Biobased adipic acid – The challenge of developing the production host

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Research review paper

Biobased adipic acid – The challenge of developing the production host

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ABSTRACT

Adipic acid is a platform chemical, and is the most important commercial dicarboxylic acid. It has been targeted for biochemical conversion as an alternative to present chemical production routes. From the perspective of bioeconomy, several kinds of raw material are of interest including the sugar platform (derived from starch, cellulose or hemicellulose), the lignin platform (aromatics) and the fatty acid platform (lipid derived). Two main biochemical-based production schemes may be employed: (i) direct fermentation to adipic acid, or (ii) fermentation to muconic or glucaric acid, followed by chemical hydrogenation (indirect fermentation). This review presents a comprehensive description of the metabolic pathways that could be constructed and analyzes their respective theoretical yields and metabolic constraints. The experimental yields and titers obtained so far are low, with the exception of processes based on palm oil and glycerol, which have been reported to yield up to 50 g and 68 g adipic acid/L, respectively. The challenges that remain to be addressed in order to achieve industrially relevant production levels include solving redox constraints, and identifying and/or engineering enzymes for parts of the metabolic pathways that have yet to be metabolically demonstrated. This review provides new insights into ways in which metabolic pathways can be constructed to achieve efficient adipic acid production. The production host provides the chassis to be engineered via an appropriate metabolic pathway, and should also have properties suitable for the industrial production of adipic acid. An acidic process pH is attractive to reduce the cost of downstream processing. The production host should exhibit high tolerance to complex raw material streams and high adipic acid concentrations at acidic pH.

1. Introduction

Greenhouse gas levels in the atmosphere have increased over recent decades to levels not previously experienced in modern times, leading to global warming which threatens to change the world's climate and life on Earth as we know it today (Figueres et al., 2017; Rockström et al., 2009). To reduce or avoid the consequences of climate change it is necessary to reduce our dependency on petrochemicals, and ultimately switch from a petrochemical-based economy to a biobased economy. In a biobased economy, biomass is used as the raw material for the production of fuels, chemicals, other materials and energy carriers. Adopting a biobased economy involves many, if not all, sectors of society. Not only the techniques and processes used for energy generation for heating and fueling of our vehicles will have to be replaced with new ones, but also those used in the chemical industry for the production of commodity chemicals. Commodity chemicals, including a

range of both organic and inorganic products, are produced on a large scale to satisfy the global market. Considerable efforts are being devoted to developing methods for the production of chemicals from biomass and to meet the demands of a growing bioeconomy. An EU survey (E4tech et al., 2015) demonstrates this rapid development, with examples of chemicals produced through biological or chemical routes, or combinations of both. Process development has reached different levels of technology readiness, and continued effort is required.

Adipic acid is considered by the International Energy Agency (IEA) to be the most important dicarboxylic acid from an industrial point of view, and has been identified as a suitable platform chemical for biobased production (de Jong et al., 2012). The amount of adipic acid produced worldwide per year is nearly 3 million tonnes, with a market value of almost USD 6 billion, growing at a compound annual growth rate of 3–5%. Most adipic acid is used in the nylon industry, while lower amounts are used in the production of plasticizers and polyurethanes,
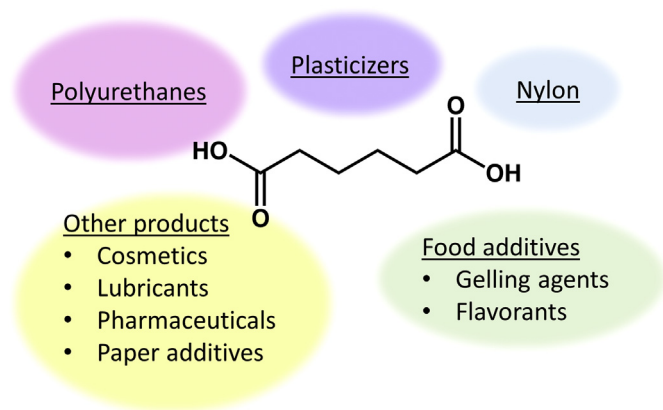
Fig. 1. The structure of adipic acid and examples of its uses.

but it is also used in other areas, including the food and pharmaceutical industries (Fig. 1). Historically, adipic acid was derived by the oxidation of various fats, thus the name adipic acid (from the Latin *adipis*, *adeps* meaning fat) (Ince, 1895). The current production routes rely on petrochemical precursors, the most common route starting with the hazardous compound benzene (Polen et al., 2013). In the ensuing reactions benzene is first reduced to cyclohexane, which is oxidized to KA oil (ketone-alcohol oil, a mixture of the ketone cyclohexanone and the alcohol cyclohexanol). The KA oil is finally reacted with nitric acid and air to yield adipic acid. During the final reaction, the highly potent greenhouse gas nitrous oxide (N_2O) is formed which is nearly 300 times more potent than carbon dioxide (CO_2). It has been estimated that replacing the current petrochemical-based process with one employing renewable precursors will reduce greenhouse gas emissions by up to 95%, depending on the process used (Aryapratama and Janssen, 2017; Diamond et al., 2014).

To realize a biobased economy, the efficient production and use of biomass are necessary. Furthermore, the raw material used in a bioeconomy must be produced in a sustainable manner. In the early development of bioethanol production, sucrose- and starch-based raw materials were used, which led to a conflict between food and feed production and fuel production. In recent years, development has focused on the use of lignocellulosic materials, such as agricultural and forestry residues and dedicated energy crops, as these do not compete with food production. Lignocellulosic material is composed of the polymers cellulose, hemicellulose and lignin. Cellulose and hemicellulose can be hydrolyzed to give a sugar platform from which the desired products can be produced. Due to its recalcitrant and heterogeneous nature, lignin has traditionally been considered a waste product in biorefineries, usually burned for energy production. However, valorization of lignin can play a key role in the viability of some biobased processes. Lignin is a polymer consisting of aromatic units, and has been tested as a feedstock for the biological production of muconic acid, which can be further converted into adipic acid (Ragauskas et al., 2014). Alternative biobased raw materials may be derived from lipids, such as palm oil, although conflict may arise if the lipid-derived source could also be used in food or feed applications.

Biobased production of adipic acid is currently being developed (Beardslee and Picataggio, 2012; Diamond et al., 2014), and three main production routes are emerging: 1) chemical processes, 2) indirect fermentation combined with chemical conversion, and 3) direct fermentation. In the first case, the company Rennovia has been producing bio-adipic acid through a chemo-catalytic process using glucose as feedstock. During this non-biotechnological process, glucose is first oxidized aerobically to glucaric acid, after which the glucaric acid undergoes hydrodeoxygenation to produce adipic acid. Although the process reached pilot scale, it resulted in a mixture of components and the yield obtained was around 60% (Boussie et al., 2013; Diamond et al., 2014). In the second kind of route, indirect fermentation, the precursors muconic or glucaric acid are produced, which then undergo chemical dehydrogenation to form adipic acid (Weber et al., 2012). In the third kind of process, direct fermentation, the raw material is directly converted to adipic acid using microbes (often referred to as the production host). Such a process is used by the company Verdezyne which used a genetically modified yeast species for the conversion of fatty acids, such as coconut oil, into adipic acid (Beardslee and Picataggio, 2012). Further development for producing adipic acid using cheaper raw materials is in a dire need especially with recent and unfortunate cease of operations and exit for both Verdezyne and Rennovia (de Guzman, 2018a, 2018b). Although several pathways have been proposed and tested for the production of adipic acid by the direct fermentation of sugars, they have been found to be less successful, with titers and yields far below those required for industrial production (Babu et al., 2015; Bart and Cavallaro, 2015; Deng et al., 2016; Deng and Mao, 2015; Kallscheuer et al., 2017; Kruyer and Peralta-Yahya, 2017; Polen et al., 2013; Turk et al., 2016; Yu et al., 2014). However, the potential of sugar-based production of adipic acid is considerable, bearing in mind the maximum theoretical yields for several of the proposed pathways, and the great abundance of carbohydrate-based raw materials. To provide guidance in the choice of a suitable metabolic pathway for adipic acid production, an in-depth analysis of the different metabolic pathways possible is useful. Not only the metabolic pathways are important, but also the choice of raw material(s) and the production host. Historically, hosts including natural and recombinant bacteria, yeasts and filamentous fungi have been identified and developed for the production of various compounds. However, there are no known efficient natural producers of adipic acid, and recombinant producers must, therefore, be developed.

The aim of this review is to provide thorough insight into microbial, biobased adipic acid production, including the selection of the production host, the raw material and metabolic pathway. The main focus is on metabolic pathways for the production of adipic acid, including an extensive and detailed analysis of all currently known metabolic pathways via direct and indirect fermentation. Pathway-specific limitations concerning, for example, redox balance and lack of enzymatic activities for each of the pathways, are identified and discussed, together with the potential for each pathway. The authors hope that aspects elucidated in this review will help to overcome obstacles and facilitate the realization of efficient, microbial biobased adipic acid production in the future.
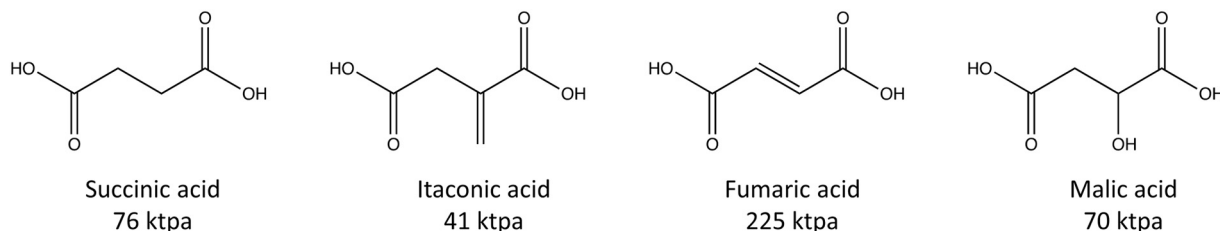

Fig. 2. Dicarboxylic acids of industrial relevance naturally produced by microorganisms. Their total global annual production volumes are given in kilo tonnes (ktpa).

2. The production host

Apart from adipic acid, other dicarboxylic acids interesting for commercial, biobased production are, fumaric, succinic, malic and itaconic acid (Fig. 2). Contrary to adipic acid, the mentioned acids are all naturally produced by microorganisms (Brown et al., 2013; Song and Lee, 2006; Wang et al., 2016; Xu et al., 2012). Although constant efforts in microbial strain engineering and improved fermentation conditions to achieve as efficient bioprocesses as possible (Chisti, 2006; Stanbury et al., 2013) fumaric and malic acids are yet not commercially produced. Although biobased commercial production of dicarboxylic acids still have many obstacles to overcome the recent commersialization of succinic acid is encouraging (BASF, 2014). In below sections we will briefly describe important requirements posed on the production host for biobased commercial production of acids as bulk chemicals, particularly adipic acid, and which microorganisms we believe are suitable to target for adipic acid production.

2.1. Requirements on industrial production host for adipic acid production

To be regarded as an interesting candidate for the industrial production of an acid, the production host must fulfill several requirements, including production at a high titer, rate and yield. For acid production, titer, rate and yield are in general required to be in the range of 50–100 g/L, 1–3 g/L h^{-1} and > 0.5 g/g respectively (Wang et al., 2016; Warnecke and Gill, 2005) to ensure an economically feasible production. High yields can be obtained when the production host can utilize all the available sugars, and the flux towards the desired acid is maximized, avoiding carbon losses in biomass and by-product formation. The requirement on reaching titers of 50–100 g/L means that the production host must tolerate the acid at such high concentrations. From the cost perspective, it is beneficial if the production host tolerates an acidic process condition, preferably around the pKa of the acid, to avoid the formation of salts when a base is added to maintain the pH thereby reducing the cost of downstream processing. The cost of downstream processing can account for $> 50\%$ of the total product cost (Wang et al., 2016), so it is important to reduce this as much as possible. For adipic acid the preferred process pH would then be ~4 since adipic acid has a pKa of 4.4.

