitrate, α-KGA's direct precursor, towards α-KGA[410] or, with more extensive engineering, towards its derivatives mesaconate,[443] *trans*-4-hydroxyproline,[471] and hydroxyleucine.[484] Additionally, the isocitrate lyase (*aceA*) in *E. coli* and *C. glutamicum* can be disrupted to prevent isocitrate from bypassing the oTCAc through the glyoxylate shunt, thus skipping the α-KGA node (Fig. 12, ③c).[406,485] Deleting *aceA* in *C. glutamicum* improves the α-KGA production titre by 67%.[406]

In *P. putida*, α-KGA may also arise from succinic acid through the unique enzyme tandem, comprising succinate semialdehyde dehydrogenase (SSADH) and α-KGA decarboxylase (KGDC) (Fig. 12, ③d).[296–298] Although the potential of these enzymes has not yet been extensively explored for α-KGA production, their implementation could unlock the possibility of producing α-KGA through the carbon-assimilating rTCAc. As such, CO_2 (in the form of bicarbonate) can be consumed, in contrast to the oTCAc[280] where CO_2 is released. This succinic acid-producing rTCAc requires pathway optimisation, which mainly focuses on replenishing NADH levels to drive the reduction reactions, implementing non-PTS systems, increasing the oxaloacetate precursor concentration, and eliminating by-product formation (*i.e.*, mixed organic acids).[475,486] NADH regeneration is accomplished by diverting the carbon flux towards the pentose phosphate pathway (PPP), which is more efficient in generating NADPH,[475,486] or by introducing one-carbon (C_1)-consuming modules from methanotrophs, such as *Bacillus methanolicus*[476,477] or *Mycobacterium vaccae*[478] (Fig. 12, ③d). To favour the PPP over glycolysis, the expression of glucose-6-phosphate dehydrogenase (*zwf*) and 6-phosphogluconate dehydrogenase (*gnd*) can be enhanced through ribosomal binding site engineering,[475] or their enzyme activities can be stimulated by relieving feedback regulation, resulting from amino acid substitutions in key residues.[486] The PPP-derived NADPH is converted into NADH using the soluble transhydrogenase (*sthA*) to support the rTCAc activity.[475,486] As a result, the succinate titre can be improved by about 27%.[486] Alternatively, heterologous methanol and formate dehydrogenases can be exploited to completely reduce C_1 substrates like methanol or formic acid into carbon dioxide, releasing NADH.[476–478] Moreover, the released CO_2 may be fixed into oxaloacetate or α-KGA in reactions catalysed by pyruvate carboxylase or KGDC from *P. putida*,[476,477] respectively.

Modeling metabolic fluxes can accelerate the identification of less obvious gene targets for optimising α-KGA production. For example, a flux balance using a genome-scale model of *Y. lipolytica* metabolism identified metabolic levers associated with citrate production. This computational approach revealed a correlation between high citrate production and the inactivity of the mitochondrial respiratory chain *via* the alternative oxidase, or AOX (Fig. 12, ③a). The AOX enzyme is involved in the cyanide resistance pathway in plants and fungi. Based on this insight, targeted inhibition of the endogenous AOX enzyme led to a two-fold increase in citric acid production in *Y. lipolytica*.[487] Given that α-KGA is only two enzymatic steps downstream of citrate, leveraging similar strategies could be promising for enhanced α-KGA yields.

5.5.4. Suppressing degradation of α-KGA towards succinic acid and glutamic acid. As discussed earlier, α-KGA is the direct precursor for (i) succinate/succinyl-CoA within the oTCAc and (ii) glutamate/glutamine within the ammonium assimilation pathway. Suppressing the activity of the α-ketoglutarate dehydrogenase (KGDH) complex and glutamate dehydrogenase through genetic engineering reduces the conversion of α-KGA into succinyl-CoA and glutamate, respectively. Inhibiting succinyl-CoA and glutamate synthesis can thus contribute to increased titres of α-KGA and α-KGA-derived products (such as putrescine, ornithine, arginine, (hydroxy)proline, and GABA)[488,489] (Table 7).

Conversion of α-KGA into succinyl-CoA throughout the oTCAc can be blocked by knocking-out the decarboxylating moiety of the KGDH complex (Fig. 13), encoded by *odhA* in *C. glutamicum*[490] or *sucA* in *E. coli* (Fig. 12, ④a).[491] These KGDH-inactivated mutants accumulate higher α-KGA levels,[406,490,492] which can also be exploited to overproduce α-KGA-derived compounds, such as glutamate,[436] GABA,[440,441] and mesaconate.[444] However, shutting down the KGDH complex perturbs the TCAc and therefore slows down microbial growth in the yeast *Y. lipolytica*[493] and in bacteria *E. coli*[492] and *C. glutamicum*.[490] An alternative approach involves attenuating or temporarily suppressing KGDH activity rather than outrightly disrupting its function. This strategy reduces excessive α-KGA degradation while concurrently mitigating adverse growth effects. In *Y. lipolytica*, *the* amino acid substitution of two active site residues (His419 and Asp423) in the dihydrolipoamide succinyltransferase subunit (*KGD2*) decreases the catalytic activity of the KGDH complex, conferring a 40% increase in α-KGA production (Fig. 12, ④a).[494] However, growth defects were not mitigated because the growth rate of the *Kgd2*^{Asp423} mutant was reduced by half compared to the wild-type growth rate. In bacterial producers, several other options have been explored, especially in the natural glutamate producer *C. glutamicum*. Like all actinomycetes, this microbe has a single, hybrid PDH-KGDH supercomplex (Fig. 13) that predominantly localises at the cell poles and performs the

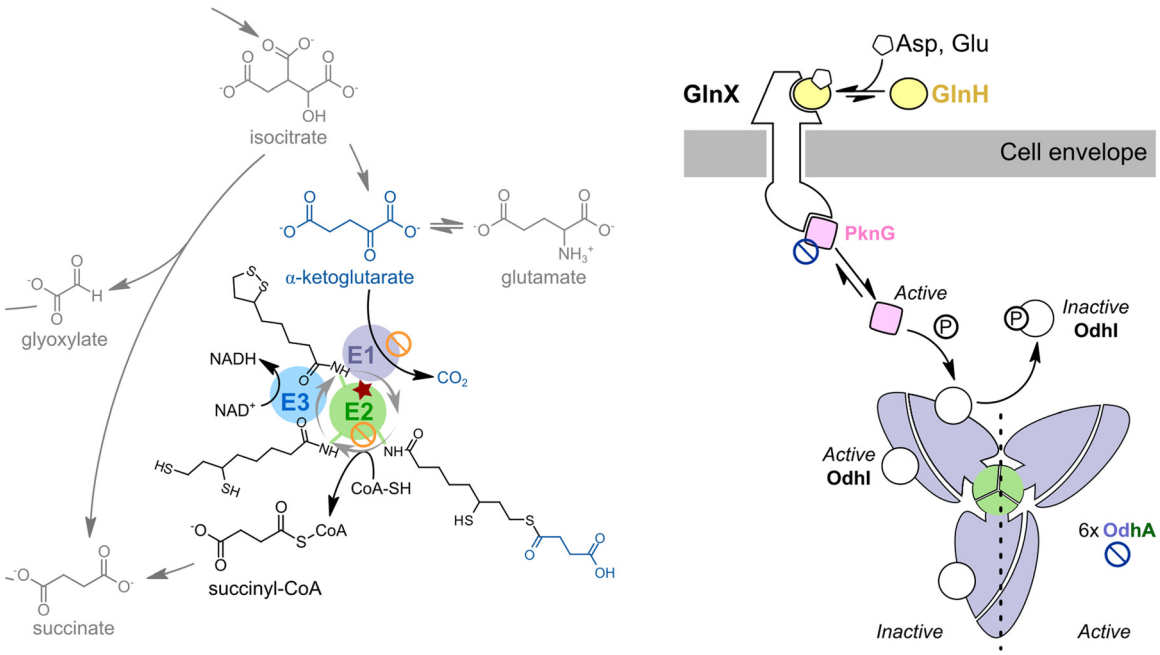


Fig. 13 The biochemistry behind the KGDH complex, its regulation, and its preferred engineering targets. (Left) The KGDH complex in the context of the oTCAc. The KGDH complex in *Y. lipolytica* and *E. coli* consists of three domains: E1, α-KGA decarboxylase, E2, lipoylated succinyl transferase, and E3, dihydrolipoyl dehydrogenase (right).[617] In contrast, *C. glutamicum* has a PDH-KGDH supercomplex, which, besides the pyruvate dehydrogenase domains (AceEF), also includes E3 and OdhA. OdhA acts as both E2 and E1 domains and assembles *in vivo* into a hexameric, three-blade propeller shape.[618] When OdhI is unphosphorylated, the inhibitor can bind to one of the OdhA domains to keep it inactive. The potential of OdhI to act as an inhibitor depends on its phosphorylation state, which, in turn, is influenced by the GlnH–GlnX–PknG signaling cascade as explained in the main text.[619] Symbols refer to the KGDH-related engineering strategies, ⊘ refers to the gene knock-out strategy. The colors of the symbols refer to the microorganism, with dark red representing *Y. lipolytica*, orange *E. coli*, and dark blue *C. glutamicum*. The figure is based on ref. 617–619.

dehydrogenase reaction on both pyruvate and α-KGA.[314] In addition, this hybrid complex is tightly regulated by a serine/threonine protein kinase G (PknG) that phosphorylates the PDH-KGDH inhibitor, OdhI (Fig. 12, ④a).[495] When PknG is active, it phosphorylates OdhI which causes the inhibitor to be released from the OdhA subunit.[496] Presumably, the activation state of PknG is regulated through the GlnH- and GlnX-mediated signaling cascade. Extracellular aspartate and glutamate bind onto the GlnH receptor which causes PknG to dissociate from GlnX and, in turn, the unleashed PknG prevents OdhI from binding to OdhA through phosphorylation (Fig. 13).[497] Interfering with this regulatory pathway provides an alternative strategy to increase α-KGA production in *C. glutamicum*. Indeed, deleting *pknG* keeps OdhI in an unphosphorylated state, allowing the inhibitor to suppress KGDH activity and thereby leading to higher α-KGA accumulation. Together with overexpressing glutamate decarboxylase (*gadB*), the *pknG*-engineered strain produces more than twice the amount of GABA than its parent, in which *pknG* was still intact.[439] Replacing the native ribosomal binding site of *odhA* with a weaker variant, or exchanging the consensus start codon for a less efficient one are other successful approaches that favour α-KGA accumulation and enhance the titres of α-KGA-derived products, such as ornithine (+16.7%),[449] arginine (+12%),[446] and putrescine (+15%)[498], in corynebacterial producers. Finally, redesigning regulatory circuits with promoters that are induced during the exponential phase or by quorum allows for the dynamic expression of the KGDH complex.[442,471] This strategy ensures proper functioning of the TCAc during the growth phase, but bypasses the α-KGA dehydrogenase reaction during the stationary phase to maximally produce α-KGA as a precursor for the production of GABA (+77% in titre) in *C. glutamicum*[442] or *trans*-4-hydroxyproline (+14.7% in titre) in *E. coli*.[471] As an alternative, a metabolite (*e.g.*, isoleucine)-responsive feedback loop can also be implemented to dynamically repress *odhA* in order to favour the α-KGA-dependent production route of 4-hydroxy isoleucine in *C. glutamicum*.[484]

Aside from being converted into succinyl-CoA, α-KGA can also be transformed into glutamate by the GS-GOGAT pathway. Disrupting glutamate dehydrogenase (*gdh*)[499] and the β-subunit of glutamate synthase (*gltB*)[500] prevents the transamination in *C. glutamicum*, resulting in a 9-fold increase of α-KGA titres (Fig. 12, ④b).[399,406] This approach has never been attempted in *Y. lipolytica*. However, the function and cofactor utilisation of the *Y. lipolytica* glutamate dehydrogenase genes (*GDH1* and *GDH2*) has been characterised recently. *GDH2* was found to be the primary dehydrogenase for glutamate assimilation. Deleting this gene did not alter growth rates in glucose media with ammonia or glutamate as nitrogen sources.[320] If *Y. lipolytica's GDH2* also performs the reversible glutamate dehydrogenation (α-KGA ↔ glutamate) as in the model yeast *S. cerevisiae*, *GDH2* deletion could be an interesting gene target to promote α-KGA accumulation (Fig. 12, ④b).

5.5.5. Preventing by-product accumulation. PEP and pyruvate are central nodes within the carbohydrate metabolism, serving as key precursors for α-KGA synthesis as well as multiple by-products. The formation of these by-products differs between species and also depends on oxygen availability. Under anaerobic conditions, some bacteria excrete succinic acid, which may serve as a precursor for α-KGA upon introduction of SSADH and KGDC, as well as ethanol and lactic, acetic, and formic acid.[501–503] *C. glutamicum* predominantly accumulates lactic acid,[501,502] whereas oxygen deprivation triggers *E. coli* to produce formic and acetic acid, primarily, and to a lesser extent ethanol and succinic acid.[462,503] Under aerobic conditions, acetate excretion is commonly detected in both *C. glutamicum* and *E. coli* cultures.[479,504]

Since excessive by-product formation drains the pyruvate, PEP, and acetyl-CoA pools and therefore competes with α-KGA production, the mixed organic acid pathways are often eliminated in re-engineered bacterial producers to enhance the yields of α-KGA (Fig. 12, ⑤) (Table 6), succinic acid (Table 8), and α-KGA- or glutamate-derived chemicals (Table 7).[410,457,463,470,472,478,505–509]

In addition to the fatty acid biosynthesis pathway, other competing acetyl-CoA-consuming pathways can be inhibited to leave more acetyl-CoA for the TCAc. For example, deleting the *Y. lipolytica* CoA-transferase gene *ACH1* eliminated acetic acid by-product formation from acetyl-CoA and, therefore, improved succinic acid production by 27% (Fig. 12, ⑤).[510] While these strategies have thus far been mainly explored for other TCA-derived carboxylic acids in *Y. lipolytica*, they may also help boost α-KGA production.

5.5.6. Improving α-KGA export. In addition to boosting its production rate, improved α-KGA yields can also be achieved by increasing product export from cell to media (also commonly called product efflux).[511] In *Y. lipolytica*, six endogenous keto-acid transporters were recently identified. The expression of additional copies of the transporter *YALI0B19470g* led to 30% less pyruvate and 28% higher extracellular α-KGA (Table 6 and Fig. 12, ⑥).[416] In addition to cell-membrane transporters, five mitochondrial carboxylic acid transporters were identified. Overexpression of all transporter genes led to improvements in extracellular succinic acid production, except for gene *YALI0E34672g*.[512] The *YALI0E34672g* mitochondrial transporter was then tested for α-KGA, where overexpression led to a 20% increase in α-KGA production in *Y. lipolytica* (Fig. 12, ⑥).[404]

5.5.7. Other untargeted strategies. Aside from rational strain engineering, untargeted methods relying on DNA mutagenesis and/or evolutionary engineering have also been used for α-KGA production. Using plasma-based random mutagenesis and pH-based bromocresol green screening methods, the selected mutant of *Y. lipolytica* strain WSH-Z06 showed an improvement of 45% α-KGA production from glycerol, reaching a final titre of 31 g L^{-1}.[417] An early patent also described a UV-mutagenised mutant that produced high titres of α-KGA, generating 185 g L^{-1} α-KGA from *n*-alkanes.[387] These untargeted methods have been widely used in the industry, especially since these mutants are not considered genetically modified organisms (GMOs).

5.5.8. Exploring different carbon substrates and expanding the microbial sugar utilisation range towards alternative feedstocks. Mono- or disaccharide hexoses, such as glucose, fructose, and sucrose, can be metabolised by most yeasts and bacteria.

These sugars are commonly sourced from hydrolysis and saccharification of plant-based starch (*e.g.*, wheat, sorghum, and potato), or sugar cane and sugar beet.[513–516] Unfortunately, using human-edible crops to produce chemicals is not a sustainable production pathway.[517] Therefore, attempts are undertaken to implement food and agro-industrial waste streams as alternative feedstocks.[518,519] Today, multiple strategies have been developed to process and extract metabolisable carbohydrates from these inexpensive resources, including glucose, xylose, arabinose, and (crude) glycerol. This review will not further describe these technologies, but more information can be found in previous reviews.[520–522] Importantly, processing waste streams often results in mixtures of carbohydrates, and the success of implementing a given agro-industrial waste stream, therefore, depends on the range of carbohydrates that the microbial species can consume. Some microorganisms can catabolise a broad range of organic carbon sources, whereas others are more selective (Fig. 14 and Table 9). However, genetic engineering efforts have unlocked the expansion of substrate utilisation ranges in *E. coli*, *C. glutamicum*, and *Y. lipolytica*, while also allowing the development of tailor-made strains adapted to specific waste stream compositions.[523–525] In this section, we summarise the inherent capabilities of the selected microbial producers for each carbohydrate category along with the engineering approaches pursued to broaden their ability to metabolise diverse carbon sources.

5.5.8.1. Alkanes. Alkanes are abundantly present in petroleum, generally reaching up to 45 (v/v)%.[526] Their biodegradation is often restricted to free-living off-shore microorganisms, which remain largely unexplored as microbial cell factories.[527,528] One exception is the yeast *Y. lipolytica*, which can degrade and thrive on *n*-alkanes as the sole carbon source owing to the secretion of emulsifiers and the activity of a cytochrome P450-based hydroxylation cascade (Fig. 14 and Table 9, reactions (25)–(28)).[529–531] Uptake and alkane catabolism in *Y. lipolytica* ultimately results in acetyl-CoA, bypassing the thiamine-dependent pyruvate dehydrogenase (PDH) that would normally replenish acetyl-CoA from glucose or glycerol.[530] Hence, even at low thiamine concentrations, a high α-KGA output can be achieved without the issue of pyruvate accumulation. The highest α-KGA production level yet (196 g L^{-1}, 0.94 g g^{-1}) was reached on a mixture of C_{12}-C_{18} alkanes by *Y. lipolytica*.[413]

While *n*-alkanes are an efficient substrate for α-KGA production, sustainability concerns are often raised when using petroleum-derived hydrocarbons as a substrate for fermentation.[26] However, *n*-alkanes can be sourced in a more sustainable way, such as through chemical recycling of plastic waste. Upon thermal pyrolysis of polyolefin plastics, one of the primary products obtained is high molecular weight hydrocarbons ($\geq C_{18}$).[532] Not only are these hydrocarbons suitable substrates for α-KGA production by *Y. lipolytica*, but they also hold promise in the transition towards a circular economy. Still, the feasibility of α-KGA production from the products of plastic pyrolysis is yet to be evaluated.

5.5.8.2. Glycerol. The use of alternative renewable carbon feedstocks that originate from the main by-product of the biodiesel industry, raw glycerol, has been explored.[26] In biodiesel production, the triglycerides in vegetable or animal fats are saponified or transesterified, releasing fatty acids (biodiesel) and glycerol as by-products. Glycerol is an attractive carbon substrate for α-KGA production in *Y. lipolytica* because this yeast grows faster on this triol (and lipid-rich substrates) than on glucose.[384,533,534] This preference for glycerol may be linked to *Y. lipolytica*'s six glycerol transporters (STL2, STL3, STL6, STL8, FPS1, and FPS2)[535] in combination with a weak catabolite repression system.[384,536] Accordingly, glycerol serves as the primary carbon substrate in most strain engineering and process optimisation studies on *Y. lipolytica* discussed in this review.

Apart from *Y. lipolytica*, *E. coli* is also a suitable microbial producer for glycerol-based bioproduction processes owing to its native glycerol metabolism. This bacterium possesses the aerobic GlpK–GldD and anaerobic GlpK–GlpABC respiratory pathways that employ ubiquinol as an electron acceptor. Alternatively, it can also use the microaerobic NAD^+-mediated GldA–DhaKLM fermentative route to transform the triol into dihydroxyacetone-phosphate (Fig. 14 and Table 9, reactions (5) and (6)).[474,537,538] Although *E. coli* is well equipped for dissimilating glycerol, growth rates on glycerol do not match those obtained with glucose-rich media.[539] However, this limitation can be overcome through ALE by evolving cells in a minimal medium supplemented with glycerol as the sole carbon source.[540] The fittest clone, isolated from the evolution experiment, consumed glycerol 46% faster than its parental strain, which improved the growth rate by nearly 40% in a glycerol-rich minimal medium. The authors identified a missense mutation in the fructose-1,6-bisphosphate allosteric inhibitor binding site of GlpK as the prime driver for enhanced glycerol uptake in the adapted clone. Furthermore, this study also demonstrated that the superior glycerol consumption phenotype can be exploited for GABA production from the α-KGA/glutamate precursor, when *gabT*, *ackA*, and *mgsA*, encoding GABA aminotransferase, acetate kinase, and methylglyoxal synthase, respectively, are also inactivated. *C. glutamicum* lacks a functional glycerol metabolism, but this limitation can be solved by introducing glycerol kinase (*glpK*) and dehydrogenase (*glpD*) from *E. coli*.[541] Interestingly, although these engineered strains showed lower substrate uptake rates on a glucose–glycerol mixture compared to growth on the corresponding single carbon sources, no diauxic growth behaviour was observed. This improved glycerol utilisation phenotype has been successfully exploited to enhance the production of the α-KGA derivatives, such as glutamate,[541] GABA,[442] and putrescine,[542] in *C. glutamicum*.

