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Research review paper

Metabolic engineering of *Saccharomyces cerevisiae* for the production of top value chemicals from biorefinery carbohydrates

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ABSTRACT

The implementation of biorefineries for a cost-effective and sustainable production of energy and chemicals from renewable carbon sources plays a fundamental role in the transition to a circular economy. The US Department of Energy identified a group of key target compounds that can be produced from biorefinery carbohydrates. In 2010, this list was revised and included organic acids (lactic, succinic, levulinic and 3-hydroxypropionic acids), sugar alcohols (xylitol and sorbitol), furans and derivatives (hydroxymethylfurfural, furfural and furandicarboxylic acid), biohydrocarbons (isoprene), and glycerol and its derivatives. The use of substrates like lignocellulosic biomass that impose harsh culture conditions drives the quest for the selection of suitable robust microorganisms. The yeast *Saccharomyces cerevisiae*, widely utilized in industrial processes, has been extensively engineered to produce high-value chemicals. For its robustness, ease of handling, genetic toolbox and fitness in an industrial context, *S. cerevisiae* is an ideal platform for the founding of sustainable bioprocesses. Taking these into account, this review focuses on metabolic engineering strategies that have been applied to *S. cerevisiae* for converting renewable resources into the previously identified chemical targets. The heterogeneity of each chemical and its manufacturing process leads to inevitable differences between the development stages of each process. Currently, 8 of 11 of these top value chemicals have been already reported to be produced by recombinant *S. cerevisiae*. While some of them are still in an early proof-of-concept stage, others, like xylitol or lactic acid, are already being produced from lignocellulosic biomass. Furthermore, the constant advances in genome-editing tools, e.g. CRISPR/Cas9, coupled with the application of innovative process concepts such as consolidated bioprocessing, will contribute for the establishment of *S. cerevisiae*-based biorefineries.

1. Introduction

In a broad sense, biorefining is described as the sustainable processing of biomass into a range of marketable biobased products and bioenergy (IEA Bioenergy, 2008). The biorefinery concept comprehends the use of a spectrum of technologies to convert renewable resources, such as lignocellulosic biomass, crude glycerol or cheese whey, into the respective building blocks that can be used for the production of biofuels, chemicals or other value-added compounds (Cherubini, 2010). Contrary to the petroleum-based refinery, where natural resources are largely exploited with tremendous waste production, biorefinery embodies a major shift by integrating systems that enable full resource usage (Cherubini, 2010). The establishment of a biorefinery fulfils two main purposes: an energy goal, which is driven by the need of renewable energy sources; and an economic goal, focusing on the development of a biobased industry capable of generating profit (Bozell and Petersen, 2010). Considering this, Bozell and Petersen (2010) presented a revised list of biobased product opportunities from renewable carbohydrates, based on the one published in 2004 by the US Department of Energy (Werpy and Petersen, 2004). Based on technological advances, the new top chemical opportunities comprise ethanol, organic acids (lactic, succinic, levulinic and 3-hydroxypropionic acids), sugar alcohols (xylitol and sorbitol), furans (hydroxymethylfurfural, furfural and furandicarboxylic acid), biohydrocarbons (isoprene), glycerol and its derivatives (Bozell and Petersen, 2010). These top value biobased chemicals were selected following specific criteria such as knowledge on conversion technology, economic value, industrial viability, size of markets and the ability of a compound to serve as a platform for the production of derivatives. These compounds have been recently the focus of a review that highlighted the recent techniques developed for

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their production (mainly chemical) within a biorefinery and the status for its commercialization (Takkellapati et al., 2018). Although the energy goal is addressed by the efforts made in the biofuel industry, fuel is considered a low value product. Despite its high volume production, it has limited returns on the funding needed to establish a biorefinery, becoming a barrier to achieve the economic goal (Bender et al., 2018). Therefore, a biorefinery able to complement biofuel production with high-value biobased products can effectively aid in the reduction of non-renewable fuel consumption and simultaneously deliver the economic incentive to expand the biorefining industry (Wu et al., 2016).

Lasure and Zhang (2003) proposed that the biorefinery future would be based on the conversion of lignocellulosic biomass into an array of useful products, where raw materials are separated into different components that can be converted into a target compound. Lignocellulosic biomass is the most available renewable resource on the planet, and it is considered an alternative to fossil carbon sources. Lignocellulosic biomass pretreatment is an essential step to break down its recalcitrant structure into cellulose, hemicellulose and lignin, enhancing the enzymatic access to cellulose and solubilization of hemicellulose into oligosaccharides and monosaccharides (Mes-Hartree et al., 1988; Romaní et al., 2014). However, this leads to the formation of inhibitory compounds like weak acids, furans and phenolic compounds (Palmqvist and Hahn-Hagerdal, 2000; Cunha et al., 2019a). In this sense, some requirements are mandatory for an integrated sustainable process: (1) identification of tolerance determinants (Gorsich et al., 2006; Mira et al., 2010; Pereira et al., 2011); (2) (over)expression of genes involved in tolerance response (Larsson et al., 2001; Cunha et al., 2015; Cunha et al., 2018a); (3) utilization of a robust microorganism able to cope with these stress factors (Tomás-Pejó et al., 2008; Pereira et al., 2014; Costa et al., 2017). Different biorefinery concepts comprising bioethanol production have been proposed (Romaní et al., 2016a, 2016b; Domínguez et al., 2017; Jesus et al., 2017; del Río et al., 2019) and significant advances have been made for the overall valorisation of lignocellulosic biomass.