In addition, to further reduce the overall production cost, the production host must be able to thrive in a simple and inexpensive medium. For instance, the addition of vitamins or essential amino acids increases the cost, and the use of a complex medium will make purification of the product costlier. Different obstacles to fermentation will be encountered depending on the source of the raw material. Lignocellulosic raw materials are regarded as attractive raw materials for fermentation since they are cheap, abundant and do not directly compete with food production. However, lignocellulosic material is very complex, containing cellulose, hemicellulose and extremely recalcitrant lignin. Therefore, pretreatment is often necessary to release the sugars (pentoses and hexoses), to make them available for fermentation, which increases the cost. Furthermore, inhibitors such as acetic acid, furfurals and phenols are normally released during pretreatment (Olsson et al., 2005). To achieve the required titer, rate and yield, the production host must also be tolerant to inhibitors present in the medium.

2.2. Microorganisms suitable for production of adipic acid

Both natural and recombinant microorganisms can be used to produce acids via fermentation. A natural producer, if it exists, must first be identified; requiring knowledge on which habitat to search, which growth conditions to apply, and feasible screening methods. For adipic acid there are no known natural producer, although the bacterium *Thermobifida fusca* has been reported to naturally possess a pathway for adipic acid. However, one endogenous enzyme was found to act as the

regulatory node and production of adipic acid was only observed in cell lysates with addition of the enzyme (Deng and Mao, 2015). Although natural producers have the advantage of producing the desired acid without the need for genetic engineering, they will not necessarily be successful on the industrial scale, as the titer may be too low, or it may be intolerant to the acid produced (Gu et al., 1998). In fact, weak acids are often used as food preservatives, and can exert product-mediated inhibition. In addition, natural producers often suffer from non-characterized genome and lack of molecular toolbox, potentially obstructing metabolic engineering strategies. Another disadvantage of natural producers is that a complex, and thus expensive, medium is often required for the organism to thrive (Mazzoli et al., 2014; Sauer et al., 2008). The alternative to a natural producer is to create a recombinant strain based on the cell chassis of a very well-characterized organism, in which the metabolic pathway for the desired acid is heterologously expressed. Metabolically engineered strains of *E. coli, C. glutamicum* and *S. cerevisiae* are attracting considerable interest for production of various dicarboxylic acids. These three microorganisms are all extremely well characterized, easy to cultivate, sequences are available for several strains, and each has a well-equipped molecular toolbox. *E. coli* is known to survive at pH down to 2, as it survives the conditions in the mammalian stomach, but it is sensitive to a decrease in pH during exponential growth. Therefore, cultivation is normally carried out at near-neutral pH, which is also the case for the gram-positive *C. glutamicum*. *S. cerevisiae,* on the other hand, tolerates lower pH with an optimal pH around 4–5 (Buzás et al., 1989). Concerning the above-mentioned requirement for low-pH cultivation for economically feasible adipic acid production, *S. cerevisiae* is an interesting microorganism to metabolically engineer for adipic acid production. In addition, *S. cerevisiae* is already a well-established microorganism in the industry and used for conversion of lignocellulosic biomass into ethanol and other products (Rødsrud et al., 2012). However, bacterial chassis have often proven superior, in terms of titer, rate and yield, to fungal chassis when metabolically engineered for the production of an array of acids (Liu and Jarboe, 2012). For direct production of adipic acid from glucose there are only reports of bacterial producers, indicating that other limitations must be overcome before *S. cerevisiae* can become an economically feasible host for production of adipic acid.

3. Metabolic pathways to adipic acid

A large number of proven and theoretical metabolic pathways for the production of adipic acids have been suggested. These include direct and indirect production of adipic acid, where precursors are formed in the latter, which are subsequently chemically transformed to render adipic acid. The proposed and proven direct pathways are presented in Figs. 3 to 5, while the indirect pathways that produce the precursors muconic acid and glucaric acid, are discussed in the following sections.

3.1. Direct pathways for adipic acid production

The pathways of direct production of adipic acid proven to work can be divided into three large pathway branches: 1) the reverse adipate degradation pathway (Babu et al., 2015; Deng and Mao, 2015; Kallscheuer et al., 2017; Yu et al., 2014; Zhao et al., 2018), 2) a combination of β-oxidation or reverse β-oxidation with the ω-oxidation pathway (Beardslee and Picattaggio, 2012; Clomburg et al., 2015), and 3) the 2-oxopimelic acid route (Turk et al., 2016) (Fig. 3).

3.1.1. The reverse adipate degradation pathway

The reverse adipate degradation pathway relies on the reverse of the adipic acid degradation pathway naturally found in *Penicillium chrysogenum* (Thykaer et al., 2002). This pathway has great potential since the maximum theoretical yield is 0.92 mol adipic acid/mol glucose, accompanied by a maximum ATP yield of 1.55 mol/mol glucose, under
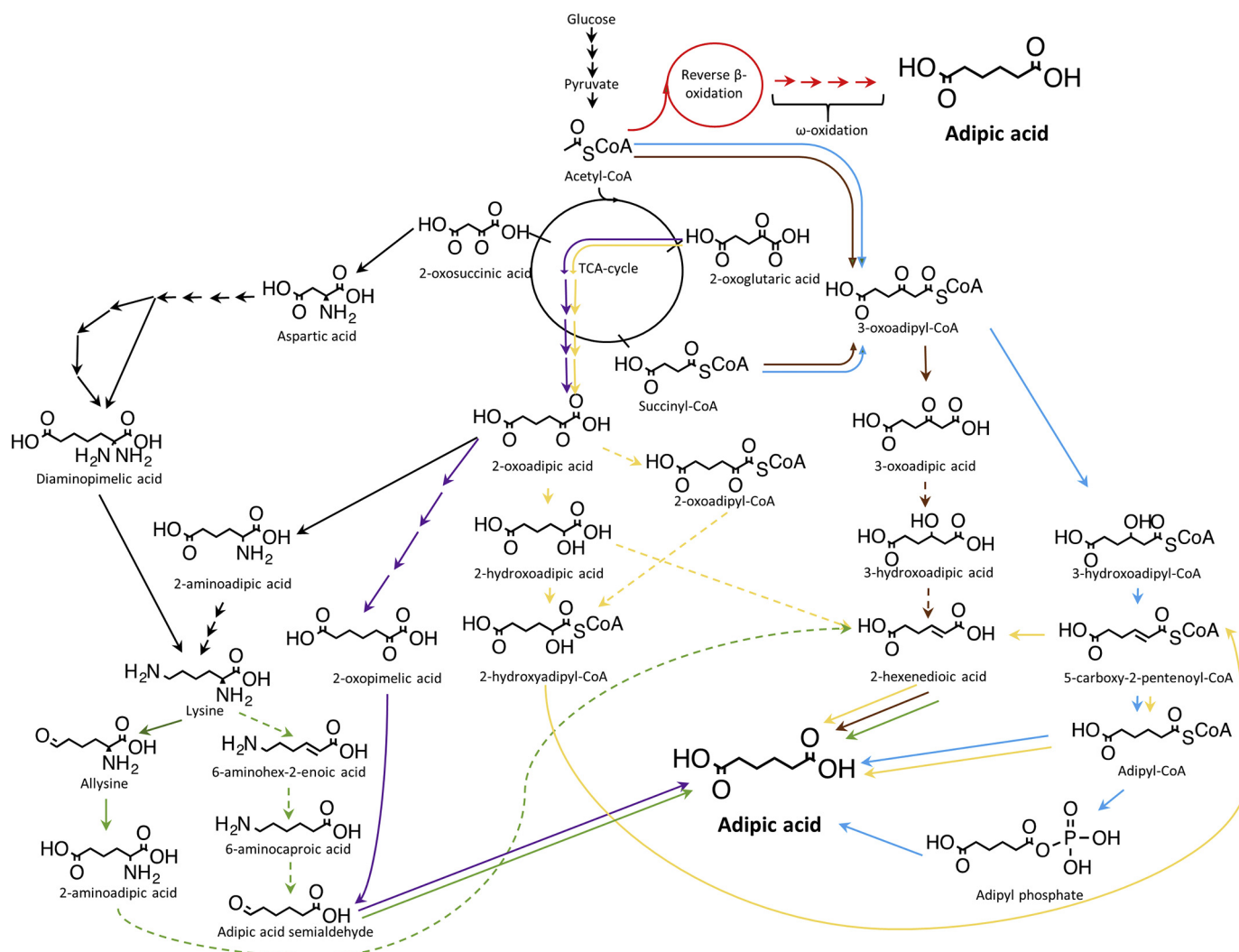
Fig. 3. Overview of the metabolic pathways for the direct production of adipic acid. Reverse adipate degradation pathway (blue), reverse β- then ω-oxidation pathway (red), 2-oxopimelic acid pathway (purple), 3-oxoadipic acid pathway (brown), 2-oxoadipic acid alternatives (yellow), lysine alternatives (green). Enzymatic activity has been demonstrated for the enzymatic steps indicated by full lines, while no proof of enzymatic activity has been demonstrated for enzymatic steps indicated by dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

both aerobic and anaerobic conditions (Burgard et al., 2016). This very high yield is possible only if the precursor succinyl-CoA is derived from the reverse operating TCA cycle, which does not form CO₂. As a result of this high theoretical yield, most efforts directed at the production of adipic acid via direct fermentation found in the literature have involved the reverse adipate degradation pathway. *E. coli* (Babu et al., 2015; Kallscheuer et al., 2017; Yu et al., 2014) and *T. fusca* (Deng and Mao, 2015) have been used as production hosts for the development of this pathway. The pathways published are identical in terms of metabolic intermediates from succinyl-CoA to adipyl-CoA, and include the condensation of succinyl-CoA and acetyl-CoA to form 3-oxoadipyl-CoA, reduction to 3-hydroxyadipyl-CoA, dehydration to 5-carboxy-2-pentenoyl-CoA, and reduction to adipyl-CoA (Fig. 3, blue pathway) (Babu et al., 2015; Deng and Mao, 2015; Kallscheuer et al., 2017; Yu et al., 2014). The enzymes able to carry out the reactions in this pathway have been described and are summarized in Table 1. This metabolic pathway is reported to be endogenous to *T. fusca* (Deng and Mao, 2015), however, the enzymatic step carried out by 5-carboxy-2-pentenoyl-CoA reductase (responsible for the reduction to adipyl-CoA) was identified as a bottleneck in this pathway. The transcription studies were carried out in order to identify this protein as the bottleneck of the pathway (Deng and Mao, 2015). Further, conversion of adipyl-CoA to adipate

has been investigated using various enzymes: 1) succinyl-CoA synthetase (Deng and Mao, 2015), 2) acyl-CoA esterase (Babu et al., 2015) and 3) by two enzymatic steps by means of phosphate butyryltransferase (phosphorylation of adipyl-CoA) and butyrate kinase (reduction of adipyl-phosphate) (Yu et al., 2014).