5.5.8.3. Fatty acids. In addition to its ability to accumulate lipids in the cell, *Y. lipolytica* can also consume lipids or aliphatic fatty acids as a carbon source (Fig. 14). Similar to alkane degradation, fatty acid catabolism in *Y. lipolytica* also connects with the TCAc *via* acetyl-CoA, bypassing the thiamine-dependent PDH. Several studies have tested growth substrates with renewable fatty acid sources, such as rapeseed oil, sunflower oil, and olive oil,[385,424] and as a mixture with glycerol.[393,394,404] At low thiamine concentrations, *Y. lipolytica* grows better on oil than on glycerol, and it produces more

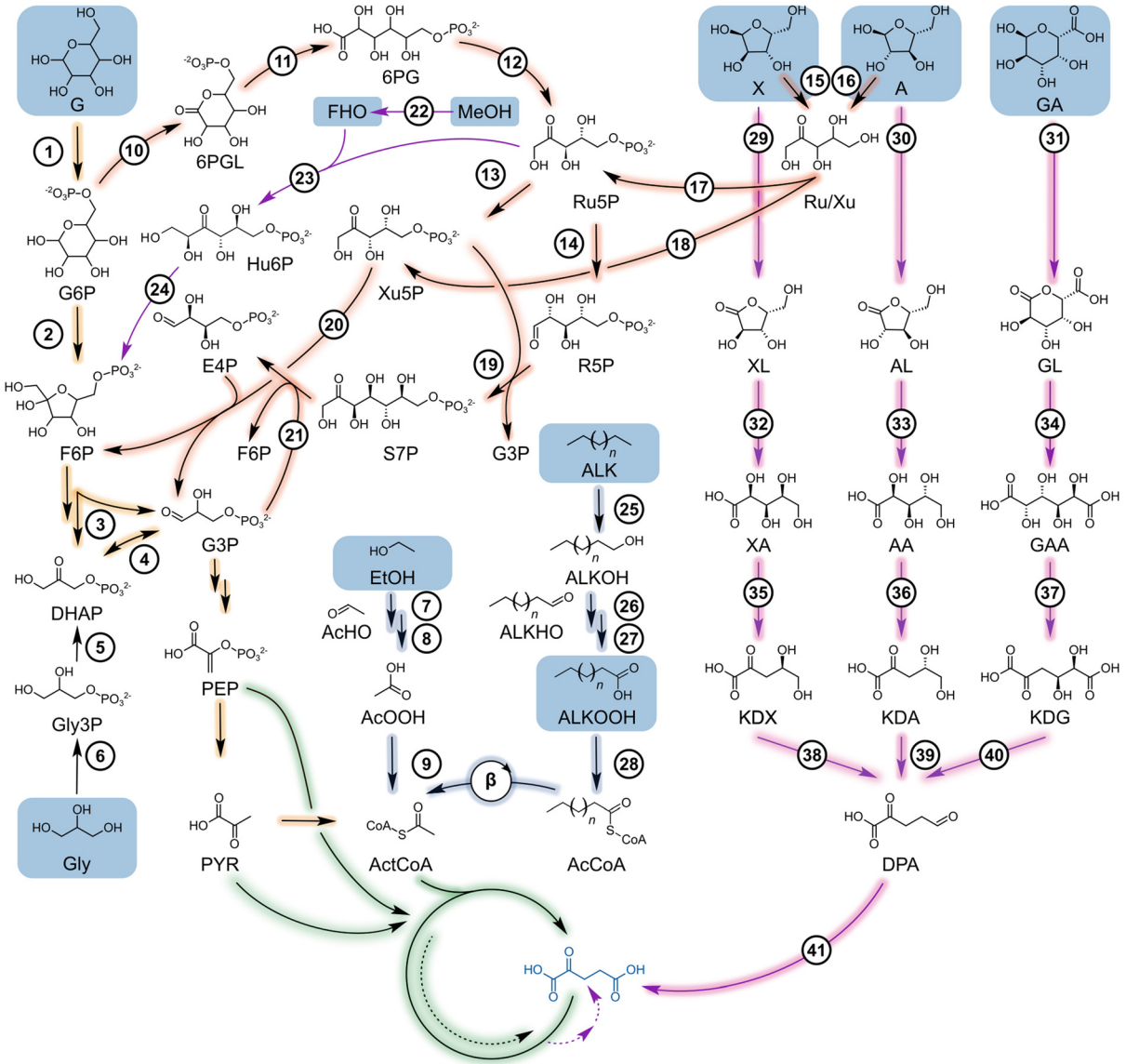


Fig. 14 Different substrate utilisation routes contribute to α-KGA production. The numbers refer to the reaction chemistry as explained in Table 9. Abbreviations: G, glucose; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde-3-phosphate; PEP, phosphoenol pyruvate; PYR, pyruvate; Gly, glycerol; Gly3P, glycerol-3-phosphate; EtOH, ethanol; MeOH, methanol; FHO, formaldehyde; AcHO, acetaldehyde; AcOOH, acetic acid; X, xylose; A, arabinose; GA, galacturonate; 6PGL, 6-phosphogluconolactone; 6PG, 6-phosphogluconate; Ru, ribulose; Xu, xylulose; Ru5P, ribulose-5-phosphate; Xu5P, xylulose-5-phosphate; R5P, ribose-5-phosphate; S7P, sedoheptulose-7-phosphate; E4P, erythrose-4-phosphate; Hu6P, hexulose-6-phosphate; ALK, *n*-alkane; ALKOH, alkanol; ALKHO, alkanal; ALKOOH, alkanoic acid; AcCoA, acyl-coenzyme A; ActCoA, acetyl-coenzyme A; XL, xylonolactone; AL, arabinolactone; GL, galactarolactone; XA, xylonic acid; AA, arabonic acid; GAA, galactaric acid; KDX, 2-keto-3-deoxy-xylonic acid; KDA, 2-keto-3-deoxy-arabonic acid; KDG, 5-keto-4-deoxy-glucaric acid; DPA, 2,5-dioxopentanoic acid; and β, fatty acid degradation (β-oxidation) pathway. Structures in blue boxes represent the primary carbon sources that originate from conventional (*e.g.*, starch-based) and alternative feedstocks (*e.g.*, vegetable oils, lignocellulose, and alcohols). The colors of the arrows correspond to the ones used in Fig. 10. The figure is based on ref. 438, 452, 530, 531, 552 and 616.

α-KGA with trace amounts of pyruvate as a by-product.[394] However, while fatty acids are generally a better substrate for α-KGA production in *Y. lipolytica*, glycerol is less expensive than vegetable oil. Additionally, mixing oil with a water-soluble substrate like glycerol can facilitate industrial processes due to easier mixing and reduced need for emulsifiers.[393]

5.5.8.4. Alcohols. Mono-alcohols, such as ethanol or methanol, can also be applied as feedstock for microbial production processes. For example, *Y. lipolytica* can be cultured on ethanol for the production of α-KGA.[388,543] Similarly to fatty acids and alkanes, ethanol also enters the TCAc *via* acetyl-CoA which bypasses the pyruvate intermediate (Fig. 14 and Table 9, reactions (7)–(9)). However, ethanol is partly oxidised into acetic acid when pH drops to 3 or lower, a condition that occurs commonly during carboxylic acid production which may limit the applicability of ethanol as feed.[388] Overall, α-KGA production titres in *Y. lipolytica* are higher when glycerol or oil is used as a substrate instead of ethanol (Table 7).

Table 9 Supplementary table to Fig. 14, describing the reactions involved in carbon utilisation. Gene names are provided in italics and species names are included in the gene accession IDs: ECK, *Escherichia coli* (*Ec*) K12-MG1655; *Corynebacterium glutamicum* (*Cg*) ATCC 13032; YALI0, *Yarrowia lipolytica* CLIB1222; BSU, *Bacillus subtilis* subsp. *subtilis* 168; BMMGA, *Bacillus methanolicus* MGA3; Bst, (*Geo*)*Bacillus stearothermophilus*; CC, *Caulobacter crescentus/vibrioides* NA1000; PP, *Pseudomonas putida* KT2440; and BMU, *Burkholderia multivorans* ATCC 17616. Gene names and related reactions are retrieved from **https://www.ecocyc.org**[309] using strain K-12 MG1655 and ref. 552 for *E. coli* or from the KEGG[311] database for all other species. Intermediates are abbreviated similar to those in Fig. 14. In addition, the subscripts out/in represent extracellular and intracellular; $P_2O_7^{4-}$, pyrophosphate. In the case of heterologous expression, the host organism is indicated after ⟾. Gene names that are found between square brackets encode for peptides that constitute a single polypeptide enzyme complex. Coloured boxes correspond to the shading of the background behind the arrows in Fig. 14

Reaction number	Ref.	Enzyme name	Gene name Gene accession ID	Reaction
1		phosphotransferase system	*ptsI* (ECK2411), *ptsG* (ECK1087), *ptsH* (ECK2410), *crr* (ECK2412) *ptsI* (cg2117), *ptsG* (cg1537), *ptsH* (cg2121)	G_{out} + PEP ↔ $G6P_{in}$ + PYR
	317	galactose permease, glucokinase inositol transporters, (polyphosphate/ATP-dependent) glucokinase	*galP* (ECK2938), *glk* (ECK2384) *iolT1,2* (cg0223, cg3387), *glk* (cg2399) or *ppgK* (cg2091)	G_{out} + H^+_{out} ↔ G_{in} + H^+_{in} + ATP ↔ G6P + ADP
2		phosphoglucose isomerase	*pgi* (ECK4017) *pgi* (cg0973) *YALI0F07711g*	G6P ↔ F6P
3		fructose-bisphosphate aldolase	*fbaA* (ECK2921) *fbaB* (ECK2090) *fda* (cg3068) *YALI0E26004g*	$F1,6P_2$ ↔ DHAP + G3P
4		triose phosphate isomerase	*tpiA* (ECK3911) *tpi* (cg1789) *YALI0F05214g*	DHAP ↔ G3P
5		glycerol kinase	*glpK* (ECK3918) *YALI0F00484g*	Gly + ATP → Gly3P + ADP
6		(anaerobic) glycerol-3-phosphate dehydrogenase	*glpD* (ECK3412)	Gly3P + Q → DHAP + QH_2
		(aerobic) glycerol-3-phosphate dehydrogenase	[*glpA, glpB, glpC*] (ECK2233, ECK2234, ECK2235)	Gly3P + MQ → DHAP + MQH_2
		NAD^+-dependent glycerol-3-phosphate dehydrogenase	*YALI0B02948g*	Gly3P + NAD^+ ↔ DHAP + NADH + H^+
		Q-dependent glycerol-3-phosphate dehydrogenase	*YALI0B13970g*	Gly3P + Q → DHAP + QH_2
7		alcohol dehydrogenase	*adhE* (ECK1235) *adhP* (ECK1472) *ADH1 (YALI0D25630g)* *ADH3 (YALI0A16379g)* *ADH7 (YALI0E07766g)*	EtOH + NAD^+ ↔ AcHO + NADH + H^+
8		aldehyde dehydrogenase	*aldB* (ECK3577) *ALDH (YALI0D07942g, YALI0E00264g, YALI0F04444g, YALI0F23793g, YALI0A17875g, YALI0B01298g, YALI0C03025g)*	AcHO + $NADP^+$ + H_2O → AcOOH + NADPH + $2H^+$ AcHO + NAD^+ + H_2O ↔ AcOOH + NADH + H^+
9	386	acetyl-CoA synthetase	*ACS1 (YALI0F05962g)*	AcOOH + ATP + CoA-SH → ActCoA + AMP + $P_2O_7^{4-}$
10		glucose-6-phosphate dehydrogenase	*zwf* (ECK1853) *zwf* (cg1778) *YALI0E22649g*	G6P + $NADP^+$ → 6PGL + NADPH + H^+
11		6-phosphogluconolactonase	*pgl* (ECK0756) *devB* (cg1780) *YALI0C19085g, YALI0E11671g*	6PGL + H_2O → 6PG + H^+

Table 9 (*continued*)

Reaction number	Ref.	Enzyme name	Gene name Gene accession ID	Reaction
12		6-phosphogluconate dehydrogenase	*gnd* (ECK2024) *gnd* (cg1643) *YALI0B15598g*	6PG + $NADP^+$ → Ru5P + CO_2 + NADPH
13		ribulose-5-phosphate 4-epimerase ribulose-phosphate 3-epimerase	*araD* (ECK0062), *ulaF* (ECK4194), or *sgbE* (ECK3572) *rpe* (ECK3373) *rpe* (cg1801) *YALI0C11880g*	Ru5P ↔ Xu5P
14		ribose-5-phosphate isomerase allose-6-phosphate isomerase	*rpiA* (ECK2910) *rpi* (cg2658) *YALI0B06941g* (A), *YALI0F01628g* (B) *rpiB* (ECK4083)	Ru5P ↔ R5P
15		xylose isomerase	*xylA* (ECK3554)	X ↔ Xu
16		arabinose isomerase	*araA* (ECK0063)	A ↔ Ru
17		ribulokinase	*araB* (ECK0064)	Ru + ATP → Ru5P + ADP + H^+
18		xylulokinase	*xylB* (ECK3553) *xylB* (cg0147) *YALI0F10923g*	Xu + ATP → Xu5P + ADP + H^+
19		transketolase	*tktA* (ECK2930), *tktB* (ECK2460) *tkt* (cg1774)	Xu5P + R5P ↔ S7P + G3P
20		transketolase	*tktA* (ECK2930), *tktB* (ECK2460) *tkt* (cg1774) *YALI0D02277g, YALI0E06479g*	Xu5P + E4P ↔ F6P + G3P
21		transaldolase	*talA* (ECK2459), *talB* (ECK0008) *tal* (cg1776) *YALI0F15587g*	S7P + G3P ↔ E4P + F6P
22 (→)	437,438	methanol dehydrogenase	*mdh* (aka *adh*) (*Bst*) ⟾ *Cg*	MeOH + NAD^+ ↔ FHO + NADH + H^+
23 (→)	437,438	3-hexulose-6-phosphate synthase	*hps* (BMMGA3_06845) ⟾ *Cg*	Ru5P + FHO ↔ Hu6P
24 (→)	437,438	6-phospho-3-hexuloisomerase	*phi* (BMMGA3_06840) ⟾ *Cg*	Hu6P ↔ F6P
25	530,531	fatty acid hydroxylase alkane monooxygenase system	*FAH* *ALK, NCP* (*Yl*)	ALK + NADPH + H^+ → ALKOH + H_2O ALK + O_2 → ALKOH + H_2O
26	530,531	fatty alcohol dehydrogenase fatty alcohol oxidase	*ADH* (*Yl*) *FAO*	ALKOH + $NAD(P)^+$ → ALKHO + NAD(P)H + H^+ ALKOH + O_2 → ALKHO + H_2O_2
27	530,531	fatty aldehyde dehydrogenase	*ALDH* (*Yl*)	ALKHO + $NAD(P)^+$ → ALKOOH + NAD(P)H + H^+
28	530,531	acetyl-CoA synthetase acyl-CoA synthetase	*ACS1* (*YALI0F05962g*, *Yl*) *ACS2* (*YALI0D17864g*, *Yl*)	ALKOOH + ATP + CoA-SH → AcCoA + AMP + $P_2O_7^{4-}$
29	411,452,561,568	D-xylose dehydrogenase myo-inositol 2-dehydrogenase	*xylB* (CCNA_00864)⟾ *Ec* or *Cg* *iolG* (cg0204)	X + NAD^+ → XL + NADH
30	452	L-arabinose dehydrogenase	*araA* (BMULJ_05320))⟾ *Ec*	A + NAD^+ → AL + NADH
31	452	D-galacturonate dehydrogenase	*udh* (PP_1171) ⟾ *Ec or Cg*	GA + NAD^+ → GL + NADH
32	452,561,568	D-xylonolactonase	*xylC* (CCNA_00863)⟾ *Ec* or *Cg*	XL + H_2O → XA + H^+
33	452	L-arabinolactonase	Spontaneous *araB* (BMULJ_05316) ⟾ *Ec*	AL + H_2O → AA + H^+
34		Spontaneous		GL + H_2O → GAA + H^+
35	452,561,568	D-xylonate dehydratase	*xylD* (CCNA_00862) ⟾ *Ec* or *Cg*	XA → KDX + H_2O
36	452	L-arabonate dehydratase	*araC* (aka *ilvD*) (BMULJ_05323) ⟾ *Ec*	AA → KDA + H_2O
37	452	D-galactarate dehydratase	*garD* (PP_3601) ⟾ *Ec*	GAA → KDG + H_2O
38	452,561,568	2-keto-3-deoxy-D-xylonate dehydratase	*xylX* (CCNA_00866) ⟾ *Ec* or *Cg*	KDX → DPA + H_2O
39	452	2-keto-3-deoxy-L-arabonate dehydratase	*araD* (aka *dapA*) (BMULJ_05321) ⟾ *Ec*	KDA → DPA + H_2O
40	452	5-keto-4-deoxy-D-glucarate dehydratase	*ycbC* (BSU02460) ⟾ *Ec*	KDG + H^+ ↔ DPA + H_2O + CO_2
41	411,452,561,452,568	2-ketoglutarate semialdehyde dehydrogenase	*xylA* (CCNA_00865) ⟾ *Ec* or *Cg* cg0535 *araE* (BMULJ_05314) ⟾ *Ec*	DPA + NAD^+ → α-KGA + NADH

Methanol has also drawn the attention of the scientific and industrial community as this alcohol can be obtained sustainably from carbon dioxide hydrogenation and biogas-based steam reforming.[544] From a biological perspective, methanol consumption is far from a universal trait and is mostly restricted to methylotrophic bacteria such as *Bacillus*, *Burkholderia*, *Methylomonas*, *Methylobacter*, and *Methylosarcina* species, and the *Candida* and *Pichia* yeasts.[545,546] Recent research efforts have focused on integrating these natural methanol assimilation pathways into industrial microbial chassis strains like *E. coli* and *C. glutamicum*. As discussed earlier, introducing methanol and formate dehydrogenases in *E. coli* considerably enhances succinate production in glucose–methanol co-feeding regimes because metabolising methanol provides both reducing equivalents and extra carbon that can be assimilated in the final product.[476,477] Furthermore, the co-consumption of xylose and methanol can be stimulated in *C. glutamicum* by introducing a methanol dehydrogenase and a synthetic ribulose monophosphate pathway.[438] This phenotype was artificially engineered by disrupting the pentose metabolism at the ribose phosphate isomerase (*rpiB*) node and transferring the 3-hexulose-6-phosphate synthase (*hps*) and 6-phospho-3-hexuloisomerase (*phi*) from *Bacillus methanolicus* to complement the anticipated defect in the ribose metabolism (Fig. 14 and Table 9, reactions (22)–(24)). Methanol utilisation was further optimised in the methanol-consuming *C. glutamicum* strain through fourteen ALE passages in methanol- and xylose-based defined medium, resulting in a 20-fold growth rate increase and a final glutamate titre of 90 mg L^{-1}.[437]

5.5.8.5. The C_5 (hemi)cellulose sugars: xylose and arabinose. In the EU, *ca.* 53 million tons of cellulose and *ca.* 36 million tons of hemicellulose are harvested annually from agricultural and forestry activities.[547] Although these biomass fractions are unsuitable for human consumption, they can serve as a carbohydrate source to support microbial growth. Alongside glucose, pretreatment of (hemi)cellulose also releases xylose and arabinose monomers that cannot be efficiently catabolised by *C. glutamicum*[548,549] and *Y. lipolytica*.[524] While *C. glutamicum* only expresses the xylulokinase (*xylB*) gene at low levels,[548] *Y. lipolytica* harbors a complete but dormant PPP, resulting in poor xylose consumption rates.[550,551] Many other microorganisms, however, are capable of converting xylose and arabinose into D-xylulose-5-phosphate through a series of oxo-reductive or isomerisation reactions that are found in fungi or (eu)bacteria such as *E. coli*, respectively (Fig. 14 and Table 9, reactions highlighted in orange). The resulting D-xylulose-5-phosphate is further metabolised throughout the non-oxidative PPP before entering the TCA at the citrate synthase node.[552–554] To enable *C. glutamicum* to consume pentose sugars, the missing enzymes from closely related species have been sourced for integration in the producer's genome. Co-overexpressing the xylose isomerase (*xylA*) from *Xanthomonas campestris* in combination with either the xylulokinase from the same foreign species or the native *xylB* facilitates pentose consumption and production of α-KGA-derived metabolites (lysine, glutamate, and diamines).[480,555] Furthermore, most *C. glutamicum* strains, except for ATCC 31831, lack the *araBAD* operon that is crucial for arabinose utilisation and encodes ribulokinase, arabinose isomerase and ribulose 5-phosphate 4-epimerase.[556] In these bacterial cells, the arabinose consumption deficiency can be resolved by introducing the *E. coli*-originated *araBAD* operon, which enables the production of α-KGA-derived amino acids, such as glutamate, ornithine, and arginine, from mixtures of glucose and arabinose.[557,558] Apart from the general phosphorylating pathway (discussed above), two other alternative pentose-metabolising and PPP-independent pathways exist in which either xylose or arabinose is converted into 2-keto-3-D-deoxy-xylonate or -arabonate, respectively.[303,554,559] These last intermediates are finally transformed into ethylene glycol or glycolic acid through the Dahms pathway[560] or, alternatively, converted *via* 2,5-dioxopentanoate into α-KGA through the Weimberg route (Fig. 14 and Table 9, reactions highlighted in purple).[305,554] Evidently, the Weimberg pathway is most relevant for this review and is often encountered in Archaebacteria as well as in certain bacterial species, including *C. crescentus* and *Burkholderia multivorans*.[303] Compared to the combination of glycolysis or PPP with TCAc, the Weimberg pathway requires fewer reaction steps to reach α-KGA and is inherently carbon-conservative (*i.e.* no emission of CO_2).[561] Besides these intrinsic biochemical advantages, introducing the foreign Weimberg pathway in *E. coli* and *C. glutamicum* circumvents carbon catabolite repression,[562–564] paving the path for the simultaneous consumption of pentose and hexose sugars when feeding lignocellulose-derived substrates.[565] Integrating the Weimberg enzyme cascade from *C. crescentus* or *B. multivorans* enables *E. coli* to efficiently produce α-KGA-derived chemicals like mesaconate[445] and butanediol[566] from xylose and/or arabinose. In addition, the Weimberg pathway can be implemented to relieve the glutamate auxotrophy that may arise during pathway optimisation of another TCAc-derived target chemical, such as itaconic acid[567] or even α-KGA. In other bacterial species, including *C. glutamicum* and *Bacillus subtilis*, the Weimberg metabolic route has been employed to produce succinic acid[568,569] or the biopolymer poly-γ-glutamic acid,[570] respectively, in a carbon-neutral way from xylose. In yeast, the Weimberg pathway has only been tested in *S. cerevisiae*, but its introduction resulted in poor xylose utilisation efficiency.[571]

6. Recovery and purification of α-KGA from the fermentation broth

During fermentation, α-KGA accumulates in the fermentation broth alongside microbial by-products, with the residual substrate and nutrients potentially still present. These impurities need to be removed in the downstream processing step, to obtain α-KGA in high yield and purity. The required purification process is far from trivial and can account for up to 40% of the total production expenses.[572,573]

Generally, (di)carboxylic acids are recovered from the fermentation broth in several steps. In the first pretreatment stage,

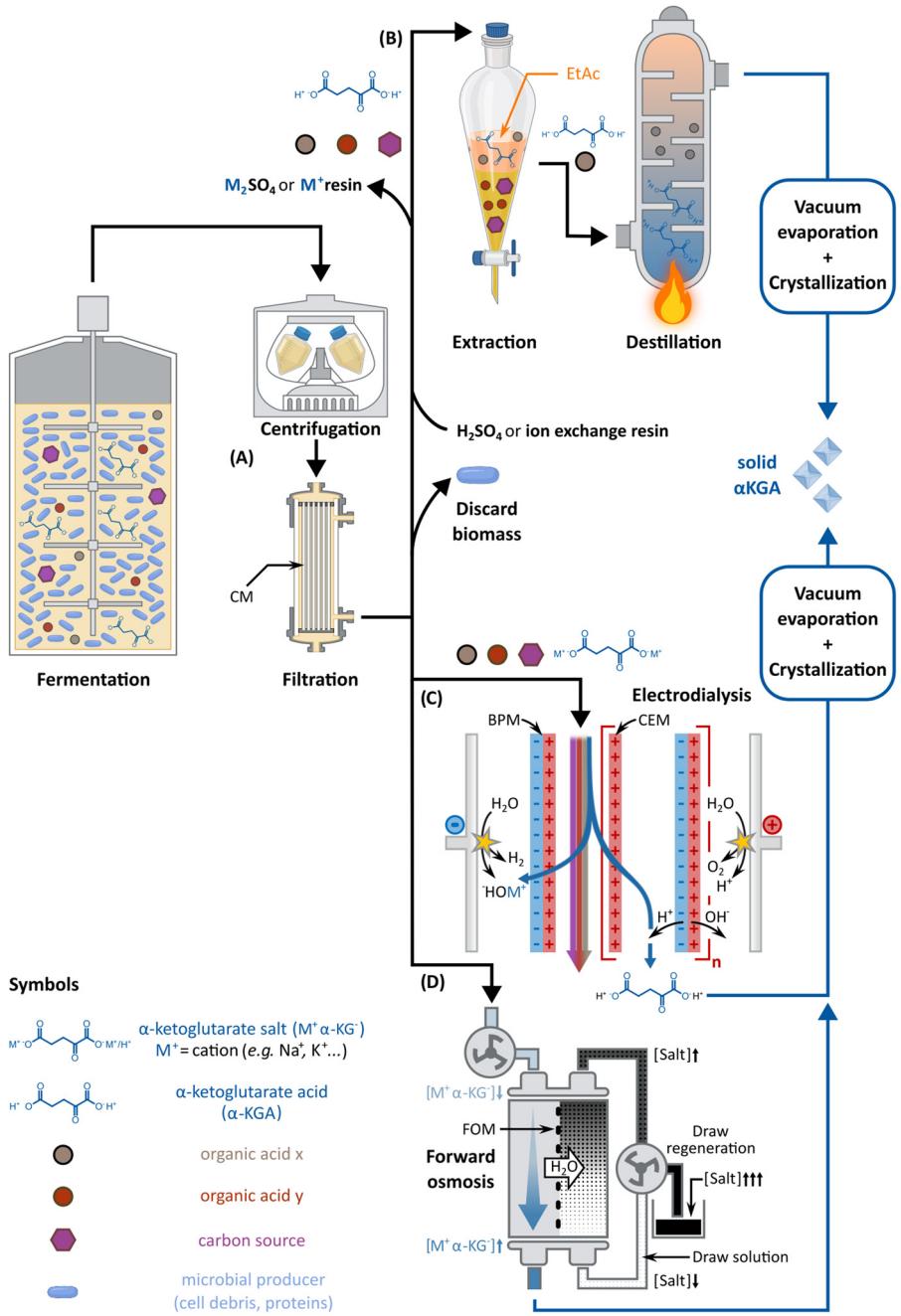


Fig. 15 Recovery strategies for purification of α-KGA from microbial fermentation. Inside the fermentation broth, α-ketoglutarate usually exists in its salt form, bound to sodium, potassium, or calcium cations. (A) Pre-treatment of the fermentation broth by centrifugation and ultra- or nanofiltration using ceramic membranes (CM) to separate the biomass (microbial cells, cell debris, and proteins) from the supernatant. Acidification is often implemented to convert the α-KG salt into α-KGA. (B) Traditionally, α-KGA is isolated and enriched from the complex fermentation broth using an ethyl acetate (EtAc)-based extraction protocol, followed by distillation. The distillate is vacuum evaporated, facilitating crystallisation to yield solid α-KGA (salt) crystals. Alternatively, α-KGA (salts) can be recovered using membrane-based setups. One approach is called bipolar membrane electrodialysis which uses a combination of an electric field and multiple membranes, both bipolar and cation exchange membranes that are organised in a stack (C). Another approach, called forward osmosis, exploits a high salt draw solution to create an osmotic gradient that withdraws water from the pre-treated fermentation broth (D). Abbreviations: EtAc, ethyl acetate; BPM, bipolar membrane; CEM, cation exchange membrane; and FOM, forward osmosis membrane. The figure is created based on ref. 574, 578 and 581.

biomass (cells and proteins) is eliminated by centrifugation and filtration. Next, the supernatant is decoloured and purified by active carbon treatment, and salts (and water) are withdrawn using ion exchange resins (Fig. 15A). Finally, the pure end-product is obtained by crystallisation (Fig. 15B).[573] Recuperating α-KGA from concentrated fermentation solutions typically involves ethyl acetate as the extraction solvent and sulphuric acid as an acidification agent to convert potassium or sodium salts of

α-KGA to organic acids (besides the use of ion exchange resins).[342,421,574] Apart from acidification, a liquid–liquid extraction system can also be used to recover α-KGA, such as an acetone/$(NH_4)_2SO_4$-solvent system, followed by crystallisation.[575,576] These methods have been industrially implemented to purify other microbially produced carboxylic acids, such as succinic acid, lactic acid, and itaconic acid,[572] whereas they have only been tested at the laboratory scale for α-KGA recovery.