In an industrial perspective, microbial production of biofuels and chemicals has been receiving increased interest, as it allows the use of renewable feedstocks and a subsequent production of building blocks at a lower cost than by traditional routes (Borodina and Nielsen, 2014). Among the compounds targeted by the chemical industry, some are not naturally produced by microorganisms or are produced with low yields and titres, with accumulation of by-products throughout the process. Advances in biotechnological production of chemicals and biofuels are motivated by innovative strategies of genetic engineering, encompassing DNA technology breakthroughs, which enable the creation of superior cell factories (Becker et al., 2015). An industrial cell factory must comprise commercial requirements for yield, productivity and titre (Hong and Nielsen, 2012). A cell factory can either be used for *de novo* synthesis, involving complex metabolic pathways to produce a compound from a simple molecule (e.g. glucose), or for biotransformation, in which a specific reaction produces a compound structurally similar to the substrate molecule. Several microorganisms including *Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae, Aspergillus niger,* among others, have been improved for commercial application (Hong and Nielsen, 2012). The yeast *S. cerevisiae* stands out as an attractive cell factory, since it is commonly used in several microbiological industrial processes, such as the production of beer, bread, wine, bioethanol, nutraceuticals, chemicals, and pharmaceuticals (Nielsen and Jewett, 2008). In addition, this yeast is generally regarded as safe (GRAS status) and has been engineered with a variety of genetic tools to be able to efficiently cope with several harsh fermentation conditions like high temperature, low pH and the presence of inhibitory compounds (Hong and Nielsen, 2012; Borodina and Nielsen, 2014). Furthermore, industrial *S. cerevisiae* isolates have received special attention due to their higher robustness, fermentation capacity and resistance to stress factors when compared with laboratory strains (Pereira et al., 2010). Particularly over the past decade, there have been joint efforts between academia and industry to develop processes for fermentation of lignocellulosic hydrolysates with engineered *S. cerevisiae* strains (Cunha et al., 2020), leading to the advent of second-generation ethanol plants, either at a demonstration or full commercial scale (Jansen et al., 2017).

Considering that ethanol has been the focus of several studies describing genetic engineering of *S. cerevisiae* to improve its production (recently and extensively reviewed by Görgens et al., 2015; Ko and Lee, 2018; Nijland and Driessen, 2020; Cunha et al., 2020), in this review we address the economic goal of the biorefinery concept, focusing on the high-value low-volume compounds previously identified by Bozell and Petersen (2010). Considering that the conversion of renewable carbon into chemicals is the most challenging and least developed step of all biorefinery operations, this review aims to enlighten the putative role of *S. cerevisiae* as a microbial cell factory for integrated biorefineries. Therefore, and bearing in mind that a decade has passed since the update of the list of top chemical opportunities from biorefinery carbohydrates, we provide an overview of the genetic engineering strategies previously applied in *S. cerevisiae* to improve the feasibility of a biorefinery implementation through the production of the identified top value compounds.

2. Top value biobased chemicals production

2.1. Organic acids: lactic, succinic, levulinic and 3-hydroxypropionic acids

Organic acids are important building block chemicals with massive market potential. They present low molecular weight, one or more acidic groups (such as carboxyl, sulfonic, alcohol, phenol, among others) and its production is mainly petroleum-based (Yin et al., 2015). Successful case studies of the different organic acids produced by engineered *S. cerevisiae* are listed in Table 1.

2.1.1. Lactic acid

Lactic acid (LA) is the most widespread hydroxycarboxylic acid in nature, being produced by many organisms by fermentation of glucose and other feedstocks (Borodina and Nielsen, 2014). LA is a valuable chemical with several applications in food, cosmetic, textile and other industries, and it is a direct product of lactate dehydrogenase (LDH), which converts pyruvate into LA (Sauer et al., 2008). LA market is evaluated in $1.25 thousand million as of 2019, with an estimated compound annual growth rate (CAGR) of 11.5% between 2020 and 2026 (Global Market Insights, 2020). Industrial-scale production of LA has been in place for a long time, starting in the late 19th century, and even though the chemical production of LA was well-established since the early 1960s (Benninga, 1990), industrial production of LA nowadays is almost entirely biotechnological (Groot et al., 2010). Several companies such as NatureWorks, Purac, Galactic, among others, produce roughly 400 thousand tonnes of LA per year (Becker et al., 2015), mostly using optimized lactobacilli and engineered yeast strains.