Enzymes from different organisms have been tested for the construction of the reverse adipate degradation pathway in *E. coli*, both *in vitro* and *in vivo* (Babu et al., 2015; Kallscheuer et al., 2017; Yu et al., 2014), and several combinations of them have been used in attempts to optimize the process. Recently, this pathway has been implemented in *E. coli* for improved adipic acid production using glycerol as the major carbon source (Zhao et al., 2018). In the mentioned study, adipic acid production with 68 g/L with 0.81 g/L h⁻¹ productivity and 0.378 g/g yield was achieved. The highest results with glucose as carbon source (0.05 mol adipic acid/mol substrate yield and 2.5 g/L titer) has been obtained by fine-tuning the natural pathway of *T. fusca* (Deng and Mao, 2015) (Table 2). It is worth noting that, NADH stoichiometry is better with glycerol than glucose especially in the microaerobic conditions (Zhao et al., 2018). This perhaps explains low titers of adipic acid production with glucose as carbon source due to an imbalance in the redox potential (Babu et al., 2015; Burgard et al., 2016; Yu et al., 2014). A limitation in the availability of either acetyl CoA or succinyl-CoA, the
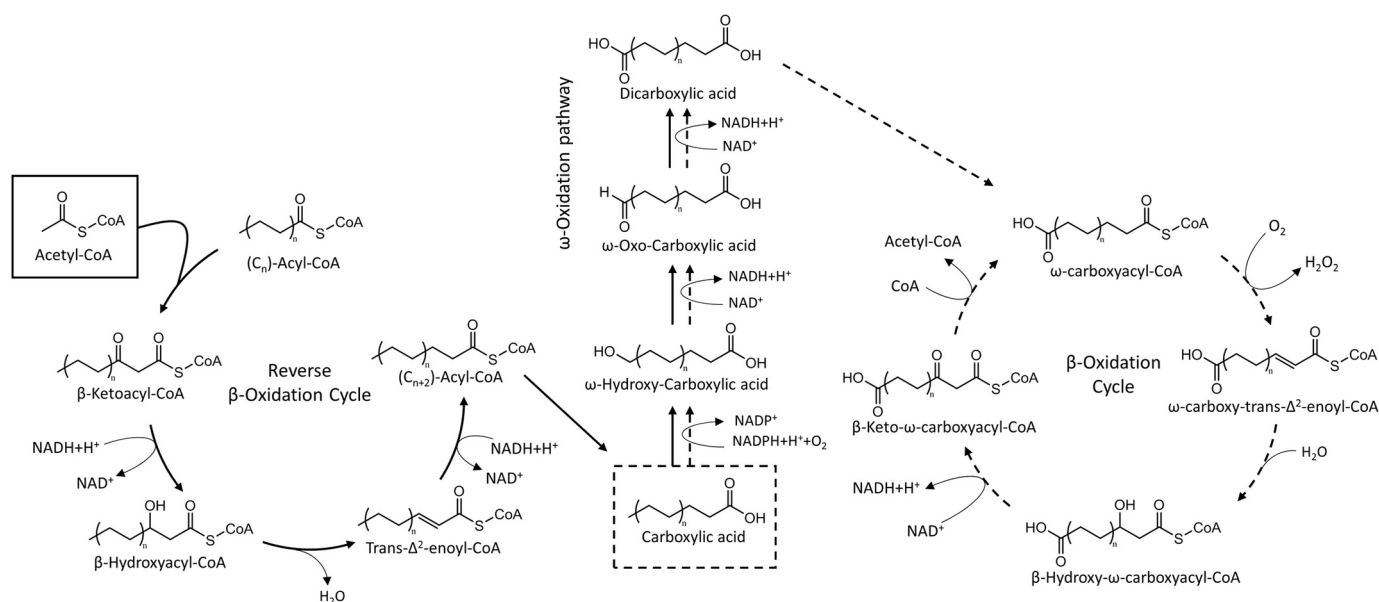
Fig. 4. Metabolic pathways for the production of adipic acid that combine the β- and ω-oxidation pathways. Full lines indicate metabolic pathways starting with acetyl-CoA which enters the reverse β-oxidation cycle to form carboxylic acid. The carboxylic acid subsequently enters the ω-oxidation pathway to form a dicarboxylic acid. Dashed lines indicate pathways starting with a fatty acid (carboxylic acid), which is converted into dicarboxylic acid by means of the ω-oxidation pathway. The dicarboxylic acid then enters the β-oxidation cycle and loses two carbons per cycle until adipic acid is formed.
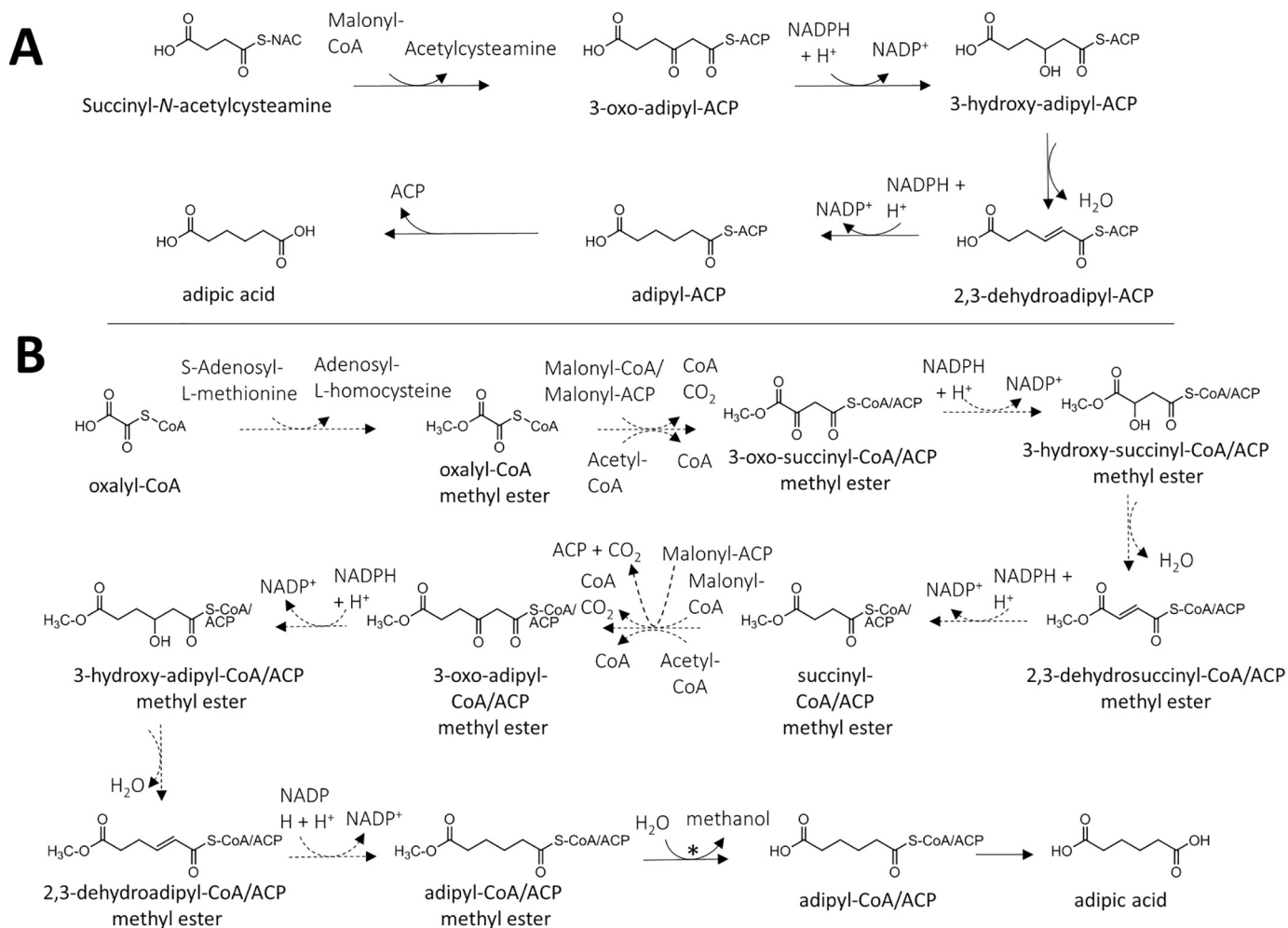
Fig. 5. A) Polyketide pathway for the production of adipic acid. B) Invista's proposed pathway for the production of adipic acid from oxalyl-CoA. ACP: acyl-carrier-protein; NAC: N-acetylcysteamine. Dashed lines indicate non-proven reactions, full lines indicate proven reactions. *The reaction has been demonstrated using adipyl-CoA-ACP as substrate.

Table 1

The enzymes used in the reverse adipate degradation pathway using *E. coli* and *T. fusca* as production hosts. (This pathway corresponds to the blue pathway in Fig. 3.)

Reaction	Substrate	Product	Enzyme	Donor	Host	References
1. Condensation	Acetyl-CoA and succinyl-CoA	3-oxoadipyl-CoA	PaaJ	*E. coli* (native)	*E. coli*	Babu et al. (2015); Kallscheuer et al. (2017); Yu et al. (2014)
			Tfu_0875	*T. fusca* (native)	*T. fusca*	Deng and Mao (2015)
			Tfu_0875	*T. fusca*	*E. coli*	Zhao et al. (2018)
2. Reduction	3-oxoadipyl-CoA	3-hydroxyadipyl-CoA	PaaH1 (3-hydroxy acyl-CoA reductase)	*R. eutropha*	*E. coli*	Yu et al., (2014)
			PaaH	*E. coli* (native)	*E. coli*	Kallscheuer et al. (2017)
			Tfu_2399	*T. fusca* (native)	*T. fusca*	Deng and Mao (2015)
			Tfu_2399	*T. fusca*	*E. coli*	Zhao et al. (2018)
3. Dehydration	3-hydroxyadipyl-CoA	5-carboxy-2-pentenoyl-CoA	Crt (crotonase)	*Clostridium acetobutylicum*	*E. coli*	Yu et al. (2014)
			Ech (enoyl CoA hydratase)	*R. eutropha*	*E. coli*	Yu et al. (2014)
			PaaF	*E. coli* (native)	*E. coli*	Kallscheuer et al. (2017)
			Tfu_0067	*T. fusca* (native)	*T. fusca*	Deng and Mao (2015)
			Tfu_0067	*T. fusca*	*E. coli*	Zhao et al. (2018)
4. Reduction	5-carboxy-2-pentenoyl-CoA	Adipyl-CoA	Ter (trans enoyl CoA reductase)	*Euglena gracilis*	*E. coli*	Yu et al. (2014)
			DcaA (acyl-CoA dehydrogenase)	*Acinetobacter baylyi*	*E. coli*	Kallscheuer et al. (2017)
			Acd (acyl-CoA dehydrogenase)	*Cupriavidus necator*	*E. coli*	Kallscheuer et al. (2017)
			Tfu_1647	*T. fusca* (native)	*T. fusca*	Deng and Mao (2015)
			Tfu_1647	*T. fusca*	*E. coli*	Zhao et al. (2018)
5. Termination	Adipyl-CoA	Adipic acid	Ptb-buk	*C. acetobutylicum*	*E. coli*	Yu et al. (2014)
			TesB (thioesterase)	*A. baylyi* ADP1	*E. coli*	Kallscheuer et al. (2017)
			TesB	*E. coli*	*E. coli*	Kallscheuer et al. (2017)
			Acd (acyl-CoA dehydrogenase)	*Thermococcus gammatolerans*	*E. coli*	Kallscheuer et al. (2017)
			Tfu_2577 and Tfu2576	*T. fusca* (native)	*T. fusca*	Deng and Mao (2015)
			Tfu_2577 and Tfu2576	*T. fusca*	*E. coli*	Zhao et al. (2018)

two substrates required at a 1:1 ratio, would also be a limiting factor for the overall rate of product formation. In fact, it has been pointed out that optimization of the native pathways and regulatory mechanisms for the formation of acetyl CoA and succinyl-CoA is crucial to increase titers of adipic acid (Babu et al., 2015).