Although high purity (99%) can be achieved through both methods described above, using concentrated mineral acids (*e.g.*, sulphuric acid) and organic solvents may generate considerable waste and harm the environment.[574] To provide a more sustainable alternative, solvent-free setups such as bipolar membrane electrodialysis or forward osmosis can be used.[577–581] The advantage of electrodialysis is that multiple bipolar membranes can be stacked in combination with anion and/or cation exchange membranes to concentrate α-KGA progressively. The application of electric power is necessary to generate an electric field that drives the purification process (Fig. 15C).[580] In the case of forward osmosis, water is withdrawn from the feed (*i.e.*, preprocessed fermentation broth) using an osmotic gradient imposed by a draw solution, rich in salts. Since establishing an osmotic gradient in this recovery method requires no energy input other than the circulation of feed and draw solutions, it may be more cost-effective (Fig. 15D). However, although high α-KGA recovery was observed (94%), the efficiency of forward osmosis depends greatly on the composition of the fermentation broth. Indeed, excessive concentrations of impurities like proteins and inorganic cations (Ca^{2+} and Mg^{2+}) are notorious for causing membrane fouling.[581] Therefore, extracting α-KGA from these complex media requires an extra pretreatment step before the fermentation broth can be fed over the membranes. This operation substantially increases the overall purification cost.[580,581]

In summary, the recovery efficiency of α-KGA and organic acids in general from fermentation broths can vary significantly, depending on the purification strategy applied, the choice of producer strain, and the type of feedstock.[582–584] Although the methods discussed above show potential, α-KGA recovery is relatively new and hence underexplored. As such, established or even recently emerged methods, such as electrodeionisation,[585] for purifying other carboxylic acids (*e.g.*, succinic acid and lactic acid) can serve as a stepping stone for developing improved purification processes for α-KGA. Overall, improved downstream processes should aim for industrial-scale recovery yield and product purity (*i.e.*, >90% and >98%, respectively).[572]

7. Concluding remarks and future perspectives

α-KGA is a key platform chemical that enables a more bio-based and sustainable production of a diverse range of products that are traditionally derived from (petro)chemical processes. The combination of an α-keto and di-acidic moiety makes α-KGA an interesting building block, either in its original form or as a precursor for chemocatalytic conversion into novel intermediates and polymers. For example, implementing α-KGA directly in copolymer synthesis opens new avenues toward fabrication of innovative polyamides and poly(keto)esters with applications in tissue engineering, drug delivery, and surface coating. Alternatively, α-KGA can be converted into highly reactive, ring-shaped building blocks (*e.g.*, lactones, carbazones, hydrazones, carbolines, and quinazolines) that serve as precursors for bioactive and pharmaceutical compounds. In medicine, α-KGA is an important intermediate of the central metabolism in living cells and plays a pivotal role in multiple cellular processes, including energy and fat metabolism, aging, and inflammation. Consequently, α-KGA has been evaluated in several studies as a nutraceutical or pharmaceutical to attenuate inflammation, accelerate muscle recovery, promote longevity, and for treating obesity, cancer, and cardiovascular pathologies. Despite the vast range of potential applications, industrial exploitation of α-KGA is still in its infancy. Specifically, the industrial use of α-KGA as a precursor for polymerisation or catalytic conversion to other derivatives and secondary chemicals remains underexplored.

Today's traditional fossil-based chemical industry will eventually need to be replaced by a circular bio-based economy. This transition will likely put α-KGA in the spotlight as one of the most promising platform chemicals because it can be produced through precision fermentation starting from sustainable plant-based biomass rich in sugars and/or fatty acids, and even from waste streams such as alcohols, alkanes, and glycerol. Moreover, recent advances in molecular biology, metabolic engineering, and process optimisation in organisms like *Y. lipolytica*, *E. coli*, and *C. glutamicum* have already enabled α-KGA production with yields up to 0.94 g g^{-1}, titres as high as 195 g L^{-1}, and spatiotemporal yields up to 1.75 g L^{-1} h^{-1}. These values are especially promising since these numbers surpass the commonly considered rule-of-thumb references of 100 g L^{-1} and 1 g L^{-1} h^{-1}. The structure of α-KGA bears resemblance to some of the highly researched organic acids included in the DOE's bio-based chemical list, such as lactic acid (C_3), succinic acid (C_4), fumaric acid (C_4), malic acid, levulinic acid (C_4), itaconic acid (C_5), and the aspartic (C_4) and glutamic (C_5) (amino)acids.[23,586–588] However, compared to these organic acids, α-KGA is perhaps more interesting owing to its unique and versatile chemical properties, broad application potential, and the progress that has been made towards its sustainable production *via* precision fermentation. In the following paragraphs, we elaborate further on these advantages, as well as the main challenges that have to be addressed to establish α-KGA as a commonly adopted platform chemical.

7.1. α-KGA as a versatile building block for the bioeconomy model

Like most other DOE-listed chemicals, α-KGA is not considered acutely toxic which limits hazards during production, storage, and transport. Owing to its safety profile and pharmacological

benefits, α-KGA, either as acid or salt, is currently tested for treating severe human diseases (*e.g.*, cardiovascular diseases,[190] cancer,[194–197] and obesity[191,233]), even in clinical trial settings.[589] To the best of our knowledge, none of the other DOE-listed chemicals has a comparable broad-range medical use to α-KGA. Only for succinic and itaconic acid, evidence suggests that both DOE molecules may be applied as potential pharmaceuticals or cosmetics in some limited cases. Succinic acid is linked to skincare[590] applications or to relieve menopausal symptoms in women,[591,592] whereas itaconic acid reduces inflammatory responses.[593,594] Because α-KGA has been demonstrated to improve the physiological state of

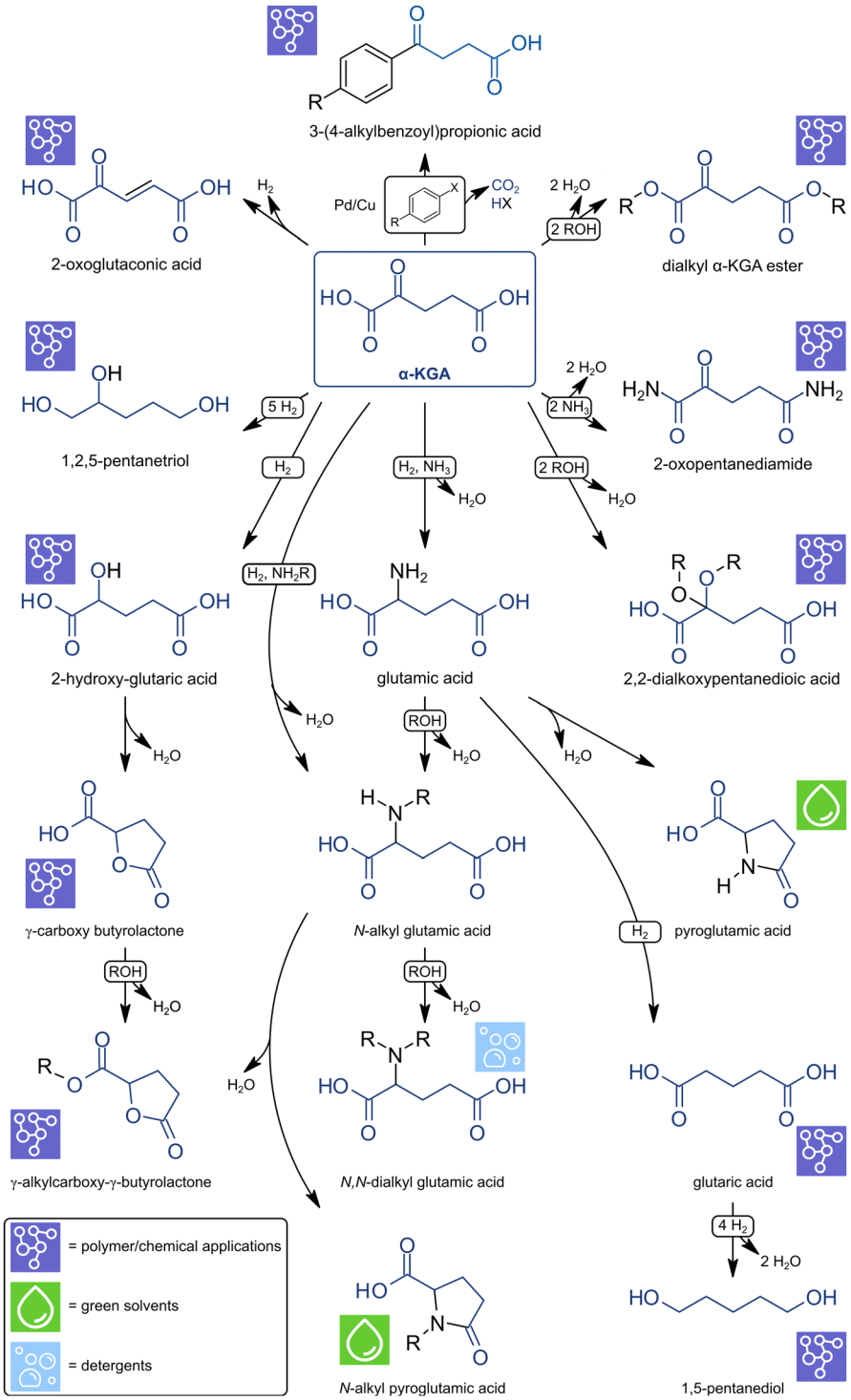


Fig. 16 Potential platform of α-KGA towards secondary derivatives for chemical/polymer, green solvent, and detergent applications. Abbreviation: Pd/Cu, palladium–copper bimetallic catalyst.

healthy individuals and patients suffering from human pathologies, we expect that this biochemical might trickle down into pharma- or nutriceutical applications.

In addition, α-KGA can serve as a precursor or additive in various chemical synthesis pathways, making it particularly suitable as a platform chemical to produce other valuable chemicals. This versatility also opens a wealth of opportunities for novel chemical derivatives and intermediates for polymer synthesis that could be derived from α-KGA (Fig. 16). While α-KGA exhibits the same di-acidic structure as most other DOE-prioritised chemicals, such as succinic, fumaric, and malic acid, it also carries a unique carbonyl group on its backbone that can be targeted for further chemical modification.[23] Moreover, the α-carboxylic acid group can positively affect the adjacent carbonyl group (through electronic effects and proton shuttle), which results in an intramolecular activation of the carbonyl group when producing novel derivatives from the α-KGA substrate (*i.e.*, electrophilic ketones; *cf.* Section 2 and Fig. 16).[54,595] The electrophilic nature of the α-carbonyl group can be completely reversed (*i.e.*, *umpolung*) when α-carboxylate salts are used instead. Subsequent decarboxylation results in a nucleophilic acyl anion which is very suitable for acylation or, in general, cross-coupling reactions, while the α-carbonyl and γ-carboxyl(ate) groups are retained.[596] Similarly, incorporating α-KGA as a co-monomer into the backbone of novel materials can expand its application potential in material engineering even more owing to the unique chemical properties of the carbonyl group. Additionally, the immediate use of the non-toxic, polar α-KGA turns the resulting polymers also more safe and environmentally friendly.[597]

The examples of novel, valuable chemicals derived from α-KGA (Fig. 16), along with the in-depth overview provided in Section 2.2, can inspire chemists and polymer scientists to explore new applications of α-KGA in the synthesis of specialised derivatives and polymers. Its potential as a polymer building block opens opportunities for entry into the plastics industry beyond its current and modest market, which is primarily focused on lactones, pharmaceuticals, and other fine chemicals.

When exploring new valorisation routes, it is of utmost importance to prioritise the use of inexpensive resources (such as hydrogen, oxygen, (di)amines, and (di)alcohols) and catalysts, while hazardous reagents for humans and the environment should be avoided. In the case of novel polymer materials, the biodegradability or recyclability aspect should also be considered. Several review papers have discussed this topic in more detail, from the different formulations for biodegradable plastics[598] to the end-of-life treatments to recycle plastics.[599] Concerning the end-of-life considerations, we have briefly discussed in this review about using pyrolysis to degrade less-recyclable plastics and implementing this mixture as feedstocks for *Y. lipolytica*. Although this field is still in its infancy, it could in time offer a sustainable upcycling opportunity in which α-KGA-based polymers are manufactured from old plastic waste.

7.2. Microbial α-KGA production guarantees sustainable production with satisfactory yields

α-KGA can be produced through microbial cell factories fed with biomass, renewable carbon substrates, or hydrocarbons from the chemical recycling of plastic, offering a sustainable alternative to the current catalytic process that relies on crude oil and toxic (chlorinated) reagents (*cf.* Fig. 7). One exception is the heterogeneously catalysed, but experimental, aldol condensation route starting from pyruvic acid and glyoxylic acid,[263] two building blocks derived from glucose metabolism, which might offer an alternative for current industrial practices.

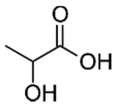
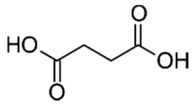
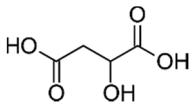
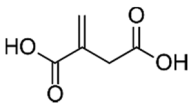
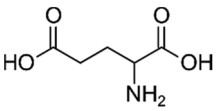
α-KGA is an intrinsic intermediate of the oTCAc respiratory pathway, a central metabolic route that is present throughout the tree of life. This implies that most microbes possess all key enzymes for α-KGA production. However, because α-KGA is an intermediate of an essential metabolic pathway, attenuating the oTCAc to accumulate α-KGA became one of the key challenges. In this review, we have summarised all strategies for high α-KGA accumulation in bacteria and yeast, from fermentation condition optimisation to metabolic pathway engineering. Many targets to manipulate the cells' metabolism towards higher α-KGA production have been tested, including engineering of genes directly linked to the TCAc. In addition, different parameters in fermentation, from pH and aeration to substrate-feeding strategies, have also been explored. We found several strategies that are most effective for α-KGA production.

The bacterium *C. glutamicum* and the yeast *Y. lipolytica* are some of the most promising microbial hosts for α-KGA production. In the case of *C. glutamicum*, disrupting the KGDH complex and the α-KGA amination systems while keeping ammonium concentrations low provides a product concentration of *ca.* 50 g L^{-1} with a yield of 0.42 g g^{-1} substrate.[406] In *Y. lipolytica*, limiting thiamine in a fed-batch cultivation system generally results in high titres (up to 195 g L^{-1}) and yields (up to 0.94 g g^{-1} substrate), regardless of the strain's genotype.[393,413,415] These values are comparable to those reported for other DOE bio-produced chemicals (Table 10), suggesting that it could become technically and economically realistic to implement α-KGA production in commercial industrial processes. Additionally, implementing a fed-batch setup for substrate feeding generally increased α-KGA production, as observed in many other industrial bioprocesses.

Based on the literature, there are three aspects that caught our attention. First, the research for microbial α-KGA production is still mostly limited to the laboratory scale. Scaling up to larger volumes often changes fermentation performance, so estimating economic viability using current parameters is difficult. Consequently, process optimisation and scale-up across the entire production chain, including the pretreatment process, bioreactor processing, and product recovery (*cf.* next subsection) at industrial biorefinery scales, should be intensively revisited.

Second, the use of inexpensive and non-edible biomass (such as lignocellulosic agricultural residues) and waste streams (*e.g.*, plastic pyrolysis or wasted vegetable oil, and crude glycerol fractions from biodiesel industries) for α-KGA production is still underexplored. The utilisation of these feedstocks can be applied in synergy in both *C. glutamicum*, which prefers hexose sugars present in plant biomass, and *Y. lipolytica*, which prefers fatty acids, alkanes, and glycerol as substrates

Table 10 Production metrics of the key biobased DOE chemicals currently on the market. LAB refers to lactic acid bacteria, including *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*, *Lactobacillus* (*para*)*casei*, *Lactobacillus amylovorus*, *Sporolactobacillus laevolacticus*, *Pediococcus acidilactici* and *Enterococcus faecalis*. Production and manufacturer data are derived from ref. 603 unless stated otherwise

Compound/structure	Microbial producer	Titre [g L^{-1}]	Productivity [g L^{-1} h^{-1}]	Global production [tonnes per year]	Manufacturers
Lactic acid 	LAB	207[604]	3–9[604]	750 000	NatureWorks (US)
	Bacillus sp.	225[604]			Corbion (NL)
	Rhizopus sp.	231[604]			Galactic (BE)
					Futerro (BE)
Succinic acid 	*Actinobacillus succinogenes*	96[605]	1.99[605]	64 000[a]	GC Innovation America (US)
					Reverdia (NL)
	Y. lipolytica	160[606]	0.40[606]		Succinity (NL, GE)
Malic acid 	*Aspergillus niger*	201[607]	0.93[607]	80–100 000[608]	Bartek (CAN)
	Trichoderma resei	220[607]	1.15[607]		Mitsubishi Corporation Life Sciences (JP)
					Anhui Sealong Biotechnology (CN)
Itaconic acid 	*E. coli*	47[609]	0.39[609]	40–70 000	Qingdao Kehai Biochemistry (CN)
	Aspergillus terreus	78[609]	1.07[609]		Cargill (US)
	Ustilago maydis	220[610]	0.74[610]		Citrique Belge (BE)
Glutamic acid 	*C. glutamicum*	120[611]	~4.6[611]	~4 000 000[612]	Ajinomoto (JP)
					Hefei TNJ Chemical Industry (CN)
					Evonik (GE)

[a] The current production volume of bio-based succinic acid is not available and is expected to be lower than the one provided in the table (corresponding to 2015). This decrease is due to the suspension of activities by BioAmber in 2018 and its successor, LCY Biosciences, in 2023.

(see Table 6). However, feeding non-edible lignocellulosic biomass remains particularly challenging since the high producers are incapable of utilising pentose sugars (*i.e.*, arabinose and xylose) natively. Therefore, one can transition to *E. coli*, a bacterium that naturally consumes pentose and hexose sugars, yet produces lower α-KGA yields. Alternatively, expanding the carbon utilisation range of *C. glutamicum* towards xylose and arabinose, which are abundantly present in hemicellulose, becomes feasible through engineering. Indeed, recently a C_5-consuming *C. glutamicum* strain was constructed that produced 0.40 g g^{-1} glutamic acid (up to 62 g L^{-1} final titre) from straw hydrolysate, approaching the metrics when glucose is used alone (0.42 g g^{-1}).[600] Although a similar example for α-KGA has not yet been reported, the authors' approach is expected to be applicable to α-KGA production.

The ability of the microbial host to metabolise lignocellulose-derived carbohydrates, however, is not the only factor that needs consideration. Lignocellulosic feedstocks are more recalcitrant and composition-wise more complex, therefore complicating the pretreatment schemes that convert hemicellulose into microbe-consumable monomers. This increases production cost and may thus threaten the profitability of the end product. Applying *reductive catalytic fractionation* (RCF) on lignocellulose, however, effectively separates this complex biomass into lignin oil and pure carbohydrate-rich pulps, which can be more readily converted into C_5 and C_6 sugar monomers through enzymatic hydrolysis.[601,602] This integrated chemoenzymatic strategy could enhance economic and environmental sustainability of existing (α-KGA) fermentation protocols.

The last point of attention is the carbon conservation during the microbial conversion processes. Although the oTCAc is the route with the highest α-KGA yield, one carbon dioxide molecule is intrinsically lost per molecule of α-KGA due to the activity of isocitrate dehydrogenase. To avoid this carbon loss, two alternative pathways have been described in the literature.