Generally, reported studies in LA production follow two main strategies: the expression of heterologous LDH that enables the transformation of pyruvate into LA (Yamada et al., 2017); and the attenuation or deletion of pyruvate decarboxylase (PDC) activity (Novy et al., 2017) to reduce carbon flux to ethanol (Fig. 1). Several studies combine these two strategies for superior LA production. Baek et al. (2016) reported a titre of 48.9 g/L of LA without neutralization and 112 g/L of LA in fed-batch under neutralizing conditions, using glucose as substrate. This was achieved by expressing the *ldhA* gene from *Leuconostoc mesenteroides* allied to the deletion of *PDC1* and *ADH1* genes to reduce ethanol production. Additional modifications were made to eliminate glycerol production (deletion of *GPD1* and *GPD2* genes), avoid glucose depletion (knockout of *DPD1* and *JEN1*) and increase LA tolerance by adaptive evolution and *HAA1* overexpression (strain JHY5320). Another strategy focused on the deletion of several alcohol dehydrogenase (ADH) genes combined with the expression of *ldhA* and the knockout of *PDC1, GPD1, GPD2* and *DPD1* genes. Adaptive laboratory evolution of the engineered

Table 1
S. cerevisiae metabolic engineering strategies for the production of organic acids (lactic, succinic and 3-hydroxypropionic)