3.1.2. Combination of β-oxidation or reverse β-oxidation with ω-oxidation pathway

The ω-, reverse β-, and β-oxidation pathways have been implemented and combined in *E. coli* and in some *Candida* yeast species (Fig. 3, red line and Fig. 4) to produce adipic acid. *E. coli* was grown on glycerol as the carbon source (Clomburg et al., 2015), while *Candida* spp. were grown on vegetable oil (e.g., coconut oil) (Beardslee and Picataggio, 2012). In theory, the order in which ω-, reverse β-, or β-oxidation takes place does not matter as long as the common goal is to obtain bi-functional groups on a six-carbon molecule. In the case of *Candida* spp. developed by Verdezyne, ω-oxidation initially takes place on fatty acids, thus the resulting molecules are di-carboxylic acid of various lengths (Fig. 4, dashed line). These di-acids are degraded via β-oxidation, which releases two carbons from the substrates per cycle, in the form of acetyl-CoA. The selection of acyl-CoA oxidase (encoded by POX5 in *Candida spp.*), the first enzyme that is involved in β-oxidation, determined the success of the strategy. This enzyme accepts di-acids of various lengths, but fails to recognize adipic acid as a substrate since it has a low affinity towards it (Beardslee and Picataggio, 2012). POX4, an isozyme of POX5, which accepts C4-C20 substrates, was deleted in the production host, thus preventing the cells from degrading adipic acid. In other words, sixteen-carbon fatty acid substrate, palmitic acid, is converted into hexadecanedioic acid via ω-oxidation, and subsequently loses two carbons in each cycle of β-oxidation until adipic acid is formed and excreted extracellularly. Moreover, in this approach, the catalytic reactions taking part in the ω-oxidation pathway were identified, and the key enzymes overexpressed in order to obtain higher productivity in the process. A titer of 50 g/L of adipic acid was achieved (Table 2). Although the maximum theoretical yield is 0.85 mol adipic

acid/mol coconut oil, the yield is reduced to 0.51 mol adipic acid/mol coconut oil when the fatty acids are oxidized. From an environmental and economic point of view any loss of carbon is undesirable. To reduce the loss of carbons the two carbons lost in each round of β-oxidation needs to be fed back to form adipic acid via other pathways, e.g. via reverse β-oxidation followed by ω-oxidation.

In the case where *E. coli* was used as the production host, elongation of acetyl-CoA via reverse β-oxidation takes place prior to ω-oxidation (Clomburg et al., 2015) (Fig. 4, continuous line). Here, the six-carbon carboxylic acid is produced first by reverse β-oxidation, and subsequently converted into adipic acid via ω-oxidation. The enzymatic activities required in this combined pathway were investigated (from known enzymes reported in the literature to act on similar substrates) and tested. Thiolase and thioesterase (enzymes responsible for the condensation step and the elimination of CoA from carboxylic acid, respectively) were considered key points in the pathway as they control the carbon length of the substrate (Clomburg et al., 2015). The selection of 3-ketoacyl-CoA thiolase encoded by bktB from *Ralstonia eutropha* governed the synthesis of C6-C10 carboxylic acids without the synthesis of longer-chain products. For thioesterase, the *E. coli* ydiI gene was selected, since it showed low specificity towards acetyl-CoA and butyryl-CoA, and a higher activity using C6, C8 and C10 acyl-CoA substrates, which favored the subsequent production of the corresponding dicarboxylic acids. However, to further increase the production of adipic acid and avoid the formation of longer di-acids, the enzyme specificities of all the aforementioned enzymes must be further optimized. This metabolic route requires that the starting metabolites acetyl-CoA and butyryl-CoA are present at equimolar concentrations. Both the metabolic pathways described in this section also suffer from redox imbalance.

3.1.3. The 2-oxopimelic acid pathway

The 2-oxopimelic acid pathway has been implemented in *E. coli* and produces adipic acid as a by-product in the biosynthetic pathway intended for the production of aminocaproic acid using glucose as

Table 2
Comparison of direct and indirect metabolic pathways for the production of adipic acid. The comparison includes both the theoretical maximum yield, Y_{max} and reported values ($Y_{reported}$) by the best producing strain of adipic acid, cis,cis-muconic acid or glucaric acid on various substrates.

Pathway	Y_{max}[a] (mol/mol)	$Y_{reported}$[a] (mol/mol)	Titer[a] (g/L)	$r_{adipic\ acid}$[a] (g/L h⁻¹)	Substrate	Host species	Limitations	Possible improvements	Reference
Direct production of adipic acid									
Reverse adipate degradation	0.92	0.054	2.23	0.034	Glucose	T. fusca	Redox imbalance Need for equimolar concentrations of starting metabolites	Enzyme specificities, expression optimization	Deng and Mao (2015)
	0.33[c]	0.24	68	0.81	Glycerol	E. coli			Zhao et al. (2018)
Reverse β- then ω-oxidation	n.r.	< 0.01	0.17	< 0.01	Glycerol	E. coli	Redox imbalance Need for equimolar concentrations of starting metabolites	Enzyme specificities, expression optimization	Clomburg et al. (2015)
ω- then β-oxidation	0.51–0.85[b]	n.r.	50	0.38	Fatty acids	Candida spp.	Redox imbalance	Enzyme specificities	Beardslee and Picataggio (2012)
2-oxopimelic acid	n.r.	n.r.	0.3	< 0.01	Glucose	E. coli	Redox imbalance	Identification of known enzymatic steps	Burgard et al. (2016)
3-oxoadipic acid	0.92	n.r.	n.r.	n.r.	Glucose	n.r.	Need for equimolar concentrations of starting metabolites	Identification of known enzymatic steps	Burgard et al. (2016)
2-oxoadipic acid	0.67/0.45	n.r.	n.r.	n.r.	Glucose	n.r.	Redox imbalance	Identification of known enzymatic steps	Burgard et al. (2016)
Lysine via 2-oxoglutaric acid	0.40/0.20	n.r.	n.r.	n.r.	Glucose	n.r.	Relatively low Y_{max}	Identification of known enzymatic steps	Burgard et al. (2016)
Lysine via aspartic acid	0.50/0.34	n.r.	n.r.	n.r.	Glucose	n.r.	Relatively low Y_{max}	Identification of known enzymatic steps	Burgard et al. (2016)
Indirect production of adipic acid									
Muconic acid									
3-dehydroshikimate	0.75	0.22	36.8	0.76	Glucose	E. coli	Oxygen-requiring enzymes, ATP yields negligible	Enzymatic conversion of muconic acid into adipic acid under aerobic conditions	Niu et al. (2002)
β-ketoadipate pathway via catechol and protocatechuate	n.r.	0.67	13.5	0.17	p-coumarate (model lignin compound)	P. putida	Oxygen-requiring enzymes	Enzymatic conversion of muconic acid into adipic acid	Vardon et al. (2015)
Glucaric acid									
D-glucuronic acid	n.r.	0.13	1.13	0.016	Glucose	E. coli	Oxygen-requiring enzymes	Enzymatic conversion of D-glucuronic acid into adipic acid, improvement of titers	Moon et al. (2009); Chen et al. (2018)
	n.r.	1.76	6.0	0.03	Myo-inositol	S. cerevisiae			

The values of Y_{max} are taken from the Genomatica patent (Burgard et al., 2016), unless stated otherwise. These values are based on glucose as the starting substrate and values are given for aerobic/anaerobic conditions (equal values under both conditions when only one value is given).

n.r. = not reported.

[a] Yields are reported as the unit product/unit substrate. Titers are reported as volumetric titers of product, r_p is the volumetric productivity rate of product. Product depends on the pathway and is adipic acid, cis,cis-muconic acid or glucaric acid. Substrate is given in the table.

[b] 0.85 $mol_{adipic\ acid}/mol_{coconut\ oil}$ was calculated from the maximum theoretical yield of 0.6 $g_{adipic\ acid}/g_{coconut\ oil}$ (Beardslee and Picataggio, 2012). 0.51 $mol_{adipic\ acid}/mol_{coconut\ oil}$ was calculated from available carbons for adipic acid production, assuming that all fatty acid taken up by the cell enters the ω- and subsequent β-oxidation pathways. For every round of β-oxidation two carbons are lost as acetyl-CoA, meaning that 6 carbons out of n carbons are available for adipic acid production, where n is the total number of carbons in the fatty acid. If the acetyl-CoA can be directed into the formation of adipic acid, e.g. via reverse β- then ω-oxidation, the number of available carbons will be increased.

[c] Value retrieved from (Zhao et al., 2018).

substrate (Turk et al., 2016) (Fig. 3, purple line). The pathway starts with 2-oxoglutaric acid, which is elongated to 2-oxoadipic acid by means of the Aks enzymes (AksA, AksD, AksE, AksF) implicated in the ketoacid elongation pathway in methanogenic archaea. While the resulting 2-oxoadipic acid is subsequently elongated in the same manner to form 2-oxopimelic acid then α-ketosuberate in these archaea, it stops at 2-oxopimelic acid and does not proceed to form α-ketosuberate in the synthetic pathway (Turk et al., 2016) (Fig. 3, purple line). Instead, the pathway was fine-tuned by the choice of the first enzyme (AksA) in the elongation cycle, partly responsible for the selection of the chain length of the intermediates in the route. The subsequent pathway is based on the degradation of branched-chain amino acids, in which aldehydes are produced by decarboxylation from the corresponding α-ketoacid. Different decarboxylases were tested for the production of adipate semialdehyde from 2-oxopimelic acid. While the intended pathway consisted of decarboxylation of 2-oxopimelic acid and subsequent amino transfer to produce 6-aminocaproic acid, an unknown endogenous enzyme was more efficient than the aminotransferase reaction (Turk et al., 2016). The higher rate of oxidation by the unknown enzyme, than aminotransferase on adipate semialdehyde, resulted in a higher amount of adipate being produced than 6-aminocaproic acid. A titer of 0.3 g/L of adipic acid was achieved with this pathway (Table 2). In this pathway, starting with 2-oxoglutarate, one net mol of NADH is consumed for every mol of adipic acid produced, resulting in an imbalance in the redox potential.

3.1.4. The polyketide pathway

Apart from metabolic engineering approaches, the production of adipic acid using only purified enzymes or cell-free extracts has also been studied in detail. While metabolic engineering enables microorganisms to produce value-added compounds *in vivo*, *in vitro* studies using enzymes provide verification of challenging steps in pathway engineering and design.

The conversion of succinyl-CoA to adipic acid using the polyketide synthase (PKS) system was reported in 2016 (Hagen et al., 2016). In this case, the borrelidin PKS system from *Streptomyces* spp. was modified for the *in vitro* synthesis of adipic acid from succinyl-CoA as the starter unit and malonyl-CoA as an extender unit (Hagen et al., 2016) (Fig. 5, A). The borrelidin PKS system was chosen because succinyl-CoA was found to be extended to yield 3-hydroxy-adipyl-ACP by the extension module of borrelidin PKS in a previous study (Hagen et al., 2014). The extension enzyme module consists of ketosynthase, acyltransferase, ketoreductase and acyl-carrier protein. In order to proceed with the dehydration of 3-hydroxy-adipyl-ACP, the dehydratase domain called BorDH2, from the second extension module from the borrelidin PKS cluster was chosen. BorDH2 is the first dehydratase domain in the borrelidin PKS cluster, and it outperformed the dehydratases from aureothin, indanomycin, nanchangmycin and spinosyn PKS clusters (Hagen et al., 2016). The ketoreductase and enoyl reductase domains were selected from the reductive loop of the spinosyn PKS cluster to replace the native ketoreductase module. Subsequent release of adipic acid from adipyl-ACP was then achieved by the thioesterase domain of the erythromycin cluster. Although the above-mentioned study of *in vitro* adipic acid production using purified enzymes reported very low production titers (~ 0.3 mg/L of adipic acid), it demonstrates an unparalleled example of pathway engineering by demonstrating a new pathway and proving its reaction schemes. Since two equivalents of NADPH are consumed for each adipic acid molecule produced, alleviating this redox imbalance will be beneficial in future metabolic engineering strategies.