The first one encompasses the anaerobic conversion of sugars and, optionally, C_1 substrates (including methanol or formic acid) into succinic acid, following the rTCAc, and subsequent transformation of succinic acid into α-KGA using a dedicated dehydrogenase–decarboxylase cascade from *P. putida*. Whereas the first segment of the pathway, leading to succinic acid, has been intensively investigated, the 'bridging' reaction from succinic acid into α-KGA has never been demonstrated in a production setting. Yet, this strategy is particularly interesting in valorising CO_2, alongside the principal carbohydrate-rich substrates, into biomolecules since two moles of carbon dioxide will be sequestered for every mole of α-KGA produced. A second alternative is the carbon-neutral Weimberg pathway, which has been recently explored for α-KGA (Fig. 14 and Table 9). With a yield of *ca.* 10 g L^{-1}, this metabolism is inferior to the traditional oTCAc in terms of total yields, but carbon dioxide

Table 11 Evaluation of α-KGA as a future-proof platform chemical[22]

Aspect	Criteria	For α-KGA
Feedstock	Scope	Broad (waste and lignocellulosic biomass)
	Cost	Cheap (*n*-alkanes, glycerol, pulps)
	Processing	Feasible (direct feed into bio-process)
Bio-process	Performance	Good production parameters
	Estimated costs	No TEA has been performed yet
	Downstream	Optimisation required at industrial levels
	Technical complexity	Straightforward and covered in extensive literature
	Integration with bio-refinery	Yes (downstream of lignin processing, saccharification, or pyrolysis)
	Scale-up	Not initiated yet
Economics	Price	Estimated in 2019: \$12–15 per kg[613]
	Market volume	Low (<kt scale, \$100 million market value)
	Market potential	Chemical, pharmaceutical, and nutritional
Market potential	Chemical functionality	Three: 2 acids, 1 carbonyl
	Drop-in	Petrochemical α-KGA, intermediate for fine chemicals
	Building block as such	Possible, several novel materials reported
	Intermediate to derivatives	Broad product portfolio, but underexplored
	Platform	New valorisation schemes → could increase market volume
Others	C_1–C_6 monomer	Yes: C_5
	No aromatic structure	Yes: linear monomer
	No super commodity	Indeed

Table 12 Comparative overview of α-KGA production strategies

Parameter	Chemical synthesis[c]	Cell-free and whole-cell biocatalysis	Microbial-based production
Feedstock[a]	• Range: limited • Cost: cheap-moderate +/−, chemical building blocks such as DEO, DES, glyoxylic acid, and pyruvic acid from well-established processes	• Range: limited • Cost: variable −, glutamate, pyrrolidone carboxylic acid	• Range: broad Cost: cheap, but processing required ++, broad: waste streams and lignocellulose
		−−, glucuronic acid	+, 1G biomass
Energy	−, high temperatures required for synthesis of building blocks (DEO, DES) or during reaction (110 °C for glyoxylic acid and pyruvic acid)	+, minimal product purification required compared to microbial-based production	+, 1G biomass (hydrolysis of sugars or no pretreatment required for ethanol and fatty acids)
	+, energy control possible during reaction		−, waste and lignocellulose (requires pretreatment)
Environmental impact	−, emission of NO_x, use of strong bases, non-green solvents and toxic reagents	+/−, containment needed for genetically modified whole-cells	++, 1G biomass with limited pretreatment/hydrolysis and non-engineered microorganisms (solely optimising fermentation conditions) +, waste streams and lignocellulose with extensive pretreatment/hydrolysis and non-engineered microorganisms (solely optimising fermentation conditions) +/−, containment needed for genetically modified microorganisms
Scalability[b]	+, established industrial processes (for DEO and DES) −, lab scale tested up to 50 mL	−, tests limited to max 3 L	++, tests up to 1500 L
Production metrics (spatio-temporal yield)	++, aldol condensation up 50 g L^{-1} h^{-1}	+, up to 14-16 g L^{-1} h^{-1}	+, up to 1.75 g L^{-1} h^{-1}, with a portion of the substrate lost for cell metabolism

[a] Feedstock parameter considers both the range of substrates, compatible for a given approach, and the cost of the substrates. [b] Scalability refers to the maximum working volume of tests that have been conducted, which could indicate how attractive or how easily the technology could be scaled-up for commercial use. [c] For chemical synthesis, both the classic condensation step between DEO and DES and the novel aldol condensation step between glyoxylic acid and pyruvic acid are considered. Legend: ++, + (advantage), +/− (neutral), −, −− (disadvantage); DEO, diethyl oxalate; DES, diethyl succinate, 1G, first generation.

emission can be considerably reduced, leading to higher efficiencies. Moreover, the reaction pathway is shorter than the oTCAc pathway and is less connected to the central carbohydrate metabolism of the production host since the entire cascade is sourced from *C. crescentus* or *Burkholderia* species. In the short term, we argue that even a suboptimal Weimberg pathway is suited for converting the C_5-fraction of lignocellulose substrates into α-KGA in a carbon-neutral way, whereas the glycolysis and oTCAc deal with the hexose sugars.

7.3. Prospects and challenges related to recovering α-KGA from complex fermentation broths

We have summarised various options for purifying α-KGA from the fermentation broth. The approaches range from conventional methods involving inorganic acids and solvents to more innovative membrane-based systems. These methods have their own advantages and disadvantages, depending on the fermentation feedstock used, the microbial species and its metabolic by-products. The purification processes discussed are relatively simple, typically involving five main steps (filtration, ion-exchange, decolouring, dehydration, and crystallisation), and are adapted from protocols used for other bio-based carboxylic acids, such as succinic acid, whose feasibility at the industrial scale has already been demonstrated.

Despite the promising production titres, current studies on α-KGA purification were conducted on a small scale, mostly benchmarked using a simplified feed composition (glucose with α-KGA and other metabolites in MQ water, with NaOH as the pH regulator, and cations) instead of a post-fermentation broth. Hence, further research is required to investigate purification from other, more realistic fermented matrices, containing by-products and impurities. Of course, the choice of the purification method may depend on the intended application domain of α-KGA: more expensive methods yielding higher purity may be suitable for nutraceutical or pharmaceutical uses, while the opposite may be true for bulk production of α-KGA for plastic polymer manufacturing or further catalytic derivatisation into novel building blocks—particularly when water is used as the solvent, allowing the dewatering step to be omitted.

Furthermore, research and development efforts in fermentation process optimisation should be conducted with downstream processing in mind as fine-tuning fermentation parameters could reduce microbial by-product levels and potentially simplify or eliminate complex purification steps. Finally, a holistic techno-economic analysis could be performed to assess economic viability and identify steps that require further optimisation.

7.4. The future of α-KGA as a potential platform chemical

Our review shows that α-KGA has received increasing attention as a compound for pharmaceutical and chemical applications, and that advances in both fermentation and downstream recovery support the emergence of an α-KGA-based bioprocess industry (see also Table 11). We therefore believe that α-KGA is a particularly promising platform chemical, with some key advantages over more known compounds like succinic acid, due to its versatile chemical reactivity and the potential for a microbial-based sustainable production route. Unfortunately, because microbial α-KGA production is not fully matured yet, it is difficult to estimate the production costs at the industrial scale for evaluating economic viability. Moreover, volatile fossil fuel prices and possible governmental (tax) incentives also greatly influence the economic perspective of a bio-based α-KGA industry. Nonetheless, we have presented an indicative overview of how the metrics of the microbial-based production route compare to those of the (bio)-catalytic production schemes (Table 12).

This review covered the state-of-the-art in α-KGA from a combined chemical-biological perspective and emphasised the application potential and the progress in terms of fermentation, downstream processing, and chemical valorisation. We hope that this review may serve as a reference and convince chemists, microbiologists, and engineers to build further on the building blocks, polymer, and therapeutic applications provided here and translate the learnings into a demo α-KGA plant as a step towards full-scale commercialisation.

Author contributions

Conceptualisation, L.-T. O. and B. F. S.; methodology (literature survey), investigation, and writing – original draft, L.-T. O., T. S., and S. Y.; writing – review & editing, L.-T. O., T. S., S. Y., K. J. V., J. M., and B. F. S.; visualisation, T. S.; supervision, project administration, and funding acquisition, K. J. V., J. M., and B. F. S.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Acknowledgements

This work was performed within SBO StrainDrops project S001421N from Research Foundation Flanders (FWO Vlaanderen) and Hybrid Moonshot project HBC.2023.0550 from Flanders Innovation & Entrepreneurship (VLAIO). L. T. O. and B. F. S. acknowledge support from FWO (S001421N) and VLAIO (HBC.2023.0550). T. S. and J. M. acknowledge support from FWO (12O1922N, 12O1917N, S001421N, G0B0420N, G0C4322N), FWO-SBO (S001421N), EOS (40007496), internal KUL funding (928 C16/17/006), and VIB. S. Y. is supported by a PhD fellowship from FWO (1S22422N). Research in the lab of K. J. V. is supported by KU Leuven, VIB, VLAIO, FWO (S001421N) and iBOF (IBOF/21/092).

References

1 S. R. Nicholson, N. A. Rorrer, T. Uekert, G. Avery, A. C. Carpenter and G. T. Beckham, *ACS Sustainable Chem. Eng.*, 2023, **11**, 2198–2208.

2 F. M. Adebiyi, *Environ. Chem. Ecotoxicol.*, 2022, **4**, 89–96.
3 H. Rajabi, M. Hadi Mosleh, P. Mandal, A. Lea-Langton and M. Sedighi, *Sci. Total Environ.*, 2020, **727**, 138654.
4 A. Juneja and V. Singh, in *Green Energy to Sustainability*, ed. A. A. Vertès, N. Qureshi, H. P. Blaschek and H. Yukawa, John Wiley & Sons, Inc., 2020, pp. 157–184.
5 P. Sudarsanam, R. Zhong, S. Van den Bosch, S. M. Coman, V. I. Parvulescu and B. F. Sels, *Chem. Soc. Rev.*, 2018, **47**, 8349–8402.
6 P. Sudarsanam, E. Peeters, E. V. Makshina, V. I. Parvulescu and B. F. Sels, *Chem. Soc. Rev.*, 2019, **48**, 2366–2421.
7 L. T. Mika, E. Cséfalvay and Á. Németh, *Chem. Rev.*, 2018, **118**, 505–613.
8 A. Corma, S. Iborra and A. Velty, *Chem. Rev.*, 2007, **107**, 2411–2502.
9 J. M. Francois, C. Alkim and N. Morin, *Biotechnol. Biofuels*, 2020, **13**, 118.
10 J. Keasling, H. Garcia Martin, T. S. Lee, A. Mukhopadhyay, S. W. Singer and E. Sundstrom, *Nat. Rev. Microbiol.*, 2021, **19**, 701–715.
11 C. Gomes, A. C. Silva, A. C. Marques, J. S. Lobo and M. H. Amaral, *Cosmetics*, 2020, **7**, 1–14.
12 C. G. Acevedo-Rocha, L. S. Gronenberg, M. Mack, F. M. Commichau and H. J. Genee, *Curr. Opin. Biotechnol.*, 2019, **56**, 18–29.
13 M. T. de Pinho Favaro, J. Atienza-Garriga, C. Martínez-Torró, E. Parladé, E. Vázquez, J. L. Corchero, N. Ferrer-Miralles and A. Villaverde, *Microb. Cell Fact.*, 2022, **21**, 1–17.
14 L. Sun, M. Gong, X. Lv, Z. Huang, Y. Gu, J. Li, G. Du and L. Liu, *Appl. Microbiol. Biotechnol.*, 2020, **104**, 9109–9124.
15 M. J. Volk, V. G. Tran, S.-I. Tan, S. Mishra, Z. Fatma, A. Boob, H. Li, P. Xue, T. A. Martin and H. Zhao, *Chem. Rev.*, 2023, **123**, 5521–5570.
16 S. Y. Lee, H. U. Kim, T. U. Chae, J. S. Cho, J. W. Kim, J. H. Shin, D. I. Kim, Y. S. Ko, W. D. Jang and Y. S. Jang, *Nat. Catal.*, 2019, **2**, 18–33.
17 I. Khalil, G. Quintens, T. Junkers and M. Dusselier, *Green Chem.*, 2020, **22**, 1517–1541.
18 M. Ventura, A. Marinas and M. E. Domine, *Top. Catal.*, 2020, **63**, 846–865.
19 M. Dusselier, P. Van Wouwe, A. Dewaele, E. Makshina and B. F. Sels, *Energy Environ. Sci.*, 2013, **6**, 1415–1442.
20 J. S. Cho, G. B. Kim, H. Eun, C. W. Moon and S. Y. Lee, *JACS Au*, 2022, **2**, 1781–1799.
21 L. T. Mika, E. Cséfalvay and Á. Németh, *Chem. Rev.*, 2018, **118**, 505–613.
22 T. Werpy and G. Petersen, *Top Value Added Chemicals from Biomass: Results of Screening for Potential Candidates from Sugars and Synthesis Gas*, U.S. Department of Energy, 2004, vol. I.
23 J. J. Bozell and G. R. Petersen, *Green Chem.*, 2010, **12**, 539–555.
24 Registered Substances: 2-oxoglutaric acid, **https://echa.europa.eu/**, (accessed 21 September 2023).
25 B. Zdzisińska, A. Żurek and M. Kandefer-Szerszeń, *Arch. Immunol. Ther. Exp.*, 2017, **65**, 21–36.
26 H. Guo, S. Su, C. Madzak, J. Zhou, H. Chen and G. Chen, *Appl. Microbiol. Biotechnol.*, 2016, **100**, 9875–9884.
27 F. Legendre, A. MacLean, V. P. Appanna and V. D. Appanna, *World J. Microbiol. Biotechnol.*, 2020, **36**, 123.
28 C. Otto, V. Yovkova and G. Barth, *Appl. Microbiol. Biotechnol.*, 2011, **92**, 689–695.
29 T. Ronzon, T. Lammens, J. Spekreijse, M. Vis and C. Parisi, *Insights into the European market for bio-based chemicals: analysis based on 10 key product categories*, Publications Office, European Commission, Joint Research Centre, 2019.
30 R. Bhattacharya, D. Kumar, K. Sugendran, S. C. Pant, R. K. Tulsawani and R. Vijayaraghavan, *J. Appl. Toxicol.*, 2001, **21**, 495–499.
31 Chemical Abstracts Service: Columbus; OH, SciFinder, **https://scifinder-n.cas.org/**, (accessed 31 May 2023).
32 C. Dudás, B. Kutus, G. Peintler, I. Pálinkó and P. Sipos, *Polyhedron*, 2018, **156**, 89–97.
33 I. Hevus, N. G. Ricapito, S. Tymoshenko, S. N. Raja and D. C. Webster, *ACS Sustainable Chem. Eng.*, 2020, **8**, 5750–5762.
34 M. Voges, F. Schmidt, D. Wolff, G. Sadowski and C. Held, *Fluid Phase Equilib.*, 2016, **422**, 87–98.
35 A. J. Copper and A. G. Redfield, *J. Biol. Chem.*, 1975, **250**, 527–532.
36 T. S. Viswanathan, R. E. Johnson and H. F. Fisher, *Biochemistry*, 1982, **21**, 339–345.
37 A. J. L. Cooper, J. Z. Ginos and A. Meister, *Chem. Rev.*, 1983, **83**, 321–358.
38 R. C. Kerber and M. S. Fernando, *J. Chem. Educ.*, 2010, **87**, 1079–1084.
39 F. D. Klingler and W. Ebertz, *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, 2000.
40 Z. Luo, S. Yu, W. Zeng and J. Zhou, *Biotechnol. Adv.*, 2021, **47**, 107706.
41 Y. Song, J. Li, H. Shin, L. Liu, G. Du and J. Chen, *Bioresour. Technol.*, 2016, **219**, 716–724.
42 U. Stottmeister, A. Aurich, H. Wilde, J. Andersch, S. Schmidt and D. Sicker, *J. Ind. Microbiol. Biotechnol.*, 2005, **32**, 651–664.
43 R. W. Hanson, *J. Chem. Educ.*, 1987, **64**, 591.
44 A. Ahmad and I. D. Spenser, *Can. J. Chem.*, 1961, **39**, 1340–1359.
45 S. L. Bartlett, Y. Sohtome, D. Hashizume, P. S. White, M. Sawamura, J. S. Johnson and M. Sodeoka, *J. Am. Chem. Soc.*, 2017, **139**, 8661–8666.
46 F. J. Hidalgo, R. M. Delgado and R. Zamora, *Food Chem.*, 2013, **141**, 1140–1146.
47 Q. Jiang, J. Jia, B. Xu, A. Zhao and C.-C. Guo, *J. Org. Chem.*, 2015, **80**, 3586–3596.
48 X. Lan, C. Tao, X. Liu, A. Zhang and B. Zhao, *Org. Lett.*, 2016, **18**, 3658–3661.
49 B. Lee, P. Kang, K. H. Lee, J. Cho, W. Nam, W. K. Lee and N. H. Hur, *Tetrahedron Lett.*, 2013, **54**, 1384–1388.
50 T. Nanjo, N. Kato and Y. Takemoto, *Org. Lett.*, 2018, **20**, 5766–5769.

51 J. Spengler, S. N. Osipov, E. Heistracher, A. Haas and K. Burger, *J. Fluorine Chem.*, 2004, **125**, 1019–1023.
52 W.-T. Xu, B. Huang, J.-J. Dai, J. Xu and H.-J. Xu, *Org. Lett.*, 2016, **18**, 3114–3117.
53 C. Zhou and D. Ma, *Chem. Commun.*, 2014, **50**, 3085–3088.
54 D. A. Klumpp, S. Lau, M. Garza, B. Schick and K. Kantardjieff, *J. Org. Chem.*, 1999, **64**, 7635–7637.
55 F. Penteado, E. F. Lopes, D. Alves, G. Perin, R. G. Jacob and E. J. Lenardão, *Chem. Rev.*, 2019, **119**, 7113–7278.
56 A. Drif, A. Pineda, D. Morvan, V. Belliere-Baca, K. De Oliveira Vigier and F. Jérôme, *Green Chem.*, 2019, **21**, 4604–4608.
57 D. G. Barrett and M. N. Yousaf, *Macromolecules*, 2008, **41**, 6347–6352.
58 D. G. Barrett and M. N. Yousaf, *Biomacromolecules*, 2008, **9**, 2029–2035.
59 H. Chao, G. Li, J. Yu, Z. Liu, Z.-W. Liu and J. Jiang, *Macromol. Chem. Phys.*, 2019, **220**, 1900004.
60 A. A. Gadgeel and S. T. Mhaske, *Prog. Org. Coat.*, 2021, **150**, 105983.
61 Z. A. Page, R. Bou Zerdan, W. R. Gutekunst, A. Anastasaki, S. Seo, A. J. McGrath, D. J. Lunn, P. G. Clark and C. J. Hawker, *J. Polym. Sci., Part A: Polym. Chem.*, 2017, **55**, 801–807.
62 D. J. Lunn, S. Seo, S.-H. Lee, R. B. Zerdan, K. M. Mattson, N. J. Treat, A. J. McGrath, W. R. Gutekunst, J. Lawrence, A. Abdilla, A. Anastasaki, A. S. Knight, B. V. K. J. Schmidt, M. W. Bates, P. G. Clark, J. P. DeRocher, A. K. Van Dyk and C. J. Hawker, *J. Polym. Sci., Part A: Polym. Chem.*, 2019, **57**, 716–725.
63 N. A. Rorrer, S. F. Notonier, B. C. Knott, B. A. Black, A. Singh, S. R. Nicholson, C. P. Kinchin, G. P. Schmidt, A. C. Carpenter, K. J. Ramirez, C. W. Johnson, D. Salvachúa, M. F. Crowley and G. T. Beckham, *Cell Rep. Phys. Sci.*, 2022, **3**, 100840.
64 S. Hashimoto, in *Amino Acid Fermentation*, ed. A. Yokota and M. Ikeda, Springer, Japan, Tokyo, 2017, pp. 15–34.
65 T. Fukushima and M. Yamauchi, *Chem. Commun.*, 2019, **55**, 14721–14724.
66 J. A. Kent, in *Riegel's Handbook of Industrial Chemistry*, ed. J. A. Kent, Springer US, Boston, MA, 2003, pp. 963–1045.
67 H. Lin, Y. Liu, C. Yang, G. Zhao, J. Song, T. Zhang and X. Huang, *Catal. Sci. Technol.*, 2022, **12**, 4057–4065.
68 A. Galkin, L. Kulakova, T. Yoshimura, K. Soda and N. Esaki, *Appl. Environ. Microbiol.*, 1997, **63**, 4651–4656.
69 K. F. Gu and T. M. S. Chang, *Biotechnol. Bioeng.*, 1988, **32**, 363–368.
70 A. Galkin, L. Kulakova, H. Yamamoto, K. Tanizawa, H. Tanaka, N. Esaki and K. Soda, *J. Ferment. Bioeng.*, 1997, **83**, 299–300.
71 C.-X. Chen, B. Jiang, C. Branford-White and L.-M. Zhu, *Biochem.*, 2009, **74**, 36–40.
72 C. Huber and G. Wächtershäuser, *Tetrahedron Lett.*, 2003, **44**, 1695–1697.
73 W. Wang, X. Liu, Y. Yang and W. Su, *Int. J. Astrobiol.*, 2013, **12**, 69–77.
74 R. Kadyrov, T. H. Riermeier, U. Dingerdissen, V. Tararov and A. Börner, *J. Org. Chem.*, 2003, **68**, 4067–4070.
75 V. Helaine and J. Bolte, *Eur. J. Org. Chem.*, 1999, 3403–3406.
76 H. Kuang, M. L. Brown, R. R. Davies, E. C. Young and M. D. Distefano, *J. Am. Chem. Soc.*, 1996, **118**, 10702–10706.
77 H. Kuang, R. R. Davies and M. D. Distefano, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2055–2060.
78 H. Kuang and M. D. Distefano, *J. Am. Chem. Soc.*, 1998, **120**, 1072–1073.
79 J. T. Slama, R. K. Satsangi, A. Simmons, V. Lynch, R. E. Bolger and J. Suttie, *J. Med. Chem.*, 1990, **33**, 824–832.
80 J. H. Kim, S. H. Lee, J. S. Lee, M. Lee and C. B. Park, *Chem. Commun.*, 2011, **47**, 10227–10229.
81 D. Mandler and I. Willner, *J. Chem. Soc., Chem. Commun.*, 1986, 851–853.
82 D. H. Nam and C. B. Park, *ChemBioChem*, 2012, **13**, 1278–1282.
83 E. Siu, K. Won and C. B. Park, *Biotechnol. Prog.*, 2007, **23**, 293–296.
84 K. Tao, B. Xue, S. Frere, I. Slutsky, Y. Cao, W. Wang and E. Gazit, *Chem. Mater.*, 2017, **29**, 4454–4460.
85 C. H. Wong and G. M. Whitesides, *J. Org. Chem.*, 1982, **47**, 2816–2818.
86 Y. Zhao, H. Liu, C. Wu, Z. Zhang, Q. Pan, F. Hu, R. Wang, P. Li, X. Huang and Z. Li, *Angew. Chem., Int. Ed.*, 2019, **58**, 5376–5381.
87 M. Zheng, S. Zhang, G. Ma and P. Wang, *J. Biotechnol.*, 2011, **154**, 274–280.
88 H. A. Ibrahim, F. M. Awadallah, H. M. Refaat and K. M. Amin, *Bioorg. Chem.*, 2018, **77**, 457–470.
89 T. Panneerselvam, S. Arumugam, M. A. Ali, K. Selvaraj, M. Indhumathy, A. Sivakumar and S. D. Joshi, *ChemistrySelect*, 2017, **2**, 2341–2347.
90 M. Rashid, *Bioorg. Chem.*, 2020, **96**, 103576.
91 M. Rashid, A. Husain, R. Mishra, S. Karim, S. Khan, M. Ahmad, N. Al-wabel, A. Husain, A. Ahmad and S. A. Khan, *Arabian J. Chem.*, 2019, **12**, 3202–3224.
92 M. Rashid, A. Husain and R. Mishra, *Eur. J. Med. Chem.*, 2012, **54**, 855–866.
93 Z. Song, X. Wang, Y. Zhang, W. Gu, A. Shen, C. Ding, H. Li, R. Xiao, M. Geng, Z. Xie and A. Zhang, *J. Med. Chem.*, 2021, **64**, 1649–1669.
94 A. Husain, M. Rashid, R. Mishra, S. Parveen, D.-S. Shin and D. Kumar, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 5438–5444.
95 T. A. Kuz'menko, V. V. Kuz'menko, A. S. Morkovnik and L. N. Divaeva, *Chem. Heterocycl. Compd.*, 2006, **42**, 648–656.
96 L. Fabian, M. F. Martini, E. S. Sarduy, D. A. Estrin and A. G. Moglioni, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 2197–2202.
97 L. Fabian, M. Taverna Porro, N. Gómez, M. Salvatori, G. Turk, D. Estrin and A. Moglioni, *Eur. J. Med. Chem.*, 2020, **188**, 111987.
98 J. Gris, R. Glisoni, L. Fabian, B. Fernández and A. G. Moglioni, *Tetrahedron Lett.*, 2008, **49**, 1053–1056.
99 E. A. Fayed, Y. A. Ammar, M. A. Saleh, A. H. Bayoumi, A. Belal, A. B. M. Mehany and A. Ragab, *J. Mol. Struct.*, 2021, **1236**, 130317.