Compound	Reference	Strategy	Main results	Substrate
Lactic acid				
	Lee et al., 2015	*ldh* expression; *Δpdc1, Δcyb2, Δgpd1, Δnde1 Δnde2*	117 g/L of LA under low pH conditions	Glucose
	Stovicek et al., 2015	*ldh* expression; Δ*pdc1*, Δpdc*5*	2.5 g/L of LA ($y_{LA/Glucose}$ 0.49 g/g)	Glucose
	Turner et al., 2015	Δ*ald6*, Δ*pho13*, expression of *ldhA*	49.1 g/L of LA from X ($y_{LA/Xylose}$ 0.60 g/g); 9.9 g/L of LA from G ($y_{LA/Glucose}$ 0.60 g/g)	Glucose and xylose
	Turner et al., 2016	Integration of *CDT-1, GH1-1, XYL1, XYL2, XYL3* and *ldhA*	83g/L of LA (yLA/Sugar 0.60 g/g)	Glucose, xylose, cellobiose, galactose, mannose and sucrose
	Lee et al., 2016	Δ*ssb1*	50 g/L of LA	Glucose
	Sugiyama et al., 2016	Overexpression of *ESBP6* strain carrying the *ldh* gene	5.5 g/L of LA (20% increase in LA production compared with the wild-type strain)	Glucose
	Baek et al., 2016	*ldhA* expression; Δ*dld1*, Δ*jen1*, Δ*pdc1*, Δ*adh1*, Δ*gpd1* and Δ*gpd2*; adaptive evolution and *HAA1* overexpression	48.9 g/L of LA without neutralization; 112 g/L of LA in fed-batch under neutralizing condition ($y_{LA/Glucose}$ = 0.80 g/g)	Glucose
	Baek et al., 2017	*ldhA* expression; Δ*dld1*, Δ*pdc1*, Δ*adh1-5*, Δ*gpd1* and Δ*gpd2*; adaptive evolution	82.6 g/L of LA ($y_{LA/Glucose}$ = 0.83 g/g) in fed-batch fermentation at pH 3.5.	Glucose
	Turner et al., 2017	Integration of *CDT-1, GH1*-1 and *ldhA*	23.77 g/L of LA from purified lactose ($y_{LA/Lactose}$ = 0.58 g/g); 10.3 g/L of LA from dairy milk-derived lactose ($y_{LA/Lactose}$ = 0.24 g/g); 15.6 g/L of LA from cheese whey-derived lactose ($y_{LA/Lactose}$ = 0.356 g/g).	Lactose, cheese whey, dairy milk
	Lian et al., 2018	Δ*ald6*, Δ*pho13*, Δ*leu2*, Δ*ura3* on a xylose-consuming LA-producing strain	Simultaneous gene deletion achieved, with a titre round 2 g/L of LA	Xylose
	Yamada et al., 2017	Expression of 12 glycolysis-related genes and *LDH* gene	The average LA production with 10 repeated batch fermentations was 60.3 g/L ($y_{LA/Glucose}$ 0.646 g/g)	Glucose
	Novy et al., 2017	Expression of *ldh* gene at the *pdc1* locus under control of the *pdc1* promotor; *pdc5* disrupted	33.8 g/L of LA (YLA/G 0.69 g/g)	Glucose and xylose
	Novy et al., 2018	Same strains as Novy et al., 2017	$y_{LA/Xylose}$ (0.27 g/g); $y_{LA/Glucose}$ (0.18 g/g) (anaerobic)	Glucose and xylose
	Ryu et al., 2018	Expression of acid stable enzymes in L-LA producing strain	17.4 g/L of LA (20% increase compared to parental strain)	Glucose
	Kim et al., 2019	Expression of *ldh* gene on a xylose-consuming LA-producing strain	11.15 g/L of LA (0.11 g lactic acid/g SGC before pretreatment)	Spent coffee grounds
Succinic acid				
	Raab et al., 2010	*Δsdh1, Δsdh2, Δidh1* and *Δidp1*	3.62 g/L of SA, a 4.8-fold increase ($y_{SA/Glucose}$ 0.11 mol/mol)	Glucose
	Otero et al., 2013	*Δsdh3* and *Δser3/ser33*; overexpression of *ICL1*	30-fold improvement in SA titre (0.9 g/L of SA; $y_{SA/Glucose}$ 0.05 g/g)	Glucose
	Ito et al., 2014	MATα *Δleu2 Δlys2 Δura3 Δadh1*::loxP Δadh2::*loxP Δadh3*::*loxP Δadh4*::*loxP Δadh5*::*loxP SDH1*::*loxPKlURA3-loxP SDH2*::*loxP-KlLEU2-loxP*	SA production was successfully improved, with a yield of 2.4% (C-mol of SA per C-mol glucose consumed)	Glucose
	Yan et al., 2014	Expression of *pyc2, mdh3, fumC, frdS1; Δhis3, Δfum1, Δgpd1, Δpdc1, Δpdc5* and *Δpdc6*	12.97 g/L of SA ($y_{SA/Glucose}$ 0.13 g/g)	Glucose
	Xiberras et al., 2020	Native L-G3P pathway replaced by an alternative NAD-dependent DHA pathway; overexpression of endogenous *MDH3* and heterologous *fumR* and *FRDg*; expression of the transporter DCT-02	10.7 g/L of SA ($y_{SA/Glycerol}$ 0.22 ± 0.01 g/g)	Glycerol
3-Hydroxypropionic acid				
	Chen et al., 2014	Overexpression of the enzymes *ALD6, ACSse, ADH2, ACC1, GAPN, CaMCR; ΔMLS1*	3-HP production was increased to 463 mg/L	Glucose
	Shi et al., 2014	Overexpression of $ACC1^{ser659ala,\ ser1157ala}$ and *CaMCR*	279 mg/L of 3-HP (~2.2-fold more than that of the wild-type ACC1)	Glucose and ethanol
	Kildegaard et al., 2015	Introduced malonyl-CoA or β-alanine pathways into a xylose-consuming yeast	7.37 ± 0.17 g of 3HP L−1 in 120 hours with an overall yield of 29 ± 1% Cmol 3HP $Cmol^{-1}$ xylose	Glucose and xylose
	Borodina et al., 2015	Constructing the β-alanine pathway by overexpressing *AAT2, PYC1, PYC2, ALT, BcBAPAT, EcHPDH*, and multiple copies of *TcPAND*	13.7 g/L of 3-HP was generated through the constructed β-alanine pathway from glucose in fed-batch fermentation at low pH	Glucose
	Li et al., 2015	Developing a malonyl-CoA biosensor based on the bacterial transcription factor *FapR* to monitor and precisely control the intracellular malonyl-CoA concentration (using Borodina et al., 2015 strain)	3-HP titre was enhanced by 120%	Glucose
	David et al., 2016	A hierarchical dynamic control strategy to control the expression level of *CaMCR* depending on the intracellular malonyl-CoA concentration	1 g/L of 3-HP (production was increased by 10-fold)	Glucose
	Jessop-Fabre et al., 2016	Expression of $ACC1^{S659A,S1157A}$ and *MCR*, PDC complex subunits (*E1α, E1β, E2*, and *E3*), as well as two genes involved in lipoylation of E2	Improvement in 3HP final titre of 19% over the basic strain in mineral medium, and 95% in the simulated fed-batch medium in laboratory strain; improvement of 23% in industrial strain	Glucose
	Kildegaard et al., 2016	Integration of multiple copies of *MCR* and *ACC1*-mutated genes; overexpressing of *PDC1, ALD6,* ACS^{L641P};	9.8 g/L of 3HP with a yield of 13% C-mol/C-mol glucose after 100 h in carbon-limited fed-batch cultivation at pH 5	Glucose

(*continued on next page*)

Table 1 (*continued*)