3.1.5. Theoretical pathways

In the previous sections, direct metabolic pathways proven to function were reviewed. However, a number of theoretical pathways have been proposed for the direct production of adipic acid, although, to the best of our knowledge, these have not been shown to function. Theoretical pathways suggested for the direct production of adipic acid will be reviewed in the following sections.

3.1.5.1. The 3-oxoadipic acid pathway.
The first enzymatic step of this pathway is identical to that in the reverse adipate degradation pathway, where acetyl-CoA and succinyl-CoA are condensed into the product 3-oxoadipyl-CoA (Fig. 3, brown line). However, the pathways differ in the second step, and in the 3-oxoadipic acid pathway, the product 3-oxoadipyl-CoA is released from CoA via 3-oxoadipyl-CoA transferase forming the intermediate product 3-oxoadipic acid. The first two enzymatic steps in this pathway are identical to the two final steps of the degradation pathway for aromatic and chloroaromatic compounds, operating in the reverse direction (Kaschabek et al., 2002; Nogales et al., 2007). The enzymatic reactions from 3-oxoadipic acid follow the same type of reactions as in the reverse adipate degradation pathway, but in this case the substrates are not condensed to CoA (Fig. 3, brown line). None of the enzymes required to convert 3-oxoadipic acid to adipic acid has yet been found in nature, however, some candidate genes have been suggested for each of these steps, although there is no experimental evidence (Burgard et al., 2016). Identifying enzymes with sufficient activity and specificity for these steps is the first serious challenge in implementing this pathway. Additional challenges are the redox imbalance and the need for equimolar concentrations of the starting substrates succinyl-CoA and acetyl-CoA. However, this pathway has considerable potential since the maximum theoretical yield is 0.92 mol adipate/mol glucose under both anaerobic and aerobic conditions, and the maximum ATP yield is 1.55 mol/mol glucose, which is unaffected by the conditions (aerobic or anaerobic) (Burgard et al., 2016). However, the high yield can only be achieved if the precursor succinyl-CoA is formed from the reverse operating TCA cycle, since two mols of carbon are lost as CO_2 for each mol succinyl-CoA formed in the TCA cycle.

3.1.5.2. 2-oxoadipic acid pathways.
One important metabolite proposed to be part of several pathways to adipic acid is 2-oxoadipic acid (Burgard et al., 2016; Parthasarathy et al., 2011; Turk et al., 2016) (Fig. 3). This metabolite is derived from 2-oxoglutaric acid via 1C elongation achieved by the enzymes homocitrate synthase, homoaconitase and homoisocitrate dehydrogenase (Burgard et al., 2016). One of the branches of this pathway proceeds through 2-oxopimelic acid, as described in section "The 2-oxopimelic acid pathway", while another branch proceeds through lysine, and is described below in the section on "Pathways starting with lysine". The remaining pathways, described in this section, are highly similar and interlinked to the reverse adipate and 3-oxoadipic acid pathways, but differ in the position of the carbon that is modified (Fig. 3, yellow line). In the 2-oxoadipic acid pathways the functional groups targeted are located on the α-carbon instead of the β-carbon, as is the case in the 3-oxoadipic acid and reverse adipate degradation pathways. Due to the loss of two CO_2 molecules in the conversion from acetyl-CoA to adipic acid, the maximum theoretical yield is 0.67 mol adipic acid/mol glucose under aerobic conditions, and is reduced to 0.45 mol/mol under anaerobic conditions (Burgard et al., 2016). Under aerobic conditions the maximum ATP yield is 6.17 mol/mol glucose, whereas under anaerobic conditions it is negligible, which requires the organism to utilize other means of generating the energy required for cell growth. Although activity has been demonstrated for some of the enzymes in the pathway proceeding through 2-hydroxyadipic acid (Parthasarathy et al., 2011; Reitman et al., 2012; Suda et al., 1977; Suda et al., 1976), none of the pathways has so far proven feasible for adipic acid production, and challenges associated with enzyme specificities must be addressed before adipic acid production via one of the 2-oxoadipic acid pathways can be realized. Another limitation in these pathways is that none of them is balanced regarding redox potential and the regeneration of redox power must be addressed via other pathways, leading to the risk of reducing the yield.

Despite these limitations, a study on the conversion of 2-oxoadipic acid to 2-hexenedioic acid was reported in 2011 (Parthasarathy et al., 2011). Three enzymes, 2-hydroxyglutarate dehydrogenase (HgdH), glutaconate CoA-transferase (GctAB) and 2-hydroxyglutaryl-CoA dehydratase (HgdC and HgdAB) collectively encoded by six genes, were used for *in vitro* conversion of 2-oxoadipic acid to 2-hexenedioic acid (Parthasarathy et al., 2011). HgdH and GctAB were obtained from *Acidaminococcus fermentans*, while HgdC and HgdAB were obtained from *Clostridium symbiosum*. The constructed pathway begins with 2-oxoadipic acid, and proceeds by reduction to 2-hydroxyadipic acid, activation to 2-hydroxyadipyl-CoA, dehydration to 5-carboxy-2-pentenoyl-CoA, and, finally, the release of CoA yields 2-hexenedioic acid. This study was inspired by the fact that very similar conversion of 2-ketoglutarate to glutaconate takes place in certain microorganisms in the orders *Clostridiales* and *Fusobacteriales* (Parthasarathy et al., 2011). Moreover, a previous report by the same group described the production of glutaconic acid (2-pentenedioic acid) by an engineered *E. coli* strain using the same gene combination, illustrating the potential for *in vivo* production (Djurdjevic et al., 2011). The final and challenging problem of the reduction of 2-hexenedioic acid to adipic acid remained unsolved for several years, but is has recently been shown that anaerobic enzymes can carry out this reaction (Joo et al., 2017).

3.1.5.3. Pathways starting with lysine. When considering a metabolic pathway starting with lysine for the production of adipic acid, one should consider the cost of lysine as the starting material. The economic feasibility of a process that involves feeding a cultivation broth with carbon sources such as hexose or glycerol together with lysine, will be compromised as lysine is considerably more expensive (van Haveren et al., 2008). However, if the pathway is used with lysine-producing industrial organisms such as *C. glutamicum* and hexose or disaccharide is used as the substrate feed, the economics should be improved, making this more feasible for industrial application. Lysine production with titers as high as 120 g/L and a productivity of $4.0 \, g/L \, h^{-1}$ has been reported previously (Becker et al., 2011; Becker and Wittmann, 2012a, 2012b).

Application of the adipic acid pathway using lysine as an intermediate has been described in a patent by Genomatica (Burgard et al., 2016). This pathway consists of four enzymatic steps; the first being the removal of the α-amino group from lysine to yield 6-aminohex-2-enoic acid, followed by reduction to yield 6-aminocaproic acid. The terminal amino group is then removed, resulting in adipic acid semialdehyde, which is oxidized to adipic acid (Fig. 3, green lines, right path). One serious challenge associated with this pathway is that only one of the four enzymes required is known to exist. In an attempt to overcome this problem, an additional pathway has been proposed starting with lysine (Karlsson et al., 2018). In this pathway, the order of the enzymatic reactions is different, resulting in other metabolites being formed. In the first enzymatic step the terminal amino group of lysine is removed yielding allysine, which is subsequently oxidized to form 2-aminoadipic acid. Thereafter, the α-amino group is removed forming hex-2-enedioic acid, which is reduced to adipic acid (Fig. 3, green lines, left path). Using this approach reduces the number of unknown enzymes to one instead of three.

Lysine can be synthesized via either of two microorganism-specific pathways, and the maximum theoretical yield depends on the pathway used. In *E. coli* and *C. glutamicum,* the native pathway to lysine is via aspartic acid, giving a maximum yield of 0.50 mol adipic acid/mol glucose under aerobic conditions. In *S. cerevisiae,* lysine is produced via 2-oxoadipic acid, giving a slightly lower maximum theoretical yield than that through aspartic acid; 0.40 mol adipic acid/mol glucose under aerobic conditions (Murthy and Janardanasarma, 1999). If lysine were to be produced under anaerobic conditions the maximum theoretical yield would be decreased (Table 2) and ATP production would be negligible. Despite the rather low maximum theoretical yield, the main advantage of these pathways compared to many of the other proposed

pathways is that they are neutral in terms of redox potential, hence the microorganism does not have to regenerate the redox cofactors via other pathways, which would risk reducing the overall yield.

A slightly different approach is described in a patent by Celexion (Baynes et al., 2015). In the Celexion patent, lysine degradation to yield 2-oxoadipic acid is described and conversion to adipic acid is described similarly as in other sections of this review.

3.1.5.4. Adipic acid via ω-oxidation of hexanoic acid from hexanoate-producing organisms. It is also theoretically possible to utilize a host microorganism that naturally produces hexanoic acid, a six-carbon monocarboxylic acid, as the product. Anaerobic microorganisms such as *Megasphaera elsdenii* are known to produce hexanoic acid as the end product (Roddick and Britz, 1997). The use of such a rumen bacteria for the production of organic acids, offering a route for nylon production, has been suggested previously (Sauer et al., 2012), but has not yet been realized. The ω-oxidation scheme can be theoretically applied to this metabolism to convert hexanoic acid into adipic acid (Fig. 4, route from carboxylic acid to dicarboxylic acid). As in the aforementioned reverse β-oxidation pathway, ω-oxidation can be incorporated into bacteria producing hexanoic acid. The problem would be that the redox balance observed in the aforementioned ω-oxidation scheme would also be present in this case. Although the metabolic engineering of *M. elsdenii* for the production of adipic acid has not been reported, it is theoretically possible, especially as it has been demonstrated in *E. coli* in the combination of ω-oxidation and reverse β-oxidation (Clomburg et al., 2015). The challenge would then be to correctly express ω-oxidation enzymes such as AlkBGT and achieve high titers under anaerobic conditions since *M. elsdenii* is an anaerobic organism. No reports on correctly functioning AlkBGT genes in *M. elsdenii* could be found in the literature.

3.1.5.5. Adipic acid via methyl-ester shielded carbon elongation. A metabolic pathway has been proposed for the production of adipic acid from the central metabolite oxalyl-CoA via two cycles of methyl-ester shielded carbon chain elongation (Botes and Van Eck Conradie, 2015). This strategy is based on the use of enzymes able to catalyze methyl-ester shielded dicarboxylic acid substrates.

This theoretical biosynthetic pathway starts with the formation of oxalyl-CoA methyl ester from oxalyl-CoA by means of a methyl-transferase (Fig. 5, B) (Lin, 2012). Oxalyl-CoA methyl ester is then condensed with malonyl-ACP to give 3-oxo-succinyl- ACP methyl ester, or with malonyl-CoA or acetyl-CoA to give 3-oxo-succinyl-CoA methyl ester (Botes and Van Eck Conradie, 2015; Slater et al., 1998). A four-carbon chain aliphatic backbone is produced by the condensation step.

The 3-oxo-succinyl-ACP methyl ester or 3-oxo-succinyl-CoA methyl ester is then subjected to a reduction reaction, dehydration and then a second reduction reaction, which renders succinyl-ACP methyl ester or succinyl-CoA methyl ester (Bond-Watts et al., 2012; Botes and Van Eck Conradie, 2015; Budde et al., 2010; Fukui et al., 1998). Another identical round of carbon elongation then takes place, which forms the six-carbon backbone (Fig. 5, B). Adipyl- ACP methyl ester or adipyl-CoA methyl ester is then obtained.