100 R. Ferreira de Freitas, R. J. Harding, I. Franzoni, M. Ravichandran, M. K. Mann, H. Ouyang, M. Lautens, V. Santhakumar, C. H. Arrowsmith and M. Schapira, *J. Med. Chem.*, 2018, **61**, 4517–4527.
101 R. J. Harding, R. Ferreira de Freitas, P. Collins, I. Franzoni, M. Ravichandran, H. Ouyang, K. A. Juarez-Ornelas, M. Lautens, M. Schapira, F. von Delft, V. Santhakumar and C. H. Arrowsmith, *J. Med. Chem.*, 2017, **60**, 9090–9096.
102 M. S. Kazunin, O. Y. Voskoboynik, O. M. Shatalova, L. N. Maloshtan and S. I. Kovalenko, *Chem. Heterocycl. Compd.*, 2019, **55**, 408–415.
103 S. Tariq, O. Alam and M. Amir, *Bioorg. Chem.*, 2018, **76**, 343–358.
104 V. L. Gein, N. A. Rassudikhina, N. V. Shepelina, M. I. Vakhrin, E. B. Babushkina and E. V. Voronina, *Pharm. Chem. J.*, 2008, **42**, 529–532.
105 K. Hachama, M. Khodja, S. Moulay, H. Boutoumi, L. Hennig and D. Sicker, *J. Heterocycl. Chem.*, 2013, **50**, 413–416.
106 M.-T. Le Bris, *J. Heterocycl. Chem.*, 1985, **22**, 1275–1280.
107 J. P. Burke, Z. Bian, S. Shaw, B. Zhao, C. M. Goodwin, J. Belmar, C. F. Browning, D. Vigil, A. Friberg, D. V. Camper, O. W. Rossanese, T. Lee, E. T. Olejniczak and S. W. Fesik, *J. Med. Chem.*, 2015, **58**, 3794–3805.
108 T. D. Kapti Cagatay and M. Balci, *Synthesis*, 2017, 1898–1904.
109 H. Yang, M. Poznik, S. Tang, P. Xue, L. Du, C. Liu, X. Chen and J. J. Chruma, *ACS Omega*, 2021, **6**, 19291–19303.
110 W. Jahnke, J.-M. Rondeau, S. Cotesta, A. Marzinzik, X. Pellé, M. Geiser, A. Strauss, M. Götte, F. Bitsch, R. Hemmig, C. Henry, S. Lehmann, J. F. Glickman, T. P. Roddy, S. J. Stout and J. R. Green, *Nat. Chem. Biol.*, 2010, **6**, 660–666.
111 P. Yu, T. Wang, J. Li and J. M. Cook, *J. Org. Chem.*, 2000, **65**, 3173–3191.
112 Z. Hou, L.-F. Zhu, X. Yu, M.-Q. Sun, F. Miao and L. Zhou, *J. Agric. Food Chem.*, 2016, **64**, 2847–2854.
113 X. Li, B. Zhang, W. Zhao, S. Yang, X. Yang and L. Zhou, *Sci. Rep.*, 2019, **9**, 1941.
114 B. Zhou, B. Zhang, X. Li, X. Liu, H. Li, D. Li, Z. Cui, H. Geng and L. Zhou, *Sci. Rep.*, 2018, **8**, 1559.
115 A. Tsotinis, M. Panoussopoulou, A. Eleutheriades, K. Davidson and D. Sugden, *Eur. J. Med. Chem.*, 2007, **42**, 1004–1013.
116 M. A. Flores and J. W. Bode, *Org. Lett.*, 2010, **12**, 1924–1927.
117 Y.-L. Huang and J. W. Bode, *Nat. Chem.*, 2014, **6**, 877–884.
118 M. M. Sheha, N. M. Mahfouz, H. Y. Hassan, A. F. Youssef, T. Mimoto and Y. Kiso, *Eur. J. Med. Chem.*, 2000, **35**, 887–894.
119 T. G. Wucherpfennig, F. Rohrbacher, V. R. Pattabiraman and J. W. Bode, *Angew. Chem., Int. Ed.*, 2014, **53**, 12244–12247.
120 C. E. Murar, F. Thuaud and J. W. Bode, *J. Am. Chem. Soc.*, 2014, **136**, 18140–18148.
121 T. Nanjo, N. Kato, X. Zhang and Y. Takemoto, *Chem. – Eur. J.*, 2019, **25**, 15504–15507.
122 M. Mihalache, O. Oprea, C. Guran and A. M. Holban, *C. R. Chim.*, 2018, **21**, 32–40.
123 R. Müller, E. Hübner and N. Burzlaff, *Eur. J. Inorg. Chem.*, 2004, 2151–2159.
124 S. Parveen, T. Premkumar, H. H. Nguyen and S. Govindarajan, *New J. Chem.*, 2019, **43**, 13371–13380.
125 A. Tavman, *Main Group Met. Chem.*, 2012, **35**, 81–89.
126 Z.-H. Zhou, H. Wang, P. Yu, M. M. Olmstead and S. P. Cramer, *J. Inorg. Biochem.*, 2013, **118**, 100–106.
127 R. C. Evans and F. Y. Wiselogle, *J. Am. Chem. Soc.*, 1945, **67**, 60–62.
128 G. B. Kline and S. H. Cox, *J. Org. Chem.*, 1961, **26**, 1854–1856.
129 H.-Y. Qian, Z.-L. Wang, X.-Y. Xie, Y.-L. Pan, G.-J. Li, X. Xie and J.-Z. Chen, *Eur. J. Med. Chem.*, 2017, **137**, 598–611.
130 M. P. Giovannoni, I. A. Schepetkin, A. Cilibrizzi, L. Crocetti, A. I. Khlebnikov, C. Dahlgren, A. Graziano, V. Dal Piaz, L. N. Kirpotina, S. Zerbinati, C. Vergelli and M. T. Quinn, *Eur. J. Med. Chem.*, 2013, **64**, 512–528.
131 A. Katrusiak, P. Piechowiak and A. Katrusiak, *J. Mol. Struct.*, 2011, **998**, 84–90.
132 S. Hussain, A. Khan, S. Gul, M. Resmini, C. S. Verma, E. W. Thomas and K. Brocklehurst, *Biochemistry*, 2011, **50**, 10732–10742.
133 J. Li, H. Yin and M. Hong, *Z. Anorg. Allg. Chem.*, 2012, **638**, 387–391.
134 S. Parveen, S. Govindarajan, H. Puschmann and R. Revathi, *Inorg. Chim. Acta*, 2018, **477**, 66–74.
135 S. Parveen, T. Premkumar, H.-H. Nguyen, S. Govindarajan, D. Manikandan, S. K. Anandasadagopan and E. Vijayakumar, *Inorg. Chim. Acta*, 2021, **516**, 120142.
136 M. Baldini, M. Belicchi-Ferrari, F. Bisceglie, S. Capacchi, G. Pelosi and P. Tarasconi, *J. Inorg. Biochem.*, 2005, **99**, 1504–1513.
137 M. Baldini, M. Belicchi-Ferrari, F. Bisceglie, P. P. Dall'Aglio, G. Pelosi, S. Pinelli and P. Tarasconi, *Inorg. Chem.*, 2004, **43**, 7170–7179.
138 Z.-Y. Yang and R.-D. Yang, *Synth. React. Inorg. Met.-Org. Chem.*, 2002, **32**, 1059–1069.
139 E. Novotná, K. Waisser, J. Kuneš, K. Palát, L. Skálová, B. Szotáková, V. Buchta, J. Stolaříková, V. Ulmann, M. Pávová, J. Weber, J. Komrsková, P. Hašková, I. Vokřál and V. Wsól, *Arch. Pharm.*, 2017, **350**, 1700020.
140 J.-K. Dai, W.-J. Dan, N. Li, H.-T. Du, J.-W. Zhang and J.-R. Wang, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 580–583.
141 J. Dai, W. Dan, Y. Zhang, M. He and J. Wang, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 3123–3128.
142 J. Dai, W. Dan, N. Li, R. Wang, Y. Zhang, N. Li, R. Wang and J. Wang, *Food Chem.*, 2018, **253**, 211–220.
143 F. Zhao, J.-K. Dai, D. Liu, S.-J. Wang and J.-R. Wang, *Molecules*, 2016, **21**, 390.
144 K. M. Czerwinski, C. A. Zificsak, J. Stevens, M. Oberbeck, C. Randlett, M. King and S. Mennen, *Synth. Commun.*, 2003, **33**, 1225–1231.
145 K. Narayanan and J. M. Cook, *Tetrahedron Lett.*, 1990, **31**, 3397–3400.

146 S. V. Ryabukhin, D. M. Panov, A. S. Plaskon, A. A. Tolmachev and R. V. Smaliy, *Monatsh. Chem.*, 2012, **143**, 1507–1517.
147 V. Stavytskyi, O. Voskoboinik, O. Antypenko, N. Krasovska, K. Shabelnyk, I. Konovalova, S. Shishkyna, S. Kholodniak and S. Kovalenko, *J. Heterocycl. Chem.*, 2020, **57**, 1249–1260.
148 Y.-P. Wang, X.-R. Ou, Y. Wang, J.-Q. Liu and X.-S. Wang, *J. Heterocycl. Chem.*, 2018, **55**, 2325–2333.
149 M.-M. Zhang, L. Lu, Y.-J. Zhou and X.-S. Wang, *Res. Chem. Intermed.*, 2013, **39**, 3327–3335.
150 D. Zicāne, Z. Tetere, I. Rāviņa and M. Turks, *Chem. Heterocycl. Compd.*, 2013, **49**, 310–316.
151 M. Beigi, S. Waltzer, A. Fries, L. Eggeling, G. A. Sprenger and M. Müller, *Org. Lett.*, 2013, **15**, 452–455.
152 A. Kurutsch, M. Richter, V. Brecht, G. A. Sprenger and M. Müller, *J. Mol. Catal. B: Enzym.*, 2009, **61**, 56–66.
153 R. Westphal, S. Waltzer, U. Mackfeld, M. Widmann, J. Pleiss, M. Beigi, M. Müller, D. Rother and M. Pohl, *Chem. Commun.*, 2013, **49**, 2061–2063.
154 R. Westphal, S. Jansen, C. Vogel, J. Pleiss, M. Müller, D. Rother and M. Pohl, *ChemCatChem*, 2014, **6**, 1082–1088.
155 R. Westphal, D. Hahn, U. Mackfeld, S. Waltzer, M. Beigi, M. Widmann, C. Vogel, J. Pleiss, M. Müller, D. Rother and M. Pohl, *ChemCatChem*, 2013, **5**, 3587–3594.
156 M. Beigi, S. Loschonsky, P. Lehwald, V. Brecht, S. L. A. Andrade, F. J. Leeper, W. Hummel and M. Müller, *Org. Biomol. Chem.*, 2013, **11**, 252–256.
157 B. B. Jarvis, K. M. Wells and T. Kaufmann, *Synthesis*, 1990, 1079–1082.
158 H. Natsugari, Y. Kawano, A. Morimoto, K. Yoshioka and M. Ochiai, *J. Chem. Soc., Chem. Commun.*, 1987, 62–63.
159 H.-B. Chen, L.-Y. Chen, P.-Q. Huang, H.-K. Zhang, Z.-H. Zhou and K.-R. Tsai, *Tetrahedron*, 2007, **63**, 2148–2152.
160 P. Singh, A. Mittal, P. Kaur and S. Kumar, *Tetrahedron*, 2006, **62**, 1063–1068.
161 K. Felföldi, K. Szöri and M. Bartók, *Appl. Catal., A*, 2003, **251**, 457–460.
162 Y. Laras, V. Hugues, Y. Chandrasekaran, M. Blanchard-Desce, F. C. Acher and N. Pietrancosta, *J. Org. Chem.*, 2012, **77**, 8294–8302.
163 S. Sasaki, H. Suzuki, H. Ouchi, T. Asakawa, M. Inai, R. Sakai, K. Shimamoto, Y. Hamashima and T. Kan, *Org. Lett.*, 2014, **16**, 564–567.
164 N. Ohgami, S. Upadhyay, A. Kabata, K. Morimoto, H. Kusakabe and H. Suzuki, *Biosens. Bioelectron.*, 2007, **22**, 1330–1336.
165 H. Irikawa, S. Ooe and Y. Okumura, *Bull. Chem. Soc. Jpn.*, 1988, **61**, 3365–3367.
166 A. P. Mityuk, A. V. Denisenko, O. O. Grygorenko and A. A. Tolmachev, *ARKIVOC*, 2012, **2012**, 226–230.
167 M. Jiang, M. Chen, Y. Cao, Y. Yang, K. H. Sze, X. Chen and Z. Guo, *Org. Lett.*, 2007, **9**, 4765–4767.
168 M. Chen, M. Jiang and Z. Guo, *Sci. China: Chem.*, 2013, **56**, 312–320.
169 A. Balakrishnan, F. Jordan and C. F. Nathan, *J. Biol. Chem.*, 2013, **288**, 21688–21702.
170 Y. A. Davidovich and S. V. Kozlov, *Pharm. Chem. J.*, 2021, **55**, 506–509.
171 Y. I. Sakhno, O. V. Radchenko, E. A. Muravyova, S. M. Sirko, S. V. Shishkina, V. I. Musatov, S. M. Desenko and V. A. Chebanov, *Chem. Heterocycl. Compd.*, 2021, **57**, 261–265.
172 J. H. Bushweller and P. A. Bartlett, *J. Org. Chem.*, 1989, **54**, 2404–2409.
173 J. G. Hubert, I. A. Stepek, H. Noda and J. W. Bode, *Chem. Sci.*, 2018, **9**, 2159–2167.
174 V. A. Kozlov, K. V. Novikov, T. G. Mokeeva and S. A. Kuz'mina, *Russ. J. Gen. Chem.*, 2013, **83**, 1467–1468.
175 N. Tibrewal and G. I. Elliott, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 517–519.
176 J. E. Baldwin, J. K. Cha and L. I. Kruse, *Tetrahedron*, 1985, **41**, 5241–5260.
177 M. A. Iqbal, A. Husain, O. Alam, S. A. Khan, A. Ahmad, M. R. Haider and M. A. Alam, *Arch. Pharm.*, 2020, **353**, 2000071.
178 2-oxoglutaric acid, **https://chem.echa.europa.eu**, (accessed 15 May 2024).
179 L. A. Cynober, *Curr. Opin. Clin. Nutr. Metab. Care*, 1999, **2**, 33–37.
180 J. E. Abusalim, K. Yamamoto, N. Miura, B. Blackman, J. R. Brender, C. Mushti, T. Seki, K. A. Camphausen, R. E. Swenson, M. C. Krishna and A. H. Kesarwala, *ACS Chem. Biol.*, 2021, **16**, 2144–2150.
181 H. Abla, M. Sollazzo, G. Gasparre, L. Iommarini and A. M. Porcelli, *Semin. Cell Dev. Biol.*, 2020, **98**, 26–33.
182 B. Gyanwali, Z. X. Lim, J. Soh, C. Lim, S. P. Guan, J. Goh, A. B. Maier and B. K. Kennedy, *Trends Endocrinol. Metab.*, 2022, **33**, 136–146.
183 F. Hammarqvist, J. Wernerman, A. von der Decken and E. Vinnars, *Surgery*, 1991, **109**, 28–36.
184 M. Wirén, J. Permert and J. Larsson, *Nutrition*, 2002, **18**, 725–728.
185 P. Iwaniak, E. Tomaszewska, S. Muszyński, M. Marszałek-Grabska, S. G. Pierzynowski and P. Dobrowolski, *Nutrients*, 2022, **14**, 2062.
186 U. Kjellman, K. Björk, R. Ekroth, H. Karlsson, R. Jagenburg, F. Nilsson, G. Svensson and J. Wernerman, *Lancet*, 1995, **345**, 553–555.
187 U. W. Kjellman, K. Björk, R. Ekroth, H. Karlsson, R. Jagenburg, F. N. Nilsson, G. Svensson and J. Wernerman, *Ann. Thorac. Surg.*, 1997, **63**, 1625–1633.
188 J. Wernerman, F. Hammarqvist and E. Vinnars, *Lancet*, 1990, **335**, 701–703.
189 A. Jeppsson, R. Ekroth, P. Friberg, K. Kirnö, I. Milocco, F. N. Nilsson, S. Svensson and J. Wernerman, *Ann. Thorac. Surg.*, 1998, **65**, 684–690.
190 N. M. Shrimali, S. Agarwal, S. Kaur, S. Bhattacharya, S. Bhattacharyya, J. T. Prchal and P. Guchhait, *eBioMedicine*, 2021, **73**, 103672.
191 S. Agarwal, R. Ghosh, G. Verma, R. Khadgawat and P. Guchhait, *Clin. Exp. Immunol.*, 2023, **XX**, 12.
192 Y. Yuan, C. Zhu, Y. Wang, J. Sun, J. Feng, Z. Ma, P. Li, W. Peng, C. Yin, G. Xu, P. Xu, Y. Jiang, Q. Jiang and G. Shu, *Sci. Adv.*, 2022, **8**, eabn2879.

193 C. W. Tseng, W. H. Kuo, S. H. Chan, H. L. Chan, K. J. Chang and L. H. Wang, *Cancer Res.*, 2018, **78**, 2799–2812.
194 K. Kaławaj, A. Sławińska-Brych, M. Mizerska-Kowalska, A. Żurek, A. Bojarska-Junak, M. Kandefer-Szerszeń and B. Zdzisińska, *Int. J. Mol. Sci.*, 2020, **21**, 1–21.
195 N. Liu, J. Zhang, M. Yan, L. Chen, J. Wu, Q. Tao, B. Yan, X. Chen and C. Peng, *Cell Death Dis.*, 2023, **14**, 170.
196 K. Matsumoto, N. Obara, M. Ema, M. Horie, A. Naka, S. Takahashi and S. Imagawa, *Cancer Sci.*, 2009, **100**, 1639–1647.
197 V. Sica, J. M. Bravo-San Pedro, V. Izzo, J. Pol, S. Pierredon, D. Enot, S. Durand, N. Bossut, A. Chery, S. Souquere, G. Pierron, E. Vartholomaiou, N. Zamzami, T. Soussi, A. Sauvat, L. Mondragón, O. Kepp, L. Galluzzi, J. C. Martinou, H. Hess-Stumpp, K. Ziegelbauer, G. Kroemer and M. C. Maiuri, *Cell Rep.*, 2019, **27**, 820–834.e9.
198 R. P. Radzki, M. Bienko and S. G. Pierzynowski, *J. Bone Miner. Metab.*, 2012, **30**, 651–659.
199 R. Bhattacharya, J. Hariharakrishnan, R. M. Satpute and R. Tulsawani, *Mol. Cell. Toxicol.*, 2012, **8**, 83–93.
200 R. Ali, G. Mittal, S. Sultana and A. Bhatnagar, *Exp. Lung Res.*, 2012, **38**, 435–444.
201 D. C. Mathangi, R. Shyamala, R. Vijayashree, K. R. Rao, A. Ruckmani, R. Vijayaraghavan and R. Bhattacharya, *Neurochem. Res.*, 2011, **36**, 540–548.
202 J. C. Norris, W. A. Utley and A. S. Hume, *Toxicology*, 1990, **62**, 275–283.
203 M. M. Bayliak, H. V. Shmihel, M. P. Lylyk, O. M. Vytvytska, J. M. Storey, K. B. Storey and V. I. Lushchak, *Environ. Toxicol. Pharmacol.*, 2015, **40**, 650–659.
204 T. Meissner, E. Mayatepek, M. Kinner and R. Santer, *Clin. Chim. Acta*, 2004, **341**, 23–26.
205 W. Harrington, A. Liu, D. Lonsdale and D. Igou, *Clin. Chim. Acta*, 1977, **74**, 247–254.
206 S. Islam, T. M. Leissing, R. Chowdhury, R. J. Hopkinson and C. J. Schofield, *Annu. Rev. Biochem.*, 2018, **87**, 585–620.
207 H. L. H. Green and A. C. Brewer, *Clin. Epigenet.*, 2020, **12**, 1–15.
208 J. A. Losman, P. Koivunen and W. G. Kaelin, *Nat. Rev. Cancer*, 2020, **20**, 710–726.
209 G. Zurlo, J. Guo, M. Takada, W. Wei and Q. Zhang, *Biochim. Biophys. Acta, Rev. Cancer*, 2016, **1866**, 208–220.
210 J. Guo, A. A. Chakraborty, P. Liu, W. Gan, X. Zheng, H. Inuzuka, B. Wang, J. Zhang, L. Zhang, M. Yuan, J. Novak, J. Q. Cheng, A. Toker, S. Signoretti, Q. Zhang, J. M. Asara, W. G. K. Jr and W. Wei, *Science*, 2016, **353**, 929–932.
211 B. W. Wong, A. Kuchnio, U. Bruning and P. Carmeliet, *Trends Biochem. Sci.*, 2013, **38**, 3–11.
212 G. Sutendra, P. Dromparis, A. Kinnaird, T. H. Stenson, A. Haromy, J. M. R. Parker, M. S. Mcmurtry and E. D. Michelakis, *Oncogene*, 2013, **32**, 1638–1650.
213 D. A. Tennant and E. Gottlieb, *J. Mol. Med.*, 2010, **88**, 839–849.
214 A. Sharma, S. Sinha and N. Shrivastava, *Front. Genet.*, 2022, **13**, 1–10.
215 B. Wang, Q. Zhao, Y. Zhang, Z. Liu, Z. Zheng, S. Liu, L. Meng, Y. Xin and X. Jiang, *J. Exp. Clin. Cancer Res.*, 2021, **40**, 1–16.
216 L. Jin, J. Chun, C. Pan, A. Kumar, G. Zhang, Y. Ha, D. Li, G. N. Alesi, Y. Kang, L. Zhou, W. M. Yu, K. R. Magliocca, F. R. Khuri, C. K. Qu, C. Metallo, T. K. Owonikoko and S. Kang, *Mol. Cell*, 2018, **69**, 87–99.e7.
217 X. Wang, R. Liu, X. Qu, H. Yu, H. Chu, Y. Zhang, W. Zhu, X. Wu, H. Gao, B. Tao, W. Li, J. Liang, G. Li and W. Yang, *Mol. Cell*, 2019, **76**, 148–162.e7.
218 L. Jin, J. Chun, C. Pan, A. Kumar, G. Zhang, Y. Ha, D. Li, G. N. Alesi, Y. Kang, L. Zhou, W. M. Yu, K. R. Magliocca, F. R. Khuri, C. K. Qu, C. Metallo, T. K. Owonikoko and S. Kang, *Mol. Cell*, 2018, **69**, 87–99.e7.
219 X. Wang, R. Liu, X. Qu, H. Yu, H. Chu, Y. Zhang, W. Zhu, X. Wu, H. Gao, B. Tao, W. Li, J. Liang, G. Li and W. Yang, *Mol. Cell*, 2019, **76**, 148–162.e7.
220 X. Wu and Y. Zhang, *Nat. Rev. Genet.*, 2017, **18**, 517–534.
221 A. Portela and M. Esteller, *Nat. Biotechnol.*, 2010, **28**, 1057–1068.
222 X. Wu and Y. Zhang, *Nat. Rev. Genet.*, 2017, **18**, 517–534.
223 W. Li, X. Zhang, X. Lu, L. You, Y. Song, Z. Luo, J. Zhang, J. Nie, W. Zheng, D. Xu, Y. Wang, Y. Dong, S. Yu, J. Hong, J. Shi, H. Hao, F. Luo, L. Hua, P. Wang, X. Qian, F. Yuan, L. Wei, M. Cui, T. Zhang, Q. Liao, M. Dai, Z. Liu, G. Chen, K. Meckel, S. Adhikari, G. Jia, M. B. Bissonnette, X. Zhang, Y. Zhao, W. Zhang, C. He and J. Liu, *Cell Res.*, 2017, **27**, 1243–1257.
224 R. Dhat, D. Mongad, S. Raji, S. Arkat, N. R. Mahapatra, N. Singhal and S. L. Sitasawad, *Epigenet. Chromatin*, 2023, **16**, 12.
225 F. Neri, D. Dettori, D. Incarnato, A. Krepelova, S. Rapelli, M. Maldotti, C. Parlato, P. Paliogiannis and S. Oliviero, *Oncogene*, 2015, **34**, 4168–4176.
226 L. Cimmino, M. M. Dawlaty, D. Ndiaye-Lobry, Y. S. Yap, S. Bakogianni, Y. Yu, S. Bhattacharyya, R. Shaknovich, H. Geng, C. Lobry, J. Mullenders, B. King, T. Trimarchi, B. Aranda-Orgilles, C. Liu, S. Shen, A. K. Verma, R. Jaenisch and I. Aifantis, *Nat. Immunol.*, 2015, **16**, 653–662.
227 J. P. Morris, J. J. Yashinskie, R. Koche, R. Chandwani, S. Tian, C. C. Chen, T. Baslan, Z. S. Marinkovic, F. J. Sánchez-Rivera, S. D. Leach, C. Carmona-Fontaine, C. B. Thompson, L. W. S. Finley and S. W. Lowe, *Nature*, 2019, **573**, 595–599.
228 T. Q. Tran, E. A. Hanse, A. N. Habowski, H. Li, M. B. I. Gabra, Y. Yang, X. H. Lowman, A. M. Ooi, S. Y. Liao, R. A. Edwards, M. L. Waterman and M. Kong, *Nat. Cancer*, 2020, **1**, 345–358.
229 T. TeSlaa, A. C. Chaikovsky, I. Lipchina, S. L. Escobar, K. Hochedlinger, J. Huang, T. G. Graeber, D. Braas and M. A. Teitell, *Cell Metab.*, 2016, **24**, 485–493.
230 B. W. Carey, L. W. S. Finley, J. R. Cross, C. D. Allis and C. B. Thompson, *Nature*, 2015, **518**, 413–416.
231 M. I. Matias, C. S. Yong, A. Foroushani, C. Goldsmith, C. Mongellaz, E. Sezgin, K. R. Levental, A. Talebi, J. Perrault, A. Rivière, J. Dehairs, O. Delos, J. Bertand-