Compound	Reference	Strategy	Main results	Substrate
		engineering of the cofactor specificity of the glyceraldehyde-3-phosphate dehydrogenase		
	Chen et al., 2017a	Manipulation the phospholipid synthesis transcriptional regulators including *Ino2p, Ino4p, Opi1p*, and a series of synthetic Ino2p variants	477 mg/L of 3-HP (production was increased by 9-fold)	Glucose
	Maury et al., 2018	A subset of glucose-dependent promoters, *pADH2, pICL1,* and *pHXT7*, were studies for dynamic control of 3-HP production	Less than 0.6 g/L of 3-HP. Regulating the 3-HP pathway by the ICL1 promoter resulted in 70% improvement of 3-HP titre in comparison to PGK1 promoter	Glucose
	Lis et al., 2019	Process optimization (advances in small-scale chemostat cultivation system) using Borodina et al., 2015 strain	3-HP yields of 15.9% C-mol and 0.45 g $gCDW^{-1}$ under C-limiting, as well as 25.6% C-mol and 0.50 g $gCDW^{-1}$ under phosphate-limiting conditions	Glucose
	Ferreira et al., 2019	Fine-tuning of gene expression to enhance endogenous metabolic fluxes toward increasing levels of acetyl-CoA and malonyl-CoA	Up to 27% increase in 3-HP production	Glucose

strain led to mutations in the *SUR1* and *ERF2* genes, resulting in improved LA tolerance and production. When compared to the previous JHY5320 strain, the evolved JHY5730 strain showed an improvement of 1.7-fold in glucose consumption and 1.5-fold in LA production from 100 g/L of glucose in shake flask fermentation. Ultimately, the evolved JHY5730 strain produced 82.6 g/L of LA, with a yield of 0.83 g/g of glucose in a fed-batch strategy under acidic conditions (Baek et al., 2017). Another study highlighted the ability of *S. cerevisiae* to cope with very low pH conditions and the requirement of coupling redox balance with metabolic engineering strategies. In addition to the expression of *ldhA* and knockout of *PDC1, CYB2* and *GPD1*, the deletion of genes involved in NADH-consuming reactions of the cytosolic NADH dehydrogenase (*NDE1* and *NDE2*) increased the availability of intracellular redox cofactor, leading to the production of 117 g/L of LA (Lee et al., 2015).

The positive correlation between lignocellulosic sugars and the production of organic acids can be observed in the engineering of lignocellulosic sugar-consuming yeast to express a LDH gene. As abovementioned, *S. cerevisiae* can produce LA by the introduction of heterologous LDH. However, the three native pyruvate decarboxylase genes (*PDC1, PDC5*, and *PDC6*), which are involved in the conversion of pyruvate into acetaldehyde (Hohmann, 1991), compete with this heterologous pathway for pyruvate, leading to higher production of ethanol than LA (Colombié et al., 2003). Therefore, as seen above, a common strategy to overcome this limitation is the deletion of these genes, which may result in a deficit of C2 compounds necessary for yeast growth (van Maris et al., 2004). Nevertheless, the integration of lactate dehydrogenase gene, *ldhA*, into a *S. cerevisiae* strain previously engineered for xylose consumption led to a clear favouring of LA production over ethanol production (< 0.01 g ethanol/g xylose). This strain produced 49.1 g/L of LA with a yield of 0.60 g/g, with xylose as sole carbon source and without any of the PDC genes deleted (Turner et al., 2015). Contrarily, when glucose was the only carbon source, ethanol yield (0.31 g/g of glucose) was higher than LA yield (0.22 g/g of glucose). The authors named this phenomenon as substrate-dependent product formation. The putative molecular mechanisms associated with this phenomenon are still unclear, but some possible explanations have arisen. One is that the slower uptake of xylose compared with glucose uptake leads to a lower intracellular accumulation of pyruvate, which in turn allows for a rapid conversion of pyruvate into LA by lactate dehydrogenase before the pyruvate concentration is high enough for the lower affinity pyruvate decarboxylase to become operative. Also, it is possible that the absence of glucose signalling when cultivated on xylose leads to weaker fluxes towards effective ethanol production, which ultimately may permit to prioritize the one-step conversion of pyruvate into LA (Turner et al., 2016). Finally, recognition of extracellular glucose by glucose sensors (such as *SNF4* or *RGT2*) is known to lead to the suppression of the *JEN1* gene, coding for a LA permease, which is otherwise induced when non-fermentable carbon sources like xylose are metabolized (Andrade and Casal, 2001; Jin et al., 2004). Given this, it is plausible to think that xylose cultivation results in the upregulation of *JEN1,* which may enable a fast secretion of LA from yeast cells. As LA can act as an allosteric inhibitor of lactate dehydrogenase, the removal of LA from the cytoplasm might increase the metabolic flux toward LA production (Turner et al., 2015). In a subsequent study, the expression of *GH1-1* and *CDT-1* genes, coding for a β-glucosidase and a cellodextrin transporter, respectively, enabled cellobiose consumption, which allied to xylose consumption led to a titre of 62 g/L of LA. Contrary to a glucose-exclusive culture, a mixture of xylose/cellobiose favoured the production of LA over ethanol. When these 3 carbon sources were cultured together, with calcium carbonate neutralization, 83 g/L of LA was achieved with a yield of 0.66 g/g of sugar, while still producing ethanol simultaneously (Turner et al., 2016).