The methyl ester group is then removed by means of pimelyl-ACP methyl ester esterase, and adipyl- ACP or adipyl-CoA is formed (Botes and Van Eck Conradie, 2015; Lin et al., 2010). Both metabolites can finally be converted to adipic acid. Different enzymes can be used for the conversion to adipic acid: a thioesterase or a CoA-transferase, or a reversible CoA-ligase or a combination of a dehydrogenase that forms adipate semialdehyde and an aldehyde dehydrogenase that forms adipic acid (Botes and Van Eck Conradie, 2015).

This proposed metabolic pathway suffers from metabolic constrains since NADH- or NADPH-dependent enzymes are used for double bond reduction. Moreover, most of the enzymatic reactions proposed have not yet been demonstrated. The second part of the pathway (once the

C6 intermediate is formed) resembles the reverse β-oxidation pathway, apart from the fact that in this case the intermediate metabolites carry the methyl ester group.

3.2. Indirect pathways for adipic acid production

In the previous section, we focused on the metabolic pathways that render adipic acid directly, whereas in the present section we discuss the metabolic pathways that have been thoroughly studied for the indirect production of adipic acid by production of the precursors muconic and glucaric acid. In indirect production an additional chemical step is required to obtain adipic acid.
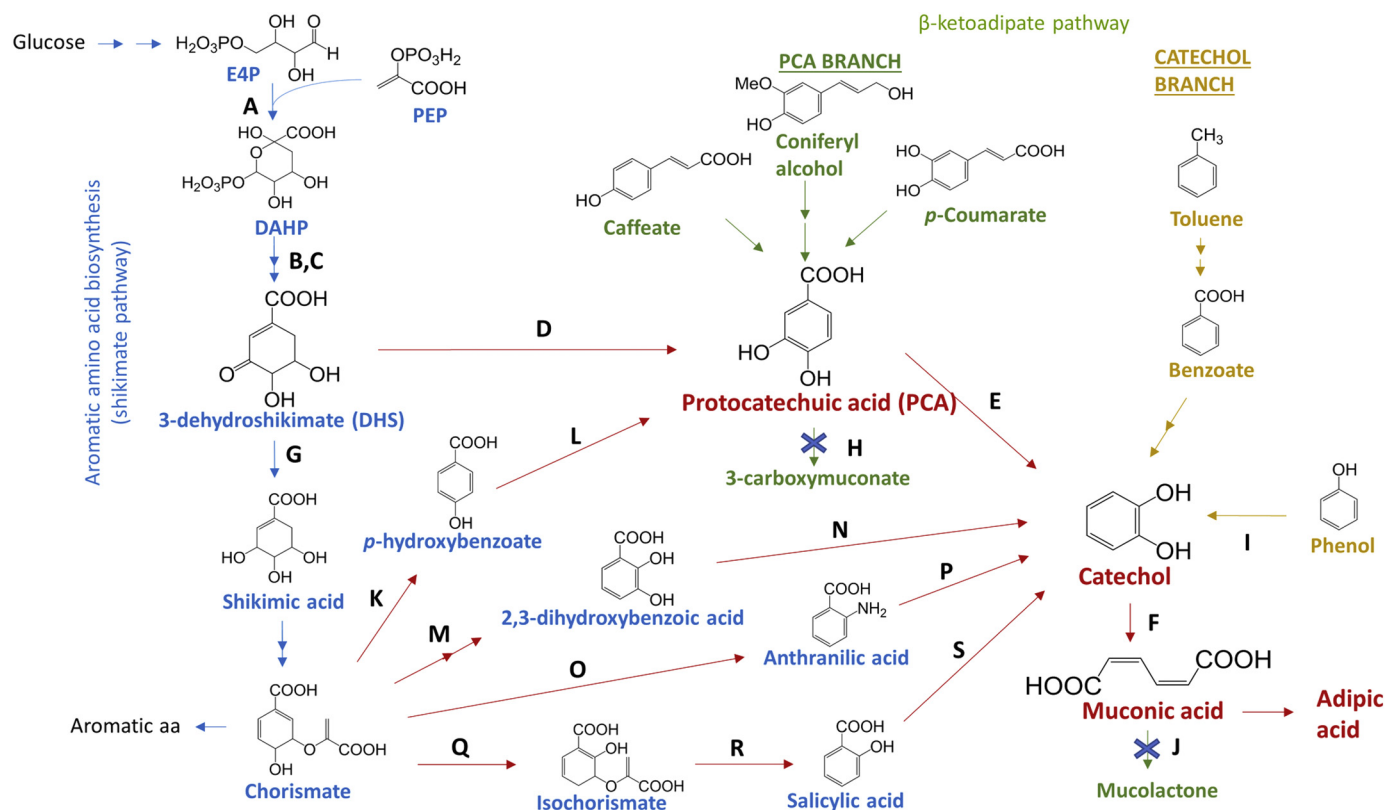
3.2.1. Production of muconic acid

Biobased production of muconic acid has been thoroughly studied as an indirect means of producing adipic acid owing to its facile conversion into adipic acid using a catalyst of platinum on activated carbon (Niu et al., 2002). However, the recent discovery of enoate reductases able to convert muconic acid to adipic acid opens the possibility to directly produce adipic acid through metabolic pathways using muconic acid, although with very low efficiency so far (Chan Joo et al., 2017; Raj et al., 2018; Sun et al., 2018).

Two main feedstocks have been used for muconic acid production, D-glucose and aromatic compounds such as benzoate or toluene (lignin-derived or petroleum-based raw materials) (Curran et al., 2013; Draths and Frost, 1994; Vardon et al., 2015; Weber et al., 2012). The first metabolic pathway studied for the production of muconic acid from glucose starts with 3-dehydroshikimate (DHS), an intermediate of the shikimate pathway for the biosynthesis of aromatic amino acids

(Fig. 6). DHS is converted to protocatechuic acid (PCA) (by means of DHS dehydratase), is subsequently transformed to catechol (by means of a PCA decarboxylase) and finally converted to muconic acid (in a step catalyzed by a catechol 1, 2-dioxygenase) (Fig. 6, reactions D-E-F). In an *in silico* evaluation of sixteen different metabolic pathways that render adipic acid, the routes based on the 3-dehydroshikimate pathway were found to be the most thermodynamically favourable over large pH range and substrate concentration (Averesch et al., 2018).

The 3-dehydroshikimate pathway has been implemented in *E. coli* and *S. cerevisiae* in which heterologous synthetic pathways have been designed, and in *Klebsiella pneumoniae*, in which the pathway occurs naturally. This pathway was first attempted in *E. coli*, in which the three heterologous genes aroZ (encoding for DHS dehydratase), aroY (encoding for PCA decarboxylase) from *K. pneumoniae*, and catA (encoding catechol 1,2-dioxygenase) from *Acinetobacter calcoaceticus* were introduced in three different plasmids (Draths and Frost, 1994). Relatively low concentrations of muconic acid were synthesized (2.4 g/L) when using shake flask cultivations. In a follow-up study using bioreactors in batch mode, the *E. coli* strain was further improved and the complexity of the microbes was reduced to constructs possessing only a single plasmid (Niu et al., 2002). The best strain engineered in this way synthetized 36.8 g/L of muconic acid, and rendered a yield of 0.22 mol/mol. The maximum theoretical yield calculated for this pathway is 0.75 mol/mol (Table 2) (Burgard et al., 2016).

The natural pathway in *K. pneumoniae* has been used for muconic acid production (Jung et al., 2015). *K. pneumoniae* has native aromatic amino acid synthesis and benzoate degradation pathways, which produce muconic acid as an intermediate. Wild-type *K. pneumoniae* does not accumulate muconic acid since the intracellular carbon flow does

Fig. 6. Metabolic pathways for muconic acid production from glucose and lignin-derived compounds. E4P, erythrose 4-phosphate; PEP, phosphoenolpyruvate; DAHP, 3-deoxy-D-arabinoheptulosonate-7-phosphate; A, DAHP synthase; B, 3-dehydroquinate (DHQ) synthase; C, DHQ dehydratase; D, DHS dehydratase; E, PCA decarboxylase; F, catechol 1,2-dioxygenase; G, 3-dehydroshikimate dehydrogenase; H, protocatechuate 3,4 dioxygenase; I, phenol monooxygenase; J, muconate cycloisomerase; K, chorismate pyruvate-lyase; L, 4-hydroxybenzoic acid hydrolase; M, isochorismate synthase, isochorismatase and 2,3-dihydro-2,3-DHBA dehydrogenase; N, 2,3-dihydroxybenzoate decarboxylase; O, anthranilate synthase; P, anthranilate 1,2-dioxygenase; Q, isochorismate synthase; R, isochorismate pyruvate lyase; S, salicylate 1-monoxygenase.

not favor it. However, metabolic engineering strategies that involved removing branched and/or degradation pathways and overexpressing catechol 1,2-dioxygenase have been used in an attempt to improve the accumulation of muconic acid. The developed strain produced 2.1 g/L of muconic acid in flask cultivation.

Finally, *S. cerevisiae* strains able to produce muconic acid were developed using the same heterologous metabolic pathway (Curran et al., 2013; Weber et al., 2012). Curran et al. introduced the genes encoding for DHS dehydratase from *Podospora anserina*, PCA decarboxylase from *Enterobacter cloacae* and catechol 1,2-dioxygenase from *Candida albicans* into yeast after assessing different candidates. A titer of 0.14 g/L was obtained from shake flask cultures. In order to improve the production of adipic acid in *S.cerevisiae,* a biosensor based evolution strategy was used to evolve a strain that was able to produce 2.1 g/L of muconic acid (Leavitt et al., 2017). Weber et al. used a slightly different means to construct the same synthetic pathway with three bacterial enzymes (DHS dehydratase from *Podospora anserina*, PCA decarboxylase from *Klebsiella pneumoniae* and catechol 1,2-dioxygenase from *Acinetobacter radioresistens*), which resulted in a final muconic acid concentration of 0.0015 g/L. However, the titers obtained with *S. cerevisiae* were much lower than those produced by *E. coli*.

Recently, the enzymatic conversion of muconic acid to adipic acid by different enoate reductases has been described (Chan Joo et al., 2017). This discovery opens the possibility of directly producing adipic acid from glucose through the 3-dehydroshikimate pathway. In this way, a proof of concept was established by the introduction of the enoate reductase from *Bacillus coagulans* in a *S. cerevisiae* strain and the development of a three-stage fermentation process that rendered low titers of adipic acid (0.0026 g/L) (Raj et al., 2018). In another attempt, 0.027 g/L of adipic acid was produced under microaerobic conditions when the enoate reductase from *Clostridium acetobutylicum* was introduced in an *E.coli* strain (Sun et al., 2018). The indirect production of adipic acid through muconic acid rendered higher titers that the direct option so far. More effort needs to be done for the direct production of adipic acid through muconic acid can be considered a competitive option.

In all the above cases, metabolic engineering that involved the redirection of carbon flow and the optimization of enzyme expression levels and activity was required to improve the yields. When intermediate metabolite concentrations were measured, high levels of PCA were detected in many cases. It thus became apparent that PCA decarboxylase activity was rate limiting (Fig. 6, D). Different strategies have been tested to improve the PCA decarboxylase activity, such as cloning into high-copy-number vectors or genomic integration (Curran et al., 2013; Weber et al., 2012), although the enzyme activity still constituted a bottleneck. Some recent studies have revealed that co-expression of PCA decarboxylase with two genetically associated proteins, one of which probably produces a flavin-derived cofactor required by the enzyme, improved muconic acid production and reduced PCA levels (Johnson et al., 2016; Sonoki et al., 2014).