Michel, J. C. Portais, M. Wong, J. C. Marie, A. Kelekar, S. Kinet, V. S. Zimmermann, I. Levental, L. Yvan-Charvet, J. V. Swinnen, S. A. Muljo, H. Hernandez-Vargas, S. Tardito, N. Taylor and V. Dardalhon, *Cell Rep.*, 2021, **37**, 109911.
232 S. H. Naeini, L. Mavaddatiyan, Z. R. Kalkhoran, S. Taherkhani and M. Talkhabi, *Exp. Gerontol.*, 2023, **175**, 112154.
233 Y. Yuan, P. Xu, Q. Jiang, X. Cai, T. Wang, W. Peng, J. Sun, C. Zhu, C. Zhang, D. Yue, Z. He, J. Yang, Y. Zeng, M. Du, F. Zhang, L. Ibrahimi, S. Schaul, Y. Jiang, J. Wang, J. Sun, Q. Wang, L. Liu, S. Wang, L. Wang, X. Zhu, P. Gao, Q. Xi, C. Yin, F. Li, G. Xu, Y. Zhang and G. Shu, *EMBO J.*, 2021, **40**, 1–30.
234 B. Campbell, M. Roberts, C. Kerksick, C. Wilborn, B. Marcello, L. Taylor, E. Nassar, B. Leutholtz, R. Bowden, C. Rasmussen, M. Greenwood and R. Kreider, *Nutrition*, 2006, **22**, 872–881.
235 E. D. Son, G. H. Choi, H. Kim, B. Lee, I. S. Chang and J. S. Hwang, *Biol. Pharm. Bull.*, 2007, **30**, 1395–1399.
236 A. Asadi Shahmirzadi, D. Edgar, C. Y. Liao, Y. M. Hsu, M. Lucanic, A. Asadi Shahmirzadi, C. D. Wiley, G. Gan, D. E. Kim, H. G. Kasler, C. Kuehnemann, B. Kaplowitz, D. Bhaumik, R. R. Riley, B. K. Kennedy and G. J. Lithgow, *Cell Metab.*, 2020, **32**, 447–456.e6.
237 R. M. Chin, X. Fu, M. Y. Pai, L. Vergnes, H. Hwang, G. Deng, S. Diep, B. Lomenick, V. S. Meli, G. C. Monsalve, E. Hu, S. A. Whelan, J. X. Wang, G. Jung, G. M. Solis, F. Fazlollahi, C. Kaweeteerawat, A. Quach, M. Nili, A. S. Krall, H. A. Godwin, H. R. Chang, K. F. Faull, F. Guo, M. Jiang, S. A. Trauger, A. Saghatelian, D. Braas, H. R. Christofk, C. F. Clarke, M. A. Teitell, M. Petrascheck, K. Reue, M. E. Jung, A. R. Frand and J. Huang, *Nature*, 2014, **510**, 397–401.
238 X. Cai, Y. Yuan, Z. Liao, K. Xing, C. Zhu, Y. Xu, L. Yu, L. Wang, S. Wang, X. Zhu, P. Gao, Y. Zhang, Q. Jiang, P. Xu and G. Shu, *FASEB J.*, 2018, **32**, 488–499.
239 M. M. Bayliak, M. P. Lylyk, O. M. Vytvytska and V. I. Lushchak, *Eur. Food Res. Technol.*, 2016, **242**, 179–188.
240 S. Liu, L. He and K. Yao, *Biomed. Res. Int.*, 2018, **2018**, 3408467.
241 S. Whillier, B. Garcia, B. E. Chapman, P. W. Kuchel and J. E. Raftos, *FEBS J.*, 2011, **278**, 3152–3163.
242 L. He, J. Wu, W. Tang, X. Zhou, Q. Lin, F. Luo, Y. Yin and T. Li, *J. Agric. Food Chem.*, 2018, **66**, 11273–11283.
243 D. An, Q. Zeng, P. Zhang, Z. Ma, H. Zhang, Z. Liu, J. Li, H. Ren and D. Xu, *Redox Biol.*, 2021, **46**, 1–14.
244 V. Matzi, J. Lindenmann, A. Muench, J. Greilberger, H. Juan, R. Wintersteiger, A. Maier and F. M. Smolle-Juettner, *Eur. J. Cardio-Thoracic Surg.*, 2007, **32**, 776–782.
245 CYL Pharmazeutika GmbH, EP1842536A1, 2007.
246 B. Tugnoli, G. Giovagnoni, A. Piva and E. Grilli, *Animals*, 2020, **10**, 134.
247 K. Lohbeck, H. Haferkorn, W. Fuhrmann and N. Fedtke, *Ullmann's Encyclopedia of Industrial Chemistry*, John Wiley & Sons, Inc., 2000.
248 B. Cornils, P. Lappe and U. Staff, *Ullmann's Encyclopedia of Industrial Chemistry*, John Wiley & Sons, Inc., 2014, pp. 1–18.
249 J. M. Pinazo, M. E. Domine, V. Parvulescu and F. Petru, *Catal. Today*, 2015, **239**, 17–24.
250 W. Riemenschneider and M. Tanifuji, *Ullmann's Encyclopedia of Industrial Chemistry*, John Wiley & Sons, Inc., 2011.
251 E. Schuler, M. Demetriou, N. R. Shiju and G.-J. M. Gruter, *ChemSusChem*, 2021, **14**, 3636–3664.
252 C. L. Mehltretter and C. E. Rist, *J. Agric. Food Chem.*, 1953, **1**, 779–783.
253 E. M. Bottorff and L. L. Moore, *Org. Synth.*, 1964, **44**, 67.
254 L. Friedman and E. Kosower, *Org. Synth.*, 1946, **26**, 42.
255 Maleic anhydride, **https://chem.echa.europa.eu**, (accessed 15 May 2024).
256 Oxalic acid, **https://chem.echa.europa.eu**, (accessed 15 May 2024).
257 Tianjin Tiancheng Pharmaceutical Co., Ltd., US8680329B2, 2014.
258 G. Koenig, E. Lohmar, N. Rupprich, M. Lison and A. Gnass, *Ullmann's Encyclopedia of Industrial Chemistry*, John Wiley & Sons, Inc., 2012.
259 E.-L. Dreher, K. K. Beutel, J. D. Myers, T. Lübbe, S. Krieger and L. H. Pottenger, *Ullmann's Encyclopedia of Industrial Chemistry*, John Wiley & Sons, Inc., 2014, pp. 1–81.
260 T. Ohara, T. Sato, N. Shimizu, G. Prescher, H. Schwind, O. Weiberg, K. Marten, H. Greim, T. D. Shaffer and P. Nandi, *Ullmann's Encyclopedia of Industrial Chemistry*, John Wiley & Sons, Inc., 2020, pp. 1–21.
261 R. J. Mayer, H. Kaur, S. A. Rauscher and J. Moran, *J. Am. Chem. Soc.*, 2021, **143**, 19099–19111.
262 H. Choudhary, S. Nishimura and K. Ebitani, *Appl. Catal., A*, 2013, **458**, 55–62.
263 C.-B. Hong, W. Hua, L. Liu and H. Liu, *Nat. Commun.*, 2025, **16**, 1245.
264 W. Yuan, Y. Du, K. Yu, S. Xu, M. Liu, S. Wang, Y. Yang, Y. Zhang and J. Sun, *Microorganisms*, 2022, **10**(12), DOI: **10.3390/microorganisms10122454**.
265 A. Karpov, C. Walsdorff, M. Siemer, G. Mattioda and A. Blanc, *Ullmann's Encyclopedia of Industrial Chemistry*, 2021, pp. 1–11.
266 H. Li, P. S. Bhadury, A. Riisager and S. Yang, *Catal. Sci. Technol.*, 2014, **4**, 4138–4168.
267 University of Iowa Research Foundation UIRF, US5221621A, 1993.
268 E. M. M. Abdelraheem, H. Busch, U. Hanefeld and F. Tonin, *React. Chem. Eng.*, 2019, **4**, 1878–1894.
269 J. B. Pyser, S. Chakrabarty, E. O. Romero and A. R. H. Narayan, *ACS Cent. Sci.*, 2021, **7**, 1105–1116.
270 B. Beer, A. Pick and V. Sieber, *Metab. Eng.*, 2017, **40**, 5–13.
271 F. Busch, J. Brummund, E. Calderini, M. Schürmann and R. Kourist, *ACS Sustainable Chem. Eng.*, 2020, **8**, 8604–8612.
272 L. Liu, G. Sakir, H. Shin, J. Li, G. Du and J. Chen, *J. Biotechnol.*, 2013, **164**, 97–104.
273 P. Ödman, W. B. Wellborn and A. S. Bommarius, *Tetrahedron: Asymmetry*, 2004, **15**, 2933–2937.
274 B. Beer, A. Pick and V. Sieber, *Metab. Eng.*, 2017, **40**, 5–13.
275 A. Al-Shameri, D. L. Siebert, S. Sutiono, L. Lauterbach and V. Sieber, *Nat. Commun.*, 2023, **14**, 2693.

276 S. Sutiono, A. Pick and V. Sieber, *Green Chem.*, 2021, **23**, 3656–3663.
277 T. Hermann, *J. Biotechnol.*, 2003, **104**, 155–172.
278 J. E. Cronan and D. Laporte, *EcoSal Plus*, 2005, 24.
279 C. Malina, C. Larsson and J. Nielsen, *FEMS Yeast Res.*, 2018, **18**, foy040.
280 H. L. Kornberg and H. A. Krebs, *Nature*, 1957, **179**, 988–991.
281 L. Jimenez-Diaz, A. Caballero and A. Segura, in *Aerobic Utilization of Hydrocarbons, Oils, and Lipids*, ed. K. N. Timmis, M. Boll, O. Geiger, H. Goldfine, T. Krell, S. Y. Lee, J. M. Terry, F. Rojo, D. Z. Sousa, A. J. M. Stams, R. Steffan, H. Wilkes and O. Geiger, Springer, Cham, 2019, pp. 291–325.
282 R. L. Anderson and W. A. Wood, *Annu. Rev. Microbiol.*, 1969, **23**, 539–578.
283 A. Beopoulos, J. M. Nicaud and C. Gaillardin, *Appl. Microbiol. Biotechnol.*, 2011, **90**, 1193–1206.
284 B. H. Kim and G. M. Gadd, *Bacterial Physiology and Metabolism*, Cambridge University Press, 2nd edn, 2019, pp. 85–125.
285 M. Inigo, S. Deja and S. C. Burgess, *Annu. Rev. Nutr.*, 2021, **41**, 19–47.
286 B. Bergdahl, D. Heer, U. Sauer, B. Hahn-hägerdal and E. W. J. Van Niel, *Biotechnol. Biofuels*, 2012, **5**, 1–19.
287 C. Thakker, I. Martínez, K. San and G. N. Bennett, *Biotechnol. J.*, 2012, **7**, 213–224.
288 A. M. Raab and C. Lang, *Bioeng. Bugs*, 2011, **2**, 120–123.
289 K. S. Vuoristo, A. E. Mars, J. P. M. Sanders, G. Eggink and R. A. Weusthuis, *Trends Biotechnol.*, 2016, **34**, 191–197.
290 J. C. Bergmann and L. P. Sallet, *Advances in Sugarcane Biorefinery*, eds. C. Anuj and M. H. Luciano Silveira, Elsevier Inc., 2018, pp. 73–95.
291 L. Steffens, E. Pettinato, T. M. Steiner, A. Mall, S. König, W. Eisenreich and I. A. Berg, *Nature*, 2021, **592**, 784–788.
292 A. Mall, J. Sobotta, C. Huber, C. Tschirner, S. Kowarschik, K. Ba, M. Mergelsberg, M. Boll, M. Hügler, W. Eisenreich and I. A. Berg, *Science*, 2018, **359**(6375), 563–567.
293 P. Mara, G. S. Fragiadakis, F. Gkountromichos and D. Alexandraki, *Microb. Cell Fact.*, 2018, **17**, 1–13.
294 R. B. Helling, *J. Bacteriol.*, 1994, **176**, 4664–4668.
295 L. Reitzer, in *EcoSal Plus*, ed. V. Stewart, American Society for Microbiology, 2004, p. 18.
296 A. A. Alhasawi, S. C. Thomas, T. Sujeethar, F. Legendre and V. D. Appanna, *Front. Microbiol.*, 2019, **10**, 1–13.
297 A. Maclean, S. Tharmalingam and V. D. Appanna, *Antioxidants*, 2022, **11**, 14.
298 R. J. Mailloux, R. Singh, G. Brewer, C. Auger, J. Lemire and V. D. Appanna, *J. Bacteriol.*, 2009, **191**(12), 3804–3810.
299 F. Oliveira, P. C. Engel and A. R. Khan, *FEBS J.*, 2013, **280**, 4681–4692.
300 G. Massad, H. U. I. Zhao and H. L. T. Mobley, *J. Bacteriol.*, 1995, **177**, 5878–5883.
301 H. Drechsel, A. Thieken, R. Reissbrodt, G. Jung, R. K. Institut and O. Chemie, *J. Bacteriol.*, 1993, **175**, 2727–2733.
302 H. Kusakabe, Y. Midorikawa, T. Fujishlma, A. Kuninaka and H. Yoshino, *Agric. Biol. Chem.*, 1983, **47**, 1323–1328.
303 K. N. G. Valdehuesa, K. R. M. Ramos, G. M. Nisola, A. B. Bañares, R. B. Cabulong, W. K. Lee, H. Liu and W. J. Chung, *Appl. Microbiol. Biotechnol.*, 2018, **102**, 7703–7716.
304 C. E. M. Nunn, U. Johnsen, P. Scho, T. Fuhrer, U. Sauer, D. W. Hough and M. J. Danson, *J. Biol. Chem.*, 2010, **285**, 33701–33709.
305 R. Weimberg, *J. Biol. Chem.*, 1961, **236**, 629–635.
306 K. A. K. Köhler, L. M. Blank, O. Frick and A. Schmid, *Environ. Microbiol.*, 2015, **17**, 156–170.
307 C. Stephens, B. Christen, T. Fuchs, V. Sundaram, K. Watanabe and U. Jenal, *J. Bacteriol.*, 2007, **189**, 2181–2185.
308 D. Liu, Y. Zhang, J. Li, W. Sun, Y. Yao and C. Tian, *Biotechnol. Biofuels Bioprod.*, 2023, **16**, 1–16.
309 P. D. Karp, S. Paley, R. Caspi, A. Kothari, M. Krummenacker, P. E. Midford, L. R. Moore, P. Subhraveti, S. Gama-Castro, V. H. Tierrafria, P. Lara, L. Muñiz-Rascado, C. Bonavides-Martinez, A. Santos-Zavaleta, A. Mackie, G. Sun, T. A. Ahn-Horst, H. Choi, M. W. Covert, J. Collado-Vides and I. Paulsen, *EcoSal Plus*, 2023, **11**(1), DOI: **10.1128/ecosalplus.esp-0002-2023eesp-0002-2023**.
310 G. L. Winsor, D. K. W. Lam, L. Fleming, R. Lo, M. D. Whiteside, N. Y. Yu, R. E. W. Hancock and F. S. L. Brinkman, *Nucleic Acids Res.*, 2011, **39**, D596–D600.
311 M. Kanehisa, Y. Sato, M. Kawashima, M. Furumichi and M. Tanabe, *Nucleic Acids Res.*, 2016, **44**, D457–D462.
312 L. Sundermeyer, J.-G. Folkerts, B. Lückel, C. Mack, M. Baumgart and M. Bott, *Microbiol. Spectrosc.*, 2023, **11**, e02668–23.
313 T. Haas, M. Graf, A. Nieß, T. Busche, J. Kalinowski, B. Blombach and R. Takors, *Front. Microbiol.*, 2019, **10**, 1–14.
314 L. Sundermeyer, J.-G. Folkerts, B. Lückel, C. Mack, M. Baumgart and M. Bott, *Microbiol. Spectrosc.*, 2023, **11**, e02668–23.
315 H. Guo, C. Madzak, G. Du, J. Zhou and J. Chen, *Appl. Microbiol. Biotechnol.*, 2014, **98**, 7003–7012.
316 Y. Guo, L. Su, Q. Liu, Y. Zhu, Z. Dai and Q. Wang, *Comput. Struct. Biotechnol. J.*, 2022, **20**, 2503–2511.
317 M. Bussmann, D. Emer, S. Hasenbein, S. Degraf, B. J. Eikmanns and M. Bott, *J. Biotechnol.*, 2009, **143**, 173–182.
318 Z. Jiang, Z. Cui, Z. Zhu, Y. Liu, Y. Jie Tang, J. Hou and Q. Qi, *Biotechnol. Biofuels*, 2021, **14**, 1–10.
319 D. Molenaar, M. E. Van der Rest, A. Drysch and R. Yüchel, *J. Bacteriol.*, 2000, **182**, 6892–6899.
320 P. J. Trotter, K. Juco, H. T. Le, K. Nelson, L. I. Tamayo, J. M. Nicaud and Y. K. Park, *Yeast*, 2020, **37**, 103–115.
321 Q. Liu, X. Ma, H. Cheng, N. Xu, J. Liu and Y. Ma, *Biotechnol. Lett.*, 2017, **39**, 913–919.
322 X. Zhang, N. Xu, J. Li, Z. Ma, L. Wei, Q. Liu and J. Liu, *Enzyme Microb. Technol.*, 2020, **136**, 109530.
323 K. Liu, Y. Liu, X. Li, X. Zhang, Z. Xue and M. Zhao, *Appl. Microbiol. Biotechnol.*, 2023, **107**, 6497–6506.
324 S. Niu, F. Liu, Y. Wang, B. Rao and Y. Wang, *Molecules*, 2024, **29**(8), 1861.
325 G. S. Hossain, J. Li, H. Dong Shin, R. R. Chen, G. Du, L. Liu and J. Chen, *J. Biotechnol.*, 2014, **169**, 112–120.