According to these findings, Novy et al. (2018) demonstrated that, in anaerobic fermentation, the LA yield on xylose (0.27 g/g of xylose) was higher than on glucose (0.18 g/g of glucose). Nevertheless, in aerobic conditions, xylose conversion to LA ceased completely, which suggests that oxygen conditions also play an important role in LA production. In xylose culture, the shift to microaerobic conditions ($\%O_2 \approx 2\%$) prevented the LA metabolization observed in fully aerobic conditions, increasing the productivity and yield. This evidence suggests that xylose does not repress the respiratory response as much as glucose, as TCA cycle reactions on xylose under aerobic conditions are stimulated, keeping the pyruvate absent from the reaction catalysed by LDH.

The necessity to improve resistance to LA itself in order to increase its production has also been addressed. An RNAi-mediated genome-wide expression knock-down approach was employed in order to quickly identify potential genetic targets that would confer increased acid tolerance to *S. cerevisiae.* Among all the identified genes, the highest increase in LA tolerance (52%) was found with the knockout of the ribosome-associated chaperone *SSB1* gene. This genetic modification in a LA-producing strain resulted in the production of over 50 g/L of LA, representing an increase of 60% when compared with the parental strain (Lee et al., 2016). Another study reported an increase of 20% in LA production by overexpressing *ESBP6* gene. Despite its unclear function, this gene appears to be involved in LA adaptation response in *S. cerevisiae*, increasing the intracellular pH in yeast (Sugiyama et al., 2016). The heterologous expression of acid stable enzymes from acidophilic archaea *Picrophilus torridus* was also found to increase glycolytic flux at low intracellular pH conditions, leading to a 20% increase in LA titre when compared to the parental strain (Ryu et al., 2018).

A one-step generation of LA-producing strains through simultaneous insertion of *ldhL* and disruption of *PDC1* and *PDC5* genes in *S. cerevisiae* genome via CRISPR/Cas9 was reported for one diploid industrial strain (Stovicek et al., 2015). Stemming on this technology, another study