Another key aspect is the deletion of the aroE gene (Draths and Frost, 1994; Jung et al., 2015; Niu et al., 2002; Weber et al., 2012), which encodes for shikimate dehydrogenase (Fig. 6, G). The interruption of the flow to the aromatic amino acid synthesis pathway at this point left DHS available for muconate production.

Other alternative pathways derived from chorismate (an intermediate in the shikimate pathway) have also been investigated. One of them produced muconic acid via anthranilate (Fig. 6, reactions O-P-F) (Sun et al., 2013), the first intermediate in the pathway for the biosynthesis of tryptophan. The introduction in *E. coli* of the gene encoding anthranilate 1,2-dioxygenase that enabled the conversion of anthranilate to catechol, and the gene encoding catechol 1,2 dioxygenase which further converts catechol to muconic acid, enabled the pathway to muconic acid. Moreover, a glutamine regeneration system was introduced (by expression of a glutamine synthase from *E. coli*). This would facilitate the activity of anthranilate synthase (Fig. 6, reaction O)

which uses glutamine as amino group donor. Titers of 0.39 g/L of muconic acid were achieved using glucose and glycerol as substrates in shake flask experiments (Sun et al., 2013).

Another similar pathway for muconic acid production was developed by the same group (Lin et al., 2014). In this approach, salicylic acid production was established in *E. coli* by the introduction of the enzymes isochorismate synthase and isochorismate pyruvate lyase (Lin et al., 2013), which convert chorismate to isochorismate and isochorismate to salicylic acid, respectively (Fig. 6, Q and R). Muconic acid production was achieved by the additional expression of the genes encoding salicylate 1-monooxygenase and catechol 1,2-dioxygenase (part of the salicylate degradation pathway, Fig. 6, S and F), leading to the production of 1.5 g/L using shake flask cultivation (Lin et al., 2014).

Yet other alternative for muconic acid production related to the shikimate pathway has been explored (Sun et al., 2014). This pathway focuses on the production of 2,3-dihydroxybenzoic acid (an intermediate in the native *E. coli* enterobactin biosynthesis pathway) from chorismate and its further conversion to catechol and muconic acid (Fig. 6, reactions M-N-F). The introduction of three genes encoding for isochorismate synthase, isochorismate and 2,3-dihydro-2,3-DHBA dehydrogenase enabled the production of 2,3-dihydroxybenzoic acid from chorismate. Further introduction of the genes encoding for 2,3-dihydroxybenzoic acid decarboxylase and catechol 1,2-dioxygenase (Sun et al., 2013) allowed the production of muconic acid, rendering 0.48 g/L of muconic acid in cultivations in shake flasks.

In another pathway, *p*-hydroxybenzoate (PHB) was used as an intermediate to produce catechol (Pugh et al., 2014; Sengupta et al., 2015). PHB is an intermediate in the ubiquinone pathway, which branches out from chorismate in the aromatic amino acid pathway. This route has been implemented in *E. coli* using glucose as substrate. In this pathway, the intermediate chorismate can be converted to PHB (Fig. 6, K). PHB is subsequently transformed to protocatechuate and to catechol (Fig. 6, L and E). A titer of 0.63 g/L of catechol was produced in batch bioreactors (Pugh et al., 2014), which can be further converted to muconic acid. The engineering of the pathway included the overexpression of the genes encoding endogenous chorismate pyruvate lyase (Fig. 6, K), the oxygen- and NADPH-dependent 4-hydroxybenzoate hydrolyase and protocatechuate decarboxylase (Fig. 6, L and E) (Pugh et al., 2014; Sengupta et al., 2015). Furthermore, deletion of the genes involved in the further transformation of chorismate via adjacent routes increased the yields obtained. A titer of 0.17 g/L of muconic acid was produced from glucose in shake flasks without any nutrient supplementation (Sengupta et al., 2015).

Most of the described routes based on the shikimate pathway were optimized by the overexpression of key genes. Overexpression and regulation of the aroB and aroD genes, encoding 3-dehydroquinate synthase and dehydratase, respectively, in the upstream pathway improved muconic acid production (Fig. 6, B and C) (Draths and Frost, 1994; Niu et al., 2002; Sengupta et al., 2015; Sun et al., 2013; Weber et al., 2012). Moreover, 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP) synthase, the first enzyme in the pathway for aromatic amino acid synthesis (Fig. 6, A), is an important regulatory node in the pathway. The enzyme is regulated via feedback inhibition, while the expression of each gene is regulated at the transcriptional level. A number of strategies have been developed to overcome both forms of regulation, such us the use of a mutated gene that encodes a feedback-resistant isozyme of DAHP synthase (Curran et al., 2013; Luttik et al., 2008; Niu et al., 2002; Weber et al., 2012), or the deletion of transcriptional regulators (Jung et al., 2015).

Increasing the expression of the tkt gene encoding for a transketolase involved in D-erythrose 4-phosphate (E4P) synthesis also had a significant impact on carbon flow into the aromatic amino acid biosynthetic pathway, presumably by increasing the availability of E4P, one of the two substrates for DAHP synthase (Fig. 6, A) (Curran et al., 2013; Lin et al., 2014; Niu et al., 2002; Sengupta et al., 2015; Sun et al., 2014). Increasing the supply of phosphoenolpyruvate (PEP) and E4P

metabolites could also increased the yields (Draths and Frost, 1994; Weber et al., 2012). Also, in the case of *Klebsiella*, the production of muconic acid was further improved by deletion of the gene encoding for muconate cycloisomerase (Fig. 6, J) (Jung et al., 2015).

The biosynthetic pathways for aromatic amino acids were blocked in some cases, leaving the key intermediates available for muconic acid production (Lin et al., 2014; Sengupta et al., 2015; Sun X., Lin Y., Huang Q., Yuan Q., 2013; Sun et al., 2014), although this led to the need for nutrient supplementation, which increased the cost of the medium and made scaling-up difficult. Most of these pathways suffered from other limitations such as intermediate toxicity (Lin et al., 2014; Pugh et al., 2014; Sun et al., 2014). Developing a more resistant host or introducing the pathway into a naturally resistant microorganism can be considered. Finally, the fact that some of the pathways described suffer from redox imbalance must also be taken into consideration.

Pseudomonas putida KT2440 has been engineered in order to produce muconic acid from lignin-derived aromatics. The resulting strain was able to catabolize different aromatics through the upper pathways in the β-ketoadipate pathway. Catechol and protocatechuic acid are central intermediates in these pathways (Fig. 6, PCA and catechol branches). A wide range of lignin-derived aromatics present in lignin, such as benzoate, caffeic acid, *p*-coumarate, coniferyl alcohol and phenol, can be converted into catechol or protocatechuic acid and, subsequently, into muconic acid using this funneling strategy (Vardon et al., 2015). To engineer the strain, PCA 3,4 dioxygenase, which metabolizes PCA to 3-carboxymuconate (Fig. 6, H), was replaced with PCA decarboxylase (aroY from *E. cloacae*), which enabled the conversion of PCA to catechol. Moreover, the gene encoding one of the catechol 1,2-dioxygenases responsible for the conversion of catechol to muconic acid was constitutively expressed by means of the tac promoter. Further metabolism of catechol was limited by the deletion of the catBC gene. Finally, genes encoding phenol monooxygenase from *Pseudomonas* CF600 were inserted in order to enable the catabolism of phenol (a major component of lignin) to muconic acid (Fig. 6, I). The performance of the engineered strain was tested in fed-batch reactors using *p*-coumarate as a model lignin monomer, giving a titer of 13.5 g/L (Table 2). Finally, muconic acid has also been produced from biomass-derived lignin in shake flask cultivation as proof of concept for lignin valorization (Vardon et al., 2015). The production of muconic acid from aromatics such as benzene obtained from non-renewable resources has also been thoroughly studied (Xie et al., 2014).

3.2.2. Production of glucaric acid

The biological production of glucaric acid has been studied as another indirect means of producing adipic acid (Fig. 7). A synthetic pathway has been designed in *E. coli* that enabled glucaric acid production using glucose as feedstock (Moon et al., 2009). The metabolic pathway included the following genes: *myo*-inositol-1-phosphate synthase (Ino1) from *S. cerevisiae*, *myo*-inositol oxygenase (MIOX) from mice and uronate dehydrogenase (Udh) from *P. syringae*. In this pathway, Ino1 (which requires NAD^+ for activity, which is regenerated during the isomerization reaction) was used to produce *myo*-inositol-1-phosphate from glucose-6-phosphate. Subsequently, *myo*-inositol-1-phosphate is hydrolytically dephosphorylated by an endogenous phosphatase to produce *myo*-inositol. MIOX can then convert *myo*-inositol to D-glucuronic acid using molecular oxygen. Finally, Udh converts D-glucuronic acid into D-glucaric acid. Udh, which showed a high activity, was able to increase the carbon flux through the pathway. However, it requires NAD^+ for catalysis, which causes an imbalance in the redox potential in this pathway. D-glucaric acid concentrations over 1 g/L were obtained (Table 2).

The activity of MIOX was found to be limiting and led to the accumulation of the intermediate *myo*-inositol. Nevertheless, glucaric acid titers were further improved (~2.5 g/L) by co-localization of the Ino1 and MIOX enzymes with protein scaffolds (which recruit the desired enzymes). The results showed that the MIOX activity depended on the

number of Ino1 molecules co-localized. Hence, the increase in the number of interaction domains targeting Ino1 led to a higher local concentration of the MIOX substrate, *myo*-inositol, which led to MIOX activation and subsequently, to higher titers of glucaric acid (Dueber et al., 2009; Moon et al., 2010).

MIOX activity was further improved by two different protein engineering approaches. The expression of MIOX with the fusion tag SUMO (Small Ubiquitin-like Modifier) led to higher stability of the enzyme and increased glucaric acid production to 4.8 g/L (Table 2). On the other hand, the directed evolution of MIOX led to the identification of a small DNA fragment (which encodes for manX mRNA), whose overexpression increased *myo*-inositol transport and thus the D-glucaric titer (4.6 g/L) (Shiue and Prather, 2014). Furthermore, knockdown of the phosphofructokinase activity, which increased the availability of glucose-6-phosphate, led to an improvement in the titer of up to 42% (Brockman et al., 2015).

Glucaric acid was produced in *S. cerevisiae* using the same metabolic pathway (Chen et al., 2018; Gupta et al., 2016). Titers of 6 g/L were achieved from *myo*-inositol when a more stable miox4 from *Arabidopsis thaliana* and the udh gene from *Pseudomonas syringae* was introduced in the genome through a high copy delta-sequence intregration strategy.

4. Discussion

Considerable efforts are being made to develop the biobased production of adipic acid in order to replace the current petrochemical production route. Although processes based on fatty acids (Beardslee and Picataggio, 2012) and glycerol (Zhao et al., 2018) have high titers, significant development is still required for the conversion of other raw materials, such as sugars. In the search for cheap and abundant, sugar-rich raw materials that do not compete with food production, lignocellulose-derived biomass has emerged as an interesting alternative. However, the use of lignocellulose as a raw material poses a number of challenges due to its recalcitrance, making it difficult to release the sugars for fermentation. It also suffers from the presence of inhibitors such as acetic acid, furfurals and phenols, which requires the identification of a suitable, robust production host. As a result of concerted research efforts over recent decades, lignocellulosic biomass is now used industrially as the raw material for the production of ethanol with yeasts (Rødsrud et al., 2012). The inherent and engineered tolerance of yeasts to lignocellulosic-derived inhibitors makes them attractive as production hosts for lignocellulosic-based processes. In addition, acids are preferably produced at acidic pH to avoid salt formation, which further complicates the costly downstream process. Acidic pH environments have long been known to be a habitat for yeasts. A recent finding, that yeasts tolerate adipic acid at industrially relevant levels under acidic conditions, in contrast to bacteria, provides further evidence that yeasts may be an interesting choice for biobased production of adipic acid (Karlsson et al., 2017). This tolerance may be due to the fact that yeasts have compartments surrounded by membranes, while bacteria do not, which may restrict transport between compartments in yeast.