326 G. S. Hossain, J. Li, H. D. Shin, L. Liu, M. Wang, G. Du and J. Chen, *J. Biotechnol.*, 2014, **187**, 71–77.
327 B. Lin and Y. Tao, *Microb. Cell Fact.*, 2017, **16**, 1–12.
328 UNIV JIANGNAN OP – CN 201410132063 A, 2014.
329 LUOYANG HUARONG BIOTECHNOLOGY CO LTD OP - CN 201410372262 A, 2014.
330 UNIV JIANGNAN OP - CN 201510179796 A, 2015.
331 HENAN JULONG BIO ENG CO LTD OP - CN 201810257243 A, 2018.
332 TIANJIN INST IND BIOTECHNOLOGY CAS OP - CN 201610637736 A, 2018.
333 UNIV JIANGNAN OP - CN 201610363824 A, 2016.
334 UNIV JIANGNAN OP - CN 201310309739 A, 2013.
335 SICHUAN JISHENG BIOPHARMACEUTICAL CO LTD OP - CN 201910030458 A, 2019.
336 INST MICROBIOLOGY CAS OP - CN 201510923517 A, 2017.
337 X. Zhang, N. Xu, J. Li, Z. Ma, L. Wei, Q. Liu and J. Liu, *Enzyme Microb. Technol.*, 2020, **136**, 109530.
338 J. Wu, X. Fan, J. Liu, Q. Luo, J. Xu and X. Chen, *Appl. Microbiol. Biotechnol.*, 2018, **102**, 4755–4764.
339 K. Liu, Y. Liu, X. Li, X. Zhang, Z. Xue and M. Zhao, *Appl. Microbiol. Biotechnol.*, 2023, **107**, 6497–6506.
340 F. Busch, F. Busch, J. Brummund, E. Calderini, M. Schürmann and R. Kourist, *ACS Sustainable Chem. Eng.*, 2020, **8**, 8604–8612.
341 K. Hideo, I. Kazutami and T. Tatsurokuro, US2786799A, 1954.
342 H. J. Koepsell, F. H. Stodola and E. S. Sharpe, US2724680A, 1952.
343 H. J. Koepsell, F. H. Stodola and E. S. Sharpe, *J. Am. Chem. Soc.*, 1952, **74**, 5142–5144.
344 L. B. Lockwood and F. H. Stodola, *J. Biol. Chem.*, 1946, **164**, 81–83.
345 K. A. G. Baritugo, H. T. Kim, Y. C. David, J. H. Choi, J. il Choi, T. W. Kim, C. Park, S. H. Hong, J. G. Na, K. J. Jeong, J. C. Joo and S. J. Park, *Biofuels, Bioprod. Biorefining*, 2018, **12**, 899–925.
346 Tianjin University Science and Technology, CN103194496A, 2013.
347 KH Neochem Co Ltd, US3450599A, 1968.
348 KH Neochem Co Ltd, US3450599A, 1967.
349 H. J. Huang, L. M. Liu, Y. Li, G. C. Du and J. Chen, *Biotechnol. Lett.*, 2006, **28**, 95–98.
350 L. Liu, Y. Li, Y. Zhu, G. Du and J. Chen, *Metab. Eng.*, 2007, **9**, 21–29.
351 D. Zhang, N. Liang, Z. Shi, L. Liu, J. Chen and G. Du, *Biotechnol. Bioprocess Eng.*, 2009, **14**, 134–139.
352 Jiangnan University, CN101153295A, 2007.
353 Qingdao University of Science and Technology, CN104195184A, 2014.
354 O. G. Chernyavskaya, N. V. Shishkanova and T. V. Finogenova, *Appl. Biochem. Microbiol.*, 1997, **33**, 300.
355 Daziran Biological Group Co Ltd, CN114507611A, 2022.
356 Z. D. Blount, *eLife*, 2015, **4**, 1–12.
357 F. R. Blattner, G. Plunkett, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau and Y. Shao, *Science*, 1997, **277**, 1453–1462.
358 M. H. Serres, S. Gopal, L. A. Nahum, P. Liang and M. Riley, *Genome Biol.*, 2001, **2**, 1–7.
359 E. Tantoso, B. Eisenhaber, S. Sinha, L. J. Jensen and F. Eisenhaber, *Biol. Direct*, 2023, **18**, 1–17.
360 S. Pontrelli, T. Y. Chiu, E. I. Lan, F. Y. H. Chen, P. Chang and J. C. Liao, *Metab. Eng.*, 2018, **50**, 16–46.
361 S. Ghatak, Z. A. King, A. Sastry and B. O. Palsson, *Nucleic Acids Res.*, 2019, **47**, 2446–2454.
362 J. Jeong, N. Cho, D. Jung and D. Bang, *Biotechnol. Adv.*, 2013, **31**, 804–810.
363 D. Yang, C. P. S. Prabowo, H. Eun, S. Y. Park, I. J. Cho, S. Jiao and S. Y. Lee, *Essays Biochem.*, 2021, **65**, 225–246.
364 C. Zhao, Y. Zhang and Y. Li, *Biotechnol. Adv.*, 2019, **37**, 107402.
365 C. Wang, B. F. Pfleger and S. W. Kim, *Curr. Opin. Biotechnol*, 2017, **45**, 92–103.
366 X. Chen, L. Zhou, K. Tian, A. Kumar, S. Singh, B. A. Prior and Z. Wang, *Biotechnol. Adv.*, 2013, **31**, 1200–1223.
367 T. N. B. T. Ibrahim, A. Bin Abas and N. F. A. Razak, in *Biomanufacturing for Sustainable Production of Biomolecules*, ed. V. Singh and P. Loke, Springer Nature, Singapore, 2023, pp. 141–163.
368 J. Yang and S. Yang, *BMC Genomics*, 2017, **18**, 940.
369 J. Kalinowski, in *Handbook of Corynebacterium glutamicum*, ed. L. Eggeling and M. Bott, CRC Press, Boca Raton, 1st edn, 2005, pp. 37–56.
370 J. Becker and C. Wittmann, in *Industrial Biotechnology: Microorganisms*, ed. C. Wittmann and J. C. Liao, Wiley-VCH Verlag GmbH & Co, 1st edn, 2017, pp. 183–219.
371 L. Eggeling and M. Bott, *Appl. Microbiol. Biotechnol.*, 2015, **99**, 3387–3394.
372 D. Ray, U. Anand, N. K. Jha, E. Korzeniewska, E. Bontempi, J. Proćków and A. Dey, *Environ. Res.*, 2022, **213**, 113622.
373 N. Stäbler, T. Oikawa, M. Bott and L. Eggeling, *J. Bacteriol.*, 2011, **193**, 1702–1709.
374 J. Becker, C. M. Rohles and C. Wittmann, *Metab. Eng.*, 2018, **50**, 122–141.
375 M. Merkel, D. Kiefer, M. Schmollack, B. Blombach, L. Lilge, M. Henkel and R. Hausmann, *Bioresour. Technol.*, 2022, **351**, 126994.
376 T. Han, G. B. Kim and S. Y. Lee, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 30328–30334.
377 X. Y. Wu, X. Y. Guo, B. Zhang, Y. Jiang and B. C. Ye, *Front. Bioeng. Biotechnol.*, 2020, **7**, 440.
378 L. Feng, Y. Zhang, J. Fu, Y. Mao, T. Chen, X. Zhao and Z. Wang, *Biotechnol. Bioeng.*, 2016, **113**, 1284–1293.
379 T. Kogure and M. Inui, *Appl. Microbiol. Biotechnol.*, 2018, **102**, 8685–8705.
380 H. N. Lee, W. S. Shin, S. Y. Seo, S. S. Choi, J. Soo Song, J. Yeon Kim, J. H. Park, D. Lee, S. Y. Kim, S. J. Lee, G. T. Chun and E. S. Kim, *Sci. Rep.*, 2018, **8**, 1–12.
381 V. F. Wendisch, L. F. Brito, M. Gil Lopez, G. Hennig, J. Pfeifenschneider, E. Sgobba and K. H. Veldmann, *J. Biotechnol.*, 2016, **234**, 139–157.

382 H. H. Liu, X. J. Ji and H. Huang, *Biotechnol. Adv.*, 2015, **33**, 1522–1546.
383 K. K. Miller and H. S. Alper, *Appl. Microbiol. Biotechnol.*, 2019, **103**, 9251–9262.
384 M. Workman, P. Holt and J. Thykaer, *AMB Express*, 2013, **3**, 1–9.
385 A. Aurich, R. Specht, R. A. Muller, U. Stottmeister, V. Yovkova, C. Otto, M. Holz, G. Barth, P. Heretsch, F. A. Thomas, D. Sicker and A. Giannis, *Monitoring microbial diversity of bioreactors using metagenomic approaches*, 2012, pp. 391–423.
386 M. Gatter, S. Ottlik, Z. Kövesi, B. Bauer, F. Matthäus and G. Barth, *Fungal Genet. Biol.*, 2016, **95**, 30–38.
387 IFP Energies Nouvelles IFPEN, US3930946A, 1972.
388 O. G. Chernyavskaya, N. V. Shishkanova, A. P. Il'chenko and T. V. Finogenova, *Appl. Microbiol. Biotechnol.*, 2000, **53**, 152–158.
389 Ajinomoto Co Inc, US3616213A, 1969.
390 S. V. Kamzolova and I. G. Morgunov, *Appl. Microbiol. Biotechnol.*, 2020, **104**, 7979–7989.
391 J. Zhou, H. Zhou, G. Du, L. Liu and J. Chen, *Lett. Appl. Microbiol.*, 2010, **51**, 264–271.
392 X. Yin, C. Madzak, G. Du, J. Zhou and J. Chen, *Appl. Microbiol. Biotechnol.*, 2012, **96**, 1527–1537.
393 L. Tomaszewska-Hetman, A. Rywińska, Z. Lazar and W. Rymowicz, *Catalysts*, 2023, **13**, 14.
394 A. Rywińska, L. Tomaszewska-Hetman, M. Rakicka-Pustułka, P. Juszczyk and W. Rymowicz, *Sustainability*, 2020, **12**, 6109.
395 S. Ogata and T. Hirasawa, *Appl. Microbiol. Biotechnol.*, 2021, **105**, 6909–6920.
396 E. Kimura, *J. Biosci. Bioeng.*, 2002, **94**, 545–551.
397 T. Hasegawa, K. I. Hashimoto, H. Kawasaki and T. Nakamatsu, *J. Biosci. Bioeng.*, 2008, **105**, 12–19.
398 E. Kimura, C. Yagoshi, Y. Kawahara, T. Ohsumi, T. Nakamatsu and H. Tokuda, *Biosci., Biotechnol., Biochem.*, 1999, **63**, 1274–1278.
399 Y. Li, L. Sun, J. Feng, R. Wu, Q. Xu, C. Zhang, N. Chen and X. Xie, *Bioprocess Biosyst. Eng.*, 2016, **39**, 967–976.
400 T. Hirasawa, M. Saito, K. Yoshikawa, C. Furusawa and H. Shmizu, *Biotechnol. J.*, 2018, **13**, 1700612.
401 Jiangsu Intelligent Workshop Technology Research Institute Co Ltd, CN102391977A, 2011.
402 Guangdong Huanxi Biotechnology Co Ltd, CN101250563B, 2008.
403 Y. Nakayama, K. Ichi Hashimoto, Y. Sawada, M. Sokabe, H. Kawasaki and B. Martinac, *Biophys. Rev.*, 2018, **10**, 1359–1369.
404 L. Tomaszewska-Hetman, A. Rywińska, Z. Lazar, P. Juszczyk, M. Rakicka-Pustułka, T. Janek, M. Kuźmińska-Bajor and W. Rymowicz, *Int. J. Mol. Sci.*, 2021, **22**, 7577.
405 K. R. Pomraning, Y. M. Kim, C. D. Nicora, R. K. Chu, E. L. Bredeweg, S. O. Purvine, D. Hu, T. O. Metz and S. E. Baker, *BMC Genomics*, 2016, **17**, 1–18.
406 Y. B. Lee, J. H. Jo, M. H. Kim, H. H. Lee and H. H. Hyun, *Biotechnol. Bioprocess Eng.*, 2013, **18**, 770–777.
407 W. C. van Heeswijk, H. V. Westerhoff and F. C. Boogerd, *Microbiol. Mol. Biol. Rev.*, 2013, **77**, 628–695.
408 Evonik Degussa Gmbh, WO2009053489A1, 2008.
409 M. Tesch, A. A. De Graaf and H. Sahm, *Appl. Environ. Microbiol.*, 1999, **65**, 1099–1109.
410 X. Chen, X. Dong, J. Liu, Q. Luo and L. Liu, *Biotechnol. Bioeng.*, 2020, **117**, 2791–2801.
411 N. Tenhaef, J. Kappelmann, A. Eich, M. Weiske, L. Brieß, C. Brüsseler, J. Marienhagen, W. Wiechert and S. Noack, *Biotechnol. J.*, 2021, **16**, 2100043.
412 R. Tsugawa and S. Okumura, *Agric. Biol. Chem.*, 1969, **33**, 676–682.
413 Akad Wissenschaften Ddr, DD267999B5, 1988.
414 J. Zhou, X. Yin, C. Madzak, G. Du and J. Chen, *J. Biotechnol.*, 2012, **161**, 257–264.
415 V. Yovkova, C. Otto, A. Aurich, S. Mauersberger and G. Barth, *Appl. Microbiol. Biotechnol.*, 2014, **98**, 2003–2013.
416 H. Guo, P. Liu, C. Madzak, G. Du, J. Zhou and J. Chen, *Sci. Rep.*, 2015, **5**, 1–10.
417 W. Zeng, G. Du, J. Chen, J. Li and J. Zhou, *Process Biochem.*, 2015, **50**, 1516–1522.
418 Y. Chen, F. Li, J. Mao, Y. Chen and J. Nielsen, *Proc. Natl. Acad. Sci. U. S. A.*, 2021, **118**, e2020154118.
419 M. S. Cyert and C. C. Philpott, *Genetics*, 2013, **193**, 677–713.
420 S. V. Kamzolova, M. N. Chiglintseva, J. N. Lunina and I. G. Morgunov, *Appl. Microbiol. Biotechnol.*, 2012, **96**, 783–791.
421 I. G. Morgunov, S. V. Kamzolova and V. A. Samoilenko, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 8711–8718.
422 Z. Yu, G. Du, J. Zhou and J. Chen, *Bioresour. Technol.*, 2012, **114**, 597–602.
423 K. Cybulski, L. Tomaszewska-Hetman, M. Rakicka, W. Łaba, W. Rymowicz and A. Rywińska, *Ind. Crops Prod.*, 2018, **119**, 102–110.
424 S. V. Kamzolova and I. G. Morgunov, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 5517–5525.
425 H. Dominguez, C. Nezondet, N. D. Lindley and M. Cocaign, *Biotechnol. Lett.*, 1993, **15**, 449–454.
426 F. Käß, S. Junne, P. Neubauer, W. Wiechert and M. Oldiges, *Microb. Cell Fact.*, 2014, **13**, 6.
427 A. Lemoine, N. Maya Martnez-Iturralde, R. Spann, P. Neubauer and S. Junne, *Biotechnol. Bioeng.*, 2015, **112**, 1220–1231.
428 H. Xu, W. Dou, H. Xu, X. Zhang, Z. Rao, Z. Shi and Z. Xu, *Biochem. Eng. J.*, 2009, **43**, 41–51.
429 M. Inui, M. Suda, S. Okino, H. Nonaka, L. G. Puskás, A. A. Vertès and H. Yukawa, *Microbiology*, 2007, **153**, 2491–2504.
430 A. R. Lara, H. Taymaz-Nikerel, M. R. Mashego, W. M. Van Gulik, J. J. Heijnen, O. T. Ramírez and W. A. Van Winden, *Biotechnol. Bioeng.*, 2009, **104**, 1153–1161.
431 A. N. Brown, M. T. Anderson, M. A. Bachman and H. L. T. Mobley, *Microbiol. Mol. Biol. Rev.*, 2022, **86**, e00110–21.
432 J. G. Koendjbiharie, R. Van Kranenburg and M. Kengen, *FEMS Microbiol. Rev.*, 2021, 1–19.
433 U. Sauer and B. J. Eikmanns, *FEMS Microbiol. Lett.*, 2005, **29**, 765–794.

434 G. Wiegand and S. J. Remington, *Annu. Rev. Biophys. Biophys. Chem.*, 1986, **15**, 97–117.
435 X. Zhang, X. Wang, K. T. Shanmugam and L. O. Ingram, *Appl. Environ. Microbiol.*, 2011, **77**, 427–434.
436 Y. Asakura, E. Kimura, Y. Usuda, Y. Kawahara, K. Matsui, T. Osumi and T. Nakamatsu, *Appl. Environ. Microbiol.*, 2007, **73**, 1308–1319.
437 Y. Wang, L. Fan, P. Tuyishime, J. Liu, K. Zhang, N. Gao, Z. Zhang, X. Ni, J. Feng, Q. Yuan, H. Ma, P. Zheng, J. Sun and Y. Ma, *Commun. Biol.*, 2020, **3**, 217.
438 P. Tuyishime, Y. Wang, L. Fan, Q. Zhang, Q. Li, P. Zheng, J. Sun and Y. Ma, *Metab. Eng.*, 2018, **49**, 220–231.
439 N. Okai, C. Takahashi, K. Hatada, C. Ogino and A. Kondo, *AMB Express*, 2014, **4**, 1–8.
440 N. Wang, Y. Ni and F. Shi, *Biotechnol. Lett.*, 2015, **37**, 1473–1481.
441 A. Zhao, X. Hu and X. Wang, *Appl. Microbiol. Biotechnol.*, 2017, **101**, 3587–3603.
442 L. Wei, J. Zhao, Y. Wang, J. Gao, M. Du, Y. Zhang, N. Xu, H. Du, J. Ju, Q. Liu and J. Liu, *Metab. Eng.*, 2022, **69**, 134–146.
443 J. Wang, X. Shen, Y. Lin, Z. Chen, Y. Yang, Q. Yuan and Y. Yan, *ACS Synth. Biol.*, 2018, **7**, 24–29.
444 J. Wang, J. Wang, Y. Shu Tai, Q. Zhang, W. Bai and K. Zhang, *Appl. Microbiol. Biotechnol.*, 2018, **102**, 7377–7388.
445 W. Bai, Y. S. Tai, J. Wang, J. Wang, P. Jambunathan, K. J. Fox and K. Zhang, *Metab. Eng.*, 2016, **38**, 285–292.
446 Z. Man, M. Xu, Z. Rao, J. Guo, T. Yang, X. Zhang and Z. Xu, *Sci. Rep.*, 2016, **6**, 1–10.
447 S. H. Park, H. U. Kim, T. Y. Kim, J. S. Park, S. S. Kim and S. Y. Lee, *Nat. Commun.*, 2014, **5**, 4618.
448 S. Y. Kim, J. Lee and S. Y. Lee, *Biotechnol. Bioeng.*, 2015, **112**, 416–421.
449 B. Zhang, M. Yu, Y. Zhou, Y. Li and B. C. Ye, *Microb. Cell Fact.*, 2017, **16**, 158.
450 B. Zhang, M. Yu, W. P. Wei and B. C. Ye, *Microb. Cell Fact.*, 2018, **17**, 91.
451 B. Zhang, G. Gao, X. H. Chu and B. C. Ye, *Bioresour. Technol.*, 2019, **284**, 204–213.
452 Y. S. Tai, M. Xiong, P. Jambunathan, J. Wang, J. Wang, C. Stapleton and K. Zhang, *Nat. Chem. Biol.*, 2016, **12**, 247–253.
453 A. Alva, A. Sabido-Ramos, A. Escalante and F. Bolívar, *Appl. Microbiol. Biotechnol.*, 2020, **104**, 1463–1479.
454 K. Jahreis, E. F. Pimentel-Schmitt, R. Brückner and F. Titgemeyer, *FEMS Microbiol. Rev.*, 2008, **32**, 891–907.
455 A. Escalante, A. S. Cervantes, G. Gosset and F. Bolívar, *Appl. Microbiol. Biotechnol.*, 2012, **94**, 1483–1494.
456 R. Chatterjee, C. S. Millard, K. Champion, D. P. Clark and M. I. Donnelly, *Appl. Environ. Microbiol.*, 2001, **67**, 148–154.
457 D. Wang, Q. Li, Z. Song, W. Zhou, Z. Su and J. Xing, *J. Chem. Technol. Biotechnol.*, 2011, **86**, 512–518.
458 H. Lin, G. N. Bennett and K. Y. San, *Metab. Eng.*, 2005, **7**, 116–127.
459 J. Z. Xu, H. B. Yu, M. Han, L. M. Liu and W. G. Zhang, *J. Ind. Microbiol. Biotechnol.*, 2019, **46**, 937–949.
460 S. C. Chung, J. S. Park, J. Yun and J. H. Park, *Metab. Eng.*, 2017, **40**, 157–164.
461 Z. Zhou, C. Wang, H. Xu, Z. Chen and H. Cai, *J. Ind. Microbiol. Biotechnol.*, 2015, **42**, 1073–1082.
462 X. Zhang, K. Jantama, K. T. Shanmugam and L. O. Ingram, *Appl. Environ. Microbiol.*, 2009, **75**, 7807–7813.
463 X. Zhang, K. Jantama, J. C. Moore, L. R. Jarboea, K. T. Shanmugam and L. O. Ingram, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**(48), 20180–20185.
464 Q. Liang, F. Zhang, Y. Li, X. Zhang, J. Li, P. Yang and Q. Qi, *Sci. Rep.*, 2015, **5**, 1–10.
465 L. F. Huergo and R. Dixon, *Microbiol. Mol. Biol. Rev.*, 2015, **79**, 419–435.
466 V. Chubukov, J. J. Desmarais, G. Wang, L. J. G. Chan, E. E. K. Baidoo, C. J. Petzold, J. D. Keasling and A. Mukhopadhyay, *npj Syst. Biol. Appl.*, 2017, **3**, 16035.
467 J. Tang, X. Zhu, J. Lu, P. Liu, H. Xu, Z. Tan and X. Zhang, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 2513–2520.
468 J. Meng, B. Wang, D. Liu, T. Chen, Z. Wang and X. Zhao, *Microb. Cell Fact.*, 2016, **15**, 141.
469 H. J. Kim, H. Jeong and S. J. Lee, *Front. Microbiol.*, 2020, **11**, 27.
470 X. Zhu, Z. Tan, H. Xu, J. Chen, J. Tang and X. Zhang, *Metab. Eng.*, 2014, **24**, 87–96.
471 M. Long, M. Xu, Z. Ma, X. Pan, J. You, M. Hu, Y. Shao, T. Yang, X. Zhang and Z. Rao, *Sci. Adv.*, 2020, **6**, 1–11.
472 K. Jantama, X. Zhang, J. C. Moore, K. T. Shanmugam, S. A. Svoronos and L. O. Ingram, *Biotechnol. Bioeng.*, 2008, **101**, 881–893.
473 K. Jantama, M. J. Haupt, S. A. Svoronos, X. Zhang, J. C. Moore, K. T. Shanmugam and L. O. Ingram, *Biotechnol. Bioeng.*, 2008, **99**, 1140–1153.
474 X. Zhang, K. T. Shanmugam and L. O. Ingram, *Appl. Environ. Microbiol.*, 2010, **76**, 2397–2401.
475 Z. Tan, J. Chen and X. Zhang, *Biotechnol. Biofuels*, 2016, **9**, 1–13.
476 W. Zhang, T. Zhang, M. Song, Z. Dai, S. Zhang, F. Xin, W. Dong, J. Ma and M. Jiang, *ACS Synth. Biol.*, 2018, **7**, 2803–2811.
477 F. Guo, M. Wu, S. Zhang, Y. Feng, Y. Jiang, W. Jiang and F. Xin, *Bioresour. Bioprocess.*, 2022, **9**, 13.
478 B. Litsanov, M. Brocker and M. Bott, *Appl. Environ. Microbiol.*, 2012, **78**, 3325–3337.
479 B. Litsanov, A. Kabus, M. Brocker and M. Bott, *Microb. Biotechnol.*, 2012, **5**, 116–128.
480 Y. Mao, G. Li, Z. Chang, R. Tao, Z. Cui, Z. Wang, Y. J. Tang, T. Chen and X. Zhao, *Biotechnol. Biofuels*, 2018, **11**, 1–17.
481 Z. Cui, C. Gao, J. Li, J. Hou, C. S. K. Lin and Q. Qi, *Metab. Eng.*, 2017, **42**, 126–133.
482 Q. Yu, Z. Cui, Y. Zheng, H. Huo, L. Meng, J. Xu and C. Gao, *Biochem. Eng. J.*, 2018, **139**, 51–56.
483 C. Otto, V. Yovkova, A. Aurich, S. Mauersberger and G. Barth, *Appl. Microbiol. Biotechnol.*, 2012, **95**, 905–917.
484 C. Zhang, Y. Li, J. Ma, Y. Liu, J. He, Y. Li, F. Zhu, J. Meng, J. Zhan, Z. Li, L. Zhao, Q. Ma, X. Fan, Q. Xu, X. Xie and N. Chen, *Metab. Eng.*, 2018, **49**, 287–298.