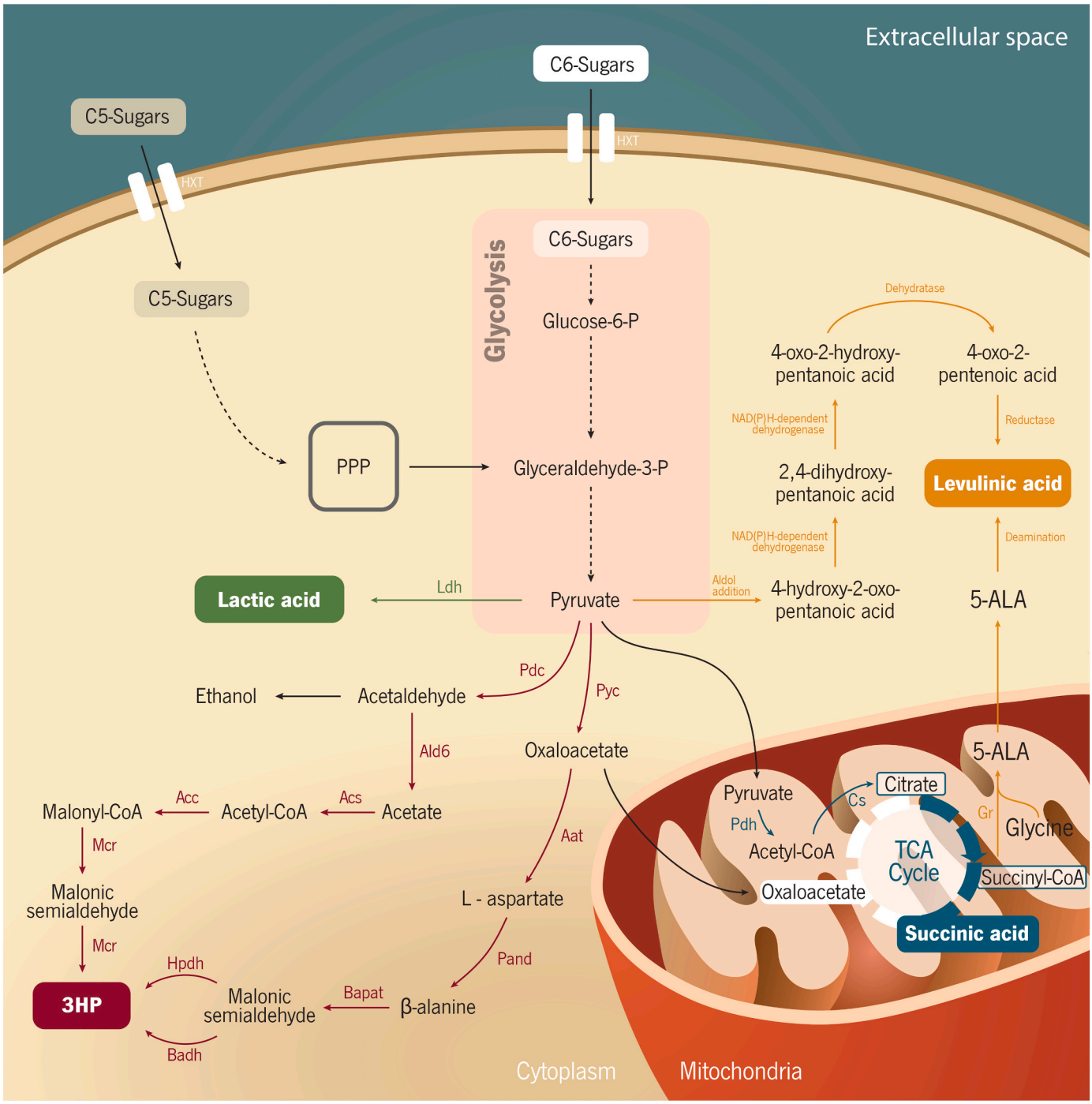

Fig. 1. Metabolic pathways for the production of organic acids in *Saccharomyces cerevisiae*: red arrows represent the reactions for the two main 3-hydroxypropionic acid pathways; green arrows for lactic acid pathway; orange arrows for levulinic acid pathway; blue arrows for succinic acid pathway. Dashed arrows indicate multiple step reactions. Abbreviations: Ldh, lactate dehydrogenase; Pyc, pyruvate carboxylase; Aat, aspartate transaminase; Pand, aspartate decarboxylase; Bapat, β-alanine–pyruvate transaminase; Hpdh, 3-hydroxypropionate dehydrogenase; Badh, 3-hydroxyisobutyrate dehydrogenase; Pdc, pyruvate decarboxylase; Ald6, aldehyde dehydrogenase; Acs, acetyl-CoA synthase; Acc, acetyl-CoA carboxylase; Mcr, malonyl-CoA reductase; Pdh, pyruvate dehydrogenase, Cs, citrate synthase; Gr, glutamyl-tRNA reductase. PPP, pentose phosphate pathway; Glucose-6-P, glucose-6-phosphate; Glyceraldehyde-3-P, glyceraldehyde-3-phosphate.

successfully knocked out four genes in a single step in diploid and triploid strains to increase LA titre in xylose-consuming LA-producing strains (Lian et al., 2018).

LA production from lignocellulosic biomass has been already reported, where a xylose-consuming strain was successfully engineered with a heterologous LDH. The recombinant strain produced 11.15 g/L of LA from spent coffee grains whole slurry, obtained by acid-pretreatment (Kim et al., 2019). Another study reported LA production from lactose. Stemming on the ability of CDT-1 transporter (*CDT-1*) to transport lactose into the cell, a strain expressing both *CDT-1* and *GH1-1* (β-glucosidase with β-galactosidade activity) alongside *ldhA* successfully produced LA from lactose, either purified (23.77 g/L) or derived from dairy milk (10.3 g/L) or cheese whey (15.6 g/L). This is relevant for the valorisation of industry by-products such as contaminated milk or cheese whey itself (Turner et al., 2017) or even for multi-waste valorisation approaches targeting lignocellulosic residues and whey (Cunha

et al., 2018b). Overall, LA production is a very well-established process in an industrial context, even though there is room for increasing its rates and yield. Nonetheless, the necessity of the industry for high purity LA drives the effort for continuous improvement of efficient and economically feasible methods for downstream processing (Ahmad et al., 2020).

2.1.2. Succinic acid

Succinic acid (SA) is a valuable platform chemical with huge potential as a building block for several products such as butanediol or nylon-type polymers (Becker et al., 2015), as well as acidity regulator in the food and beverage industry (Borodina and Nielsen, 2014). As of 2019, SA market is valued at $185.6 million, with a CAGR of 15.7% for the upcoming 2020-2026 period (PR Newswire, 2020). SA can be produced either by chemical or biotechnological synthesis. Several chemical processes have been developed in the past, such as electrolytic reduction of maleic acid or maleic anhydride, or paraffin oxidation and catalytic hydrogenation (Muzumdar et al., 2004; Cok et al., 2014). Due to its high conversion yield and efficiency, biotechnological production of SA is considered to be economically feasible and competitive in comparison to its petrochemical synthesis (Hermann et al., 2007). The annual manufacture of microbial-based SA in Europe and North America represents already nearly half of worldwide overall production (Kumar et al., 2020a). The bacteria *E. coli* (Wang et al., 2011) and *Corynebacterium glutamicum* (Okino et al., 2008; Litsanov et al., 2012) are the most used microorganisms as recombinant hosts for SA production, yielding concentrations of SA from glucose above 100 g/L. The yeast *Yarrowia lipolytica* has also been successfully used for SA production from glycerol, with a maximum reported titre of 198.2 g/L (Li et al., 2017). Several companies, such as Biosuccinium, already commercialize microbial-based SA, using *S. cerevisiae* strains to produce SA from starch/sugar (Mancini et al., 2019).