Despite the lower tolerance for acidic conditions there are known bacteria capable of producing high titers of succinic acid (Okino et al., 2008; Vemuri et al., 2002). Another interesting host could be the fungus *R. arrhizus* already producing high titers of fumaric acid (Xu et al., 2012). These fumaric acid or succinic acid producing hosts can be further equipped with reverse degradation pathway so that high titer succinic acid or fumaric acid can be fluxed towards adipic acid. These hosts are known to produce > 100 g/L succinic acid or fumaric acid and thus can be great potential candidates for producing adipic acid once the flux is efficiently re-directed. So far, no efficient natural producer of adipic acid has been found, and it therefore appears to be necessary to engineer a recombinant producer to endow it with a metabolic pathway for the production of adipic acid.

Assuming glucose is used as the starting metabolite, we have
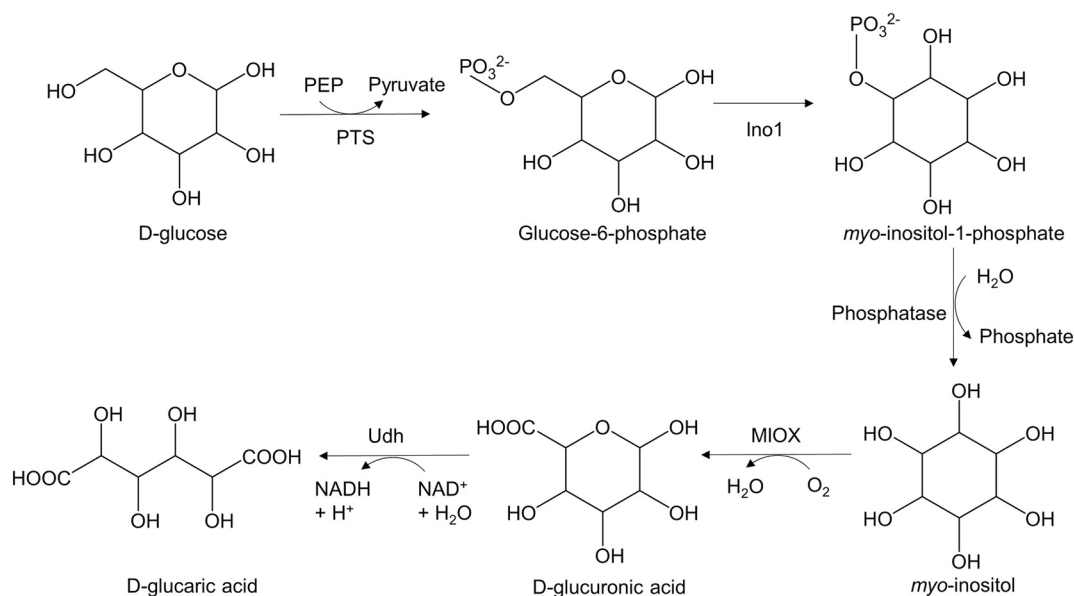
Fig. 7. Metabolic pathway for the production of D-glucaric acid from glucose. PTS, phosphoenolpyruvate-dependent phosphotransferase; PEP, phosphoenolpyruvate; Ino1, *myo*-inositol-1-phosphate synthase; MIOX, *myo*-inositol oxygenase; Udh, uronate dehydrogenase.

identified at least 8 different direct and indirect metabolic pathways with several variants from the literature. Several of these pathways have significant parts in common, and the suggested pathways typically consist of a combination of inherent metabolic reactions (depending on the host), with other steps that must be conferred heterologously. When selecting the metabolic pathway to be engineered, one should thus consider the inherent metabolic pathways of the production host. For example, a host known for its overproduction of lysine should be selected when the intention is to introduce a pathway for the production of this metabolite. The indirect production of adipic acid, from glucaric and muconic acid, has been successful when using sugars or lignin derivatives as substrates. However, both require aeration, and additional downstream chemical processing after biological production to obtain adipic acid. This is less attractive since these extra steps increase the production cost. For the direct production of adipic acid from glucose, the 2-oxopimelic pathway and reverse degradation pathway have been functionally demonstrated. However, the reported titer, production rates and yields for both these pathways are still far too low to be industrially relevant, despite the high potential indicated by the maximum theoretical yield, at least for the reverse adipate degradation pathway. Other direct pathways identified are still only theoretical, and have not yet been functionally demonstrated.

All of the metabolic pathways presented here pose different challenges. One of the main bottlenecks identified in direct metabolic pathways is the lack of suitable enzymes that can perform the desired reactions efficiently. In fact, several of the proposed, but not functionally proven pathways, include enzymatic reactions where the enzyme required has not yet been identified. The emergence of massive amounts of genomic information in recent years will hopefully facilitate the identification and characterization of the novel enzyme activities required in the suggested pathways. Protein engineering techniques may also be useful in optimizing the activity or specificity of known enzymes.

During the course of this literature survey, one enzymatic reaction, namely the reduction of 2-hexenedioic acid to adipic acid, emerged as a key reaction in four different pathways: the 3-oxoadipic acid pathway, two of the alternative 2-oxoadipic acid pathways, and one of the lysine pathway alternatives (Fig. 3). Until recently, there was no known enzyme for the reduction of 2-hexenedioic acid, which could be due to the fact that 2-hexenedioic acid is rarely, if ever, found in nature. Recently, Jeong Chan Joo and coworkers were the first to demonstrate the

reduction of 2-hexenedioic acid using enoate reductases (Joo et al., 2017). They also demonstrated the conversion of muconic acid to adipic acid, which could potentially lead to the direct production of adipic acid using a pathway for muconic acid production, hence avoiding a costly, additional purification step (Warnecke and Gill, 2005). However, the experiments were performed under anaerobic conditions, which could limit the usefulness of the enoate reductases, depending on the oxygen requirements of the selected pathway and host.

Another common problem we identified are the poor specificities of thiolases and thioesterases for the generation of C6 metabolites, which are involved in several of the experimentally demonstrated metabolic pathways based on glucose. In the reverse adipate degradation and 3-oxoadipic acid pathways, the thiolase responsible for the condensation of the starting metabolites must exhibit specificity towards acetyl-CoA and succinyl-CoA, whereas the thiolase in the reverse β-, and ω-oxidation pathways must exhibit specificity towards acetyl-CoA and butyryl-CoA. The thioesterase responsible for the link between reverse β-, and ω-oxidation must be specific to hexanoyl-CoA. Poorly defined specificities will render products of various chain lengths, as has been found when attempting to introduce the reverse adipate degradation and reverse β-, and ω-oxidation pathways (Cheong et al., 2016; Clomburg et al., 2015). Therefore, careful selection of enzymes that render C6 products is essential for adipic acid production. In addition, the starting metabolites must be available at an equimolar ratio to ensure a steady flux through the reverse adipate degradation, 3-oxoadipic acid and reverse β-, and ω-oxidation pathways. The flux will thus be limited by the generation of the starting metabolite that is metabolized at the slower rate. Also, the starting metabolite succinyl-CoA must be generated by the reverse TCA cycle to allow for the high yields estimated for the reverse adipate degradation and 3-oxoadipic acid pathways (Burgard et al., 2016), otherwise, the formation of succinyl-CoA from the TCA cycle will lead to the loss of two carbons as CO_2 for each succinyl-CoA molecule formed.

Another important finding of the present study is that some of the successfully demonstrated metabolic pathways utilize CoA-bound metabolic intermediates. Whether or not the use of CoA cofactors is advantageous for the host in the production of adipic acid has not been elucidated, but it could be hypothesized that the high-energy thioester linkage between CoA and the metabolite could facilitate energetically unfavorable reactions. Another interesting aspect is that enzymes with specificities for CoA-bound metabolites are known to be synthesized in

the cell, e.g. in the synthesis of fatty acids, and are therefore available and known to perform the desired chemical reaction. Pathways utilizing CoA-unbound metabolites have been less successful, which could partially be due to the fact that they require as yet unidentified enzymes to perform the reaction in question.

Several of the direct metabolic pathways suffer from redox imbalance as they often utilize the cofactors NADH/NADPH as a source of reducing power. For a pathway to be efficient the redox potential must be balanced, as an imbalance in the pathway must be counteracted elsewhere, via other pathways, potentially reducing the overall yield, and resulting in undesirable by-product formation. To overcome this problem, the reducing power can be fine-tuned so as to recirculate the cofactors in order to achieve a better redox balance. If the recirculation of cofactors is coupled with product formation, by-product formation can be avoided.

One parameter often evaluated when selecting a metabolic pathway is the maximum theoretical yield. The maximum theoretical yield gives valuable information on whether a pathway has the potential to reach the efficiency required. However, the maximum theoretical yield can be determined in different ways, using different model organisms and different mathematical models and assumptions. Therefore, the maximum theoretical yield is not well defined. In this review, most of the maximum theoretical yields given were obtained from the Genomatica patent (Burgard et al., 2016). However, other values reported for the same pathway may be based on the use of a different model organism (Frost and Draths, 1995). To ensure an objective comparison between the potential of different pathways when using the maximum theoretical yield, one should thus compare values based on the selected target host, using the same model and assumptions for all pathways.

5. Conclusions

No efficient naturally occurring microorganisms for the production of adipic acid have yet been identified. In this review, we have analyzed several options for the production of adipic acid using pathway engineering, suggesting maximum theoretical yields ranging from 0.20 to 0.92 mol adipic acid/mol substrate, depending on the metabolic pathway and substrate. A careful analysis of the possible metabolic pathways revealed that they are highly interlinked, which offers the possibility of new combinations of metabolic pathways for the production of adipic acid. However, most of these metabolic pathways suffer from substantial challenges due to redox imbalance, manipulation of central carbon metabolic pathways that may influence cellular fitness, or the fact that one or several enzymes in the pathway have not yet been identified. In many cases, the specificity of the enzyme employed in the pathway needs to be improved.

Some adipic-acid-producing pathways have been experimentally demonstrated using sugar as the substrate, although with limited success. However, a fermentation process using *E. coli* as host and glycerol as substrate produced up to 68 g/L of adipic acid. Also, a palm-oil-based process has shown a titer of up to 50 g/L of adipic acid, although this process has limitations in terms of sustainability. A common factor in the experimentally demonstrated pathways is that most of them use CoA-activated carbons in several metabolic conversion steps. We have discussed several other options for direct adipic acid production, where a careful analysis of these pathways indicates that enoate reductase is a key enzyme. The recent discovery of enoate reductases able to produce adipic acid from muconic acid and 2-hexenedioic acid introduces other alternative pathways for adipic acid production, although more effort need to be done in order to achieve efficient titers. Additionally, many metabolic pathways or variants thereof harbor metabolites of industrial interest which can also be targeted.

The route towards an efficient fermentation pathway is not obvious, and we conclude that the starting point should be the capability of the chosen production host, and that one metabolic pathway should be introduced through extensive strain development. The choice of microbial production host should be based on the intended raw material, the robustness of the microorganism in relation to the raw material and its tolerance to adipic acid, its inherent metabolic capability concerning the pathway in question, and on detailed knowledge about the host. Although the development of efficient production host for adipic acid production is a challenging task, the successful implementation of biobased production of adipic acid will make an important contribution to the development of a biobased economy. It is hoped that this review will provide the research community with a comprehensive analysis of the state of the art regarding metabolic capabilities in this area.

Acknowledgements

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