485 E. Theodosiou, M. Breisch, M. K. Julsing, F. Falcioni, B. Bühler and A. Schmid, *Biotechnol. Bioeng.*, 2017, **114**, 1511–1520.
486 J. Meng, B. Wang, D. Liu, T. Chen, Z. Wang and X. Zhao, *Microb. Cell Fact.*, 2016, **15**, 141.
487 J. da Veiga Moreira, M. Jolicoeur, L. Schwartz and S. Peres, *Sci. Rep.*, 2021, **11**, 1–11.
488 K. Thongbhubate, K. Irie, Y. Sakai, A. Itoh and H. Suzuki, *AMB Express*, 2021, **11**, 168.
489 Z. Zhang, P. Liu, W. Su, H. Zhang, W. Xu and X. Chu, *Microb. Cell Fact.*, 2021, **20**, 1–15.
490 M. Hoffelder, K. Raasch, J. Van Ooyen and L. Eggeling, *J. Bacteriol.*, 2010, **192**, 5203–5211.
491 C. A. Steginsky, K. J. Gruys and P. A. Frey, *J. Biol. Chem.*, 1985, **260**, 13690–13693.
492 M. Li, P. Y. Ho, S. Yao and K. Shimizu, *Biochem. Eng. J.*, 2006, **30**, 286–296.
493 T. V. Yuzbashev, E. Y. Yuzbasheva, T. I. Sobolevskaya, I. A. Laptev, T. V. Vybornaya, A. S. Larina, K. Matsui, K. Fukui and S. P. Sineoky, *Biotechnol. Bioeng.*, 2010, **107**, 673–682.
494 H. Guo, C. Madzak, G. Du and J. Zhou, *Appl. Microbiol. Biotechnol.*, 2016, **100**, 649–659.
495 A. Niebisch, A. Kabus, C. Schultz, B. Weil and M. Bott, *J. Biol. Chem.*, 2006, **281**, 12300–12307.
496 M. Bott, *Trends Microbiol.*, 2007, **15**, 417–425.
497 L. Sundermeyer, G. Bosco, S. Gujar, M. Brocker, M. Baumgart, D. Willbold, O. H. Weiergräber, M. Bellinzoni and M. Bott, *Microbiol. Spectrosc.*, 2022, **10**, e02677–22.
498 A. Q. D. Nguyen, J. Schneider, G. K. Reddy and V. F. Wendisch, *Metabolites*, 2015, **5**, 211–231.
499 E. R. B. E. Kholy, B. J. Eikmanns, M. Gutmann and H. Sahm, *Appl. Environ. Microbiol.*, 1993, **59**, 2329–2331.
500 G. Beckers, L. Nolden and A. Burkovski, *Microbiology*, 2001, **147**, 2961–2970.
501 A. Michel, A. Koch-Koerfges, K. Krumbach, M. Brocker and M. Bott, *Appl. Environ. Microbiol.*, 2015, **81**, 7496–7508.
502 S. Okino, M. Inui and H. Yukawa, *Appl. Microbiol. Biotechnol.*, 2005, **68**, 475–480.
503 D. P. Clark, *FEMS Microbiol. Lett.*, 1989, **63**, 223–234.
504 N. Zhu, H. Xia, Z. Wang, X. Zhao and T. Chen, *PLoS One*, 2013, **8**, e60659.
505 L. Jiang, J. Pang, L. Yang, W. Li, L. Duan, G. Zhang and Y. Luo, *J. Biotechnol.*, 2021, **329**, 104–117.
506 S. Okino, R. Noburyu, M. Suda, T. Jojima, M. Inui and H. Yukawa, *Appl. Microbiol. Biotechnol.*, 2008, **81**, 459–464.
507 Y. Mao, G. Li, Z. Chang, R. Tao, Z. Cui, Z. Wang, Y. J. Tang, T. Chen and X. Zhao, *Biotechnol. Biofuels*, 2018, **11**, 1–17.
508 H. Lin, G. N. Bennett and K. Y. San, *Biotechnol. Bioeng.*, 2005, **89**, 148–156.
509 A. M. Sánchez, G. N. Bennett and K. Y. San, *Metab. Eng.*, 2006, **8**, 209–226.
510 Z. Cui, C. Gao, J. Li, J. Hou, C. S. K. Lin and Q. Qi, *Metab. Eng.*, 2017, **42**, 126–133.
511 M. S. Ahmed, K. J. Lauersen, S. Ikram and C. Li, *ACS Synth. Biol.*, 2021, **10**, 646–669.
512 Z. Jiang, Z. Cui, Z. Zhu, Y. Liu, Y. Jie Tang, J. Hou and Q. Qi, *Biotechnol. Biofuels*, 2021, **14**, 1–10.
513 S. K. Mohanty and M. R. Swain, in *Bioethanol Production from Food Crops*, ed. R. C. Ray and S. Ramachandran, Elsevier Inc., 2019, pp. 45–59.
514 R. C. Ray, K. B. Uppuluri, C. Trilokesh and C. Lareo, in *Bioethanol Production from Food Crops*, ed. R. C. Ray and S. Ramachandran, Elsevier Inc., 2019, pp. 81–100.
515 C. Laluce, G. R. Leite, B. Z. Zavitoski, T. T. Zamai and R. Ventura, *Sugarcane-based Biofuels and Bioproducts*, 2016, pp. 53–86.
516 C. Marzo, A. B. Díaz and I. Caro, in *Bioethanol Production from Food Crops*, ed. R. C. Ray and S. Ramachandran, Elsevier Inc., 2019, pp. 61–79.
517 J. Valentine, J. Clifton-Brown, A. Hastings, P. Robson, G. Allison and P. Smith, *GCB Bioenergy*, 2012, **4**, 1–19.
518 T. Arpit Singh, M. Sharma, M. Sharma, G. Dutt Sharma, A. Kumar Passari and S. Bhasin, *Fuel*, 2022, **322**, 124284.
519 T. Raj, K. Chandrasekhar, R. Morya, A. Kumar Pandey, J. H. Jung, D. Kumar, R. R. Singhania and S. H. Kim, *Bioresour. Technol.*, 2022, **360**, 127512.
520 S. Periyasamy, J. Beula Isabel, S. Kavitha, V. Karthik, B. A. Mohamed, D. G. Gizaw, P. Sivashanmugam and T. M. Aminabhavi, *Chem. Eng. J.*, 2023, **453**, 139783.
521 S. Rezania, B. Oryani, J. Cho, A. Talaiekhozani, F. Sabbagh, B. Hashemi, P. F. Rupani and A. A. Mohammadi, *Energy*, 2020, **199**, 117457.
522 L. R. Kumar, S. K. Yellapu, R. D. Tyagi and X. Zhang, *Bioresour. Technol.*, 2019, **293**, 122155.
523 N. Zhao, L. Qian, G. Luo and S. Zheng, *Appl. Microbiol. Biotechnol.*, 2018, **102**, 9517–9529.
524 R. Ledesma-Amaro and J. M. Nicaud, *Trends Biotechnol.*, 2016, **34**, 798–809.
525 C. Zhang, C. Ottenheim, M. Weingarten and L. H. Ji, *Front. Bioeng. Biotechnol.*, 2022, **10**, 1–22.
526 F. D. Rossinil, *J. Chem. Educ.*, 1960, **37**, 554–561.
527 J. Son, S. H. Lim, Y. J. Kim, H. J. Lim, J. Y. Lee, S. Jeong, C. Park and S. J. Park, *Bioresour. Technol.*, 2023, **371**, 128607.
528 M. Ganesan, R. Mani, S. Sai, G. Kasivelu, M. K. Awasthi, R. Rajagopal, N. I. Wan Azelee, P. K. Selvi, S. W. Chang and B. Ravindran, *Chemosphere*, 2022, **303**, 134956.
529 R. Fukuda and A. Ohta, *Aerobic Utilization of Hydrocarbons, Oils and Lipids*, 2017, pp. 1–14.
530 P. Fickers, Y. Waché, A. Marty, S. Mauersberger, M. S. Smit, P.-H. Benetti and J.-M. Nicaud, *FEMS Yeast Res.*, 2005, **5**, 527–543.
531 R. Fukuda, *World J. Microbiol. Biotechnol.*, 2023, **39**, 1–11.
532 J. Van Waeyenberg, K. Vikanova, B. Smeyers, T. Van Vaerenbergh, M. Aerts, Z. Zhang, S. Sivanandan, H. Van Leuven, X. Wu and B. Sels, *ACS Sustainable Chem. Eng.*, 2024, **12**, 11074–11092.
533 P. Mishra, N. R. Lee, M. Lakshmanan, M. Kim, B. G. Kim and D. Y. Lee, *BMC Syst. Biol.*, 2018, **12**, 12.
534 M. Klein, S. Swinnen, J. M. Thevelein and E. Nevoigt, *Environ. Microbiol.*, 2017, **19**, 878–893.

535 A. M. Erian, M. Egermeier, H. Marx and M. Sauer, *Yeast*, 2022, **39**, 323–336.
536 P. Hapeta, P. Szczepańska, C. Neuvéglise and Z. Lazar, *Sci. Rep.*, 2021, 1–14.
537 G. Durnin, J. Clomburg, Z. Yeates, P. J. J. Alvarez, K. Zygourakis, P. Campbell and R. Gonzalez, *Biotechnol. Bioeng.*, 2009, **103**, 148–161.
538 M. D. Blankschien, J. M. Clomburg and R. Gonzalez, *Metab. Eng.*, 2010, **12**, 409–419.
539 K. B. Andersen and K. V. O. N. Meyenburg, *J. Bacteriol.*, 1980, **144**, 114–123.
540 K. Kim, C. Y. Hou, D. Choe, M. Kang, S. Cho, B. H. Sung, D. H. Lee, S. G. Lee, T. J. Kang and B. K. Cho, *Metab. Eng.*, 2022, **69**, 59–72.
541 D. Rittmann, S. N. Lindner and V. F. Wendisch, *Appl. Environ. Microbiol.*, 2008, **74**, 6216–6222.
542 T. M. Meiswinkel, D. Rittmann, S. N. Lindner and V. F. Wendisch, *Bioresour. Technol.*, 2013, **145**, 254–258.
543 A. P. Il'chenko, O. G. Cherniavskaia, N. V. Shishkanova and T. V. Finogenova, *Mikrobiologiia*, 2002, **71**, 316–322.
544 E. Delikonstantis, E. Igos, S. A. Theofanidis, E. Benetto, G. B. Marin, K. Van Geem and G. D. Stefanidis, *Green Chem.*, 2021, **23**, 7243–7258.
545 A. Gęsicka, P. Oleskowicz-Popiel and M. Łężyk, *Biotechnol. Adv.*, 2021, **53**, 107861.
546 V. Wegat, J. T. Fabarius and V. Sieber, *Biotechnol. Biofuels Bioprod.*, 2022, **15**, 1–19.
547 A. Thorenz, L. Wietschel, D. Stindt and A. Tuma, *J. Cleaner Prod.*, 2018, **176**, 348–359.
548 J. W. Choi, E. J. Jeon and K. J. Jeong, *Curr. Opin. Biotechnol*, 2019, **57**, 17–24.
549 B. Blombach and G. M. Seibold, *Appl. Microbiol. Biotechnol.*, 2010, **86**, 1313–1322.
550 S. Ryu, J. Hipp and C. T. Trinh, *Appl. Environ. Microbiol.*, 2016, **52**, 621–631.
551 S. Ryu and C. T. Trinh, *Appl. Environ. Microbiol.*, 2018, **84**, e02146–17.
552 G. A. Sprenger, *Arch. Microbiol.*, 1995, **164**, 324–330.
553 A. Masi, R. L. Mach and A. R. Mach-Aigner, *Appl. Microbiol. Biotechnol.*, 2021, **105**, 4017–4031.
554 J. M. Francois, C. Alkim and N. Morin, *Biotechnol. Biofuels*, 2020, **13**, 118.
555 T. M. Meiswinkel, V. Gopinath, S. N. Lindner, K. M. Nampoothiri and V. F. Wendisch, *Microb. Biotechnol.*, 2013, **6**, 131–140.
556 H. Kawaguchi, M. Sasaki, A. A. Vertès, M. Inui and H. Yukawa, *Appl. Environ. Microbiol.*, 2009, **75**, 3419–3429.
557 H. Kawaguchi, M. Sasaki, A. A. Vertès, M. Inui and H. Yukawa, *Appl. Microbiol. Biotechnol.*, 2008, **77**, 1053–1062.
558 J. Schneider, K. Niermann and V. F. Wendisch, *J. Biotechnol.*, 2011, **154**, 191–198.
559 J. Kim, S. Hwang and S. Lee, *Metab. Eng.*, 2022, **71**, 2–12.
560 S. A. Dahms, *Biochem. Biophys. Res. Commun.*, 1974, **60**, 1433–1439.
561 L. Shen, M. Kohlhaas, J. Enoki, R. Meier, B. Schönenberger, R. Wohlgemuth, R. Kourist, F. Niemeyer, D. van Niekerk, C. Bräsen, J. Niemeyer, J. Snoep and B. Siebers, *Nat. Commun.*, 2020, **11**, 1098.
562 A. Nair and S. J. Sarma, *Microbiol. Res.*, 2021, **251**, 9.
563 J. M. Gancedo, *Microbiol. Mol. Biol. Rev.*, 1998, **62**, 334–361.
564 B. Görke and J. Stülke, *Nat. Rev. Microbiol.*, 2008, **6**, 613–624.
565 V. Narisetty, R. Cox, R. Bommareddy and D. Agrawal, *Sustainable Energy Fuels*, 2022, **6**, 29–65.
566 Y.-S. Tai, M. Xiong, P. Jambunathan, J. Wang, J. Wang, C. Stapleton and K. Zhang, *Nat. Chem. Biol.*, 2016, **12**, 247–253.
567 K. W. Lu, C. T. Wang, H. Chang, R. S. Wang and C. R. Shen, *Metab. Eng. Commun.*, 2021, **13**, 9.
568 C. Brüsseler, A. Sp, S. Sokolowsky and J. Marienhagen, *Metab. Eng. Commun.*, 2019, **9**, 1–7.
569 K. Li, C. Li, X. Zhao, C. Liu and F. Bai, *Bioresour. Technol.*, 2023, **378**, 128991.
570 B. Halmschlag, K. Hoffmann, R. Hanke, S. P. Putri, E. Fukusaki, J. Büchs and L. M. Blank, *Front. Bioeng. Biotechnol.*, 2020, **7**, 476.
571 C. Borgström, L. Wasserstrom, H. Almqvist, K. Broberg, B. Klein, S. Noack, G. Lidén and M. F. Gorwa-Grauslund, *Metab. Eng.*, 2019, **55**, 1–11.
572 C. S. López-Garzón and A. J. J. Straathof, *Biotechnol. Adv.*, 2014, **32**, 873–904.
573 E. M. Karp, R. M. Cywar, L. P. Manker, P. O. Saboe, C. T. Nimlos, D. Salvachúa, X. Wang, B. A. Black, M. L. Reed, W. E. Michener, N. A. Rorrer and G. T. Beckham, *ACS Sustainable Chem. Eng.*, 2018, **6**, 15273–15283.
574 W. Zeng, S. Xu, G. Du, S. Liu and J. Zhou, *Bioprocess Biosyst. Eng.*, 2018, **41**, 1519–1527.
575 X. Shi, W. Su, H. Zhang, J. Fang, N. Xu, Y. Jiang and H. Li, *J. Ind. Eng. Chem.*, 2022, **115**, 528–536.
576 X. Shi, M. Yang, A. Chu, F. Zhang, J. Fang, N. Xu, Y. Jiang and H. Li, *AIChE J.*, 2022, **68**, e17814.
577 M. Szczygiełda and K. Prochaska, *J. Membr. Sci.*, 2017, **536**, 37–43.
578 M. Szczygiełda and K. Prochaska, *Process Biochem.*, 2020, **96**, 38–48.
579 M. Szczygiełda and K. Prochaska, *Sep. Sci. Technol.*, 2020, **55**, 165–175.
580 M. Szczygiełda and K. Prochaska, *Biochem. Eng. J.*, 2021, **166**, 1–10.
581 M. Szczygiełda, M. Krajewska, L. Zheng, L. D. Nghiem and K. Prochaska, *J. Membr. Sci.*, 2021, **637**, 119593.
582 W. Dessie, X. Luo, G. J. Duns, M. Wang and Z. Qin, *Environ. Technol. Innovation*, 2023, **32**, 103243.
583 C. S. López-Garzón and A. J. J. Straathof, *Biotechnol. Adv.*, 2014, **32**, 873–904.
584 C. Chen, X. Zhang, C. Liu, Y. Wu, G. Zheng and Y. Chen, *Bioresour. Technol.*, 2022, **346**, 126609.
585 B. Rukowicz, *ACS Sustainable Chem. Eng.*, 2023, **11**, 11459–11469.
586 S. Choi, C. W. Song, J. H. Shin and S. Y. Lee, *Metab. Eng.*, 2015, **28**, 223–239.
587 E. de Jong, H. Stichnothe, G. Bell and H. Jørgensen, *IEA Bioenergy*, 2020, 79.
588 L. Winstel, Top Value Added Chemicals: The Biobased Economy 12 Years Later, **https://communities.acs.org/t5/**

GCI-Nexus-Blog/Top-Value-Added-Chemicals-The-Biobased-Economy-12-Years-Later/ba-p/15759, (accessed 21 May 2024).
589 S.N., No Title, https://clinicaltrials.gov/search?term=alpha-ketoglutarate, (accessed 15 May 2024).
590 T. Papurina, O. Barsukov, O. Zabuga, D. Krasnienkov and E. Denis, *Biochem. Biophys. Rep.*, 2023, **33**, 101429.
591 V. E. Radzinsky, Y. Uspenskaya, L. P. Shulman and I. V. Kuznetsova, *Obstet. Gynecol. Int.*, 2019, **2019**, 1572196.
592 V. E. Radzinskii, I. V. Kuznetsova, Y. B. Uspenskaya, N. B. Repina, Y. K. Gusak, O. M. Zubova, D. I. Burchakov and A. A. Osmakova, *Gynecol. Endocrinol.*, 2016, **32**, 64–68.
593 X. Zhu, Y. Guo, Z. Liu, J. Yang, H. Tang and Y. Wang, *Sci. Rep.*, 2021, **11**, 1–9.
594 E. L. Mills, D. G. Ryan, H. A. Prag, D. Dikovskaya, D. Menon, Z. Zaslona, M. P. Jedrychowski, A. S. H. Costa, M. Higgins, E. Hams, J. Szpyt, M. C. Runtsch, M. S. King, J. F. McGouran, R. Fischer, B. M. Kessler, A. F. McGettrick, M. M. Hughes, R. G. Carroll, L. M. Booty, E. V. Knatko, P. J. Meakin, M. L. J. Ashford, L. K. Modis, G. Brunori, D. C. Sévin, P. G. Fallon, S. T. Caldwell, E. R. S. Kunji, E. T. Chouchani, C. Frezza, A. T. Dinkova-Kostova, R. C. Hartley, M. P. Murphy and L. A. O'Neill, *Nature*, 2018, **556**, 113–117.
595 J. Clayden, N. Greeves and S. G. Warren, *Organic Chemistry*, Oxford University Press, Oxford, 2nd edn, 2012.
596 L. J. Gooßen, F. Rudolphi, C. Oppel and N. Rodríguez, *Angew. Chem., Int. Ed.*, 2008, **47**, 3043–3045.
597 S. Tian, Q. Yue, C. Liu, M. Li, M. Yin, Y. Gao, F. Meng, B. Z. Tang and L. Luo, *J. Am. Chem. Soc.*, 2021, **143**, 10054–10058.
598 M. S. Kim, H. Chang, L. Zheng, Q. Yan, B. F. Pfleger, J. Klier, K. Nelson, E. L. W. Majumder and G. W. Huber, *Chem. Rev.*, 2023, **123**, 9915–9939.
599 J. G. Rosenboom, R. Langer and G. Traverso, *Nat. Rev. Mater.*, 2022, **7**, 117–137.
600 C. Jin, Z. Huang and J. Bao, *ACS Sustainable Chem. Eng.*, 2020, **8**, 6315–6322.
601 T. Nicolaï, W. Arts, S. Calderon-Ardila, R. Smets, M. Van Der Borght, J. M. Thevelein and B. F. Sels, *ACS Sustainable Chem. Eng.*, 2024, **12**, 8353–8365.
602 S. Van den Bosch, T. Renders, S. Kennis, S.-F. Koelewijn, G. Van den Bossche, T. Vangeel, A. Deneyer, D. Depuydt, C. M. Courtin, J. M. Thevelein, W. Schutyser and B. F. Sels, *Green Chem.*, 2017, **19**, 3313–3326.
603 T. A. Ewing, N. Nouse, M. van Lint, J. van Haveren, J. Hugenholtz and D. S. van Es, *Green Chem.*, 2022, **24**, 6373–6405.
604 E. Abedi and S. M. B. Hashemi, *Heliyon*, 2020, **6**, e04974.
605 P. Zheng, K. Zhang, Q. Yan, Y. Xu and Z. Sun, *J. Ind. Microbiol. Biotechnol.*, 2013, **40**, 831–840.
606 L. Mitrea, B. E. Teleky, S. A. Nemes, D. Plamada, R. A. Varvara, M. S. Pascuta, C. Ciont, A. M. Cocean, M. Medeleanu, A. Nistor, A. M. Rotar, C. R. Pop and D. C. Vodnar, *Heliyon*, 2024, **10**, 1–12.
607 Y. Xi, F. Fan and X. Zhang, *Green Carbon*, 2023, **1**, 118–132.
608 A. Kövilein, C. Kubisch, L. Cai and K. Ochsenreither, *J. Chem. Technol. Biotechnol.*, 2020, **95**, 513–526.
609 R. Zhang, H. Liu, Y. Ning, Y. Yu, L. Deng and F. Wang, *Fermentation*, 2023, **9**, 71.
610 H. Hosseinpour Tehrani, J. Becker, I. Bator, K. Saur, S. Meyer, A. C. Rodrigues Lóia, L. M. Blank and N. Wierckx, *Biotechnol. Biofuels*, 2019, **12**, 1–11.
611 D. Zhang, D. Guan, J. Liang, C. Guo, X. Xie, C. Zhang, Q. Xu and N. Chen, *Braz. J. Microbiol.*, 2014, **45**, 1477–1483.
612 D. Yang, H. Li, X. Jia, F. Yu, G. Wang, Y. Zhang, W. Wang, L. Zang and F. Shi, *Chem. Eng. Trans.*, 2023, **103**, 739–744.
613 Q. Lei, W. Zeng, J. Zhou and G. Du, *Bioresour. Technol.*, 2019, **292**, 121897.
614 R. Li, H. G. Sakir, J. Li, H. D. Shin, G. Du, J. Chen and L. Liu, *RSC Adv.*, 2017, **7**, 6615–6621.
615 C. Otto, V. Yovkova and G. Barth, *Appl. Microbiol. Biotechnol.*, 2011, **92**, 689–695.
616 P. D. Karp, R. Billington, R. Caspi, C. A. Fulcher, M. Latendresse, A. Kothari, I. M. Keseler, M. Krummenacker, P. E. Midford, Q. Ong, W. K. Ong, S. M. Paley and P. Subhraveti, *Briefings Bioinf.*, 2018, **20**, 1085–1093.
617 R. A. W. Frank, A. J. Price, F. D. Northrop, R. N. Perham and B. F. Luisi, *J. Mol. Biol.*, 2007, **368**, 639–651.
618 L. Yang, T. Wagner, A. Mechaly, A. Boyko, E. M. Bruch, D. Megrian, F. Gubellini, P. M. Alzari and M. Bellinzoni, *Nat. Commun.*, 2023, **14**, 4851.
619 L. Sundermeyer, G. Bosco, S. Gujar, M. Brocker, M. Baumgart, D. Willbold, O. H. Weiergräber, M. Bellinzoni and M. Bott, *Microbiol. Spectrosc.*, 2022, **10**, e02677–22.