SA is an intermediate of the tricarboxylic acid (TCA) cycle, naturally occurring in *S. cerevisiae* (Fig. 1). Strategies to enable SA accumulation rely on the deletion of genes encoding for succinate dehydrogenase (SDH) subunits (*SDH1, SDH2, SDH3* and *SDH4*), which leads to an increase in SA production in aerobic conditions by interrupting the TCA cycle and, therefore, avoiding SA depletion (Kubo et al., 2000). Raab et al. (2010) reported a production of 3.62 g/L of SA from glucose by deleting *SDH1* and *SDH2* allied to the elimination of *IDH1* and *IDP1*, both encoding for isoenzymes of isocitrate dehydrogenase. This enabled a redirection of carbon flux to the glyoxylate cycle that ultimately allowed the accumulation of SA as final product, representing a 4.8-fold increase when comparing to the wild-type strain. In another study, the production of SA was 30-fold improved, compared to the wild-type strain. The combination of directed evolution strategies with the knockout of *SDH3* gene, overexpression of native *ICL1* (encoding isocitrate lyase), and deletion of the genes encoding both isoenzymes encoding 3-phosphoglycerate dehydrogenase (*Ser3p/Ser33p*) led to the interruption of serine formation from glycolysis. L-serine is required for biomass formation, and can be alternatively synthesized from L-glycine, which is coupled to SA production through the glyoxylate pathway. By its turn, glyoxylate is produced from isocitrate, by isocitrate lyase action, producing equimolar amounts of succinate. Therefore, as biomass increases, so does the demand for L-glycine and L-serine, which ultimately leads to a SA production process coupled with biomass (Otero et al., 2013). Ito et al. (2014), in addition to the deletion of *SDH1* and *SDH2* genes, accomplished the elimination of ethanol biosynthesis pathways (*ADH1* to *ADH5* genes knockout), which led to an accumulation of intracellular SA. By enhancing SA export to the outside of the cell through heterologous expression of *mae1* gene (encoding a malic acid transporter), a SA yield of 2.4% (C-mol per C-mol glucose consumed) was attained.

Another study based its strategy on the deletion of PDC genes, blocking ethanol production and channelling carbon flux through the TCA cycle, allied to the knockout of *FUM1* and *GPD1* to avoid malate and glycerol production, respectively, and lastly expressing *PYC2* to boost SA production from pyruvate. This led to the production of 12.97 g/L of SA, under optimal supplemental CO_2 conditions in a bioreactor (Yan et al., 2014). Recently, Xiberras et al. (2020) reported the production of SA from glycerol. For full exploitation of the higher reducing power of glycerol, the authors used a previously engineered strain with the native L-glycerol 3-phosphate (L-G3P) pathway replaced by an alternative NAD-dependent DHA pathway for glycerol catabolism (Klein et al., 2016). This was followed by the (over)expression of the endogenous peroxisomal malate dehydrogenase (*MDH3*), which is responsible for oxaloacetate reduction, the heterologous cytosolic fumarase (*fumR*) from *Rhizopus oryzae* for conversion of malate to fumarate, and the peroxisomal fumarate reductase (*FRDg*) from *Trypanosoma brucei* for fumarate reduction. These modifications together with the expression of the dicarboxylic acid transporter DCT-02 from *Aspergillus niger* resulted in a maximum SA titre of 10.7 g/L and a yield of 0.22 g/g of glycerol in shake batch culture. The shift to an exclusive microbial-based SA production is still pendent on further improvements focusing on economic competitiveness against the petrochemical synthesis. Process optimization and use of low-cost raw materials (Kumar et al., 2020a) such as lignocellulosic materials or crude glycerol can help in attaining this goal, and *S. cerevisiae* can indeed play an important role in this field. Nonetheless, the titres reported so far are still very low when compared to the production levels attained with several other microorganisms, and additional investigation of metabolic mechanisms and reactions should be an immediate priority.

2.1.3. Hydroxypropionic acid

3-Hydroxypropionic acid (3HP) is a carboxylic acid with enormous market potential as a precursor for acrylic acid (Becker et al., 2015). 3HP comprises two functional groups, a carboxyl group and a β-hydroxyl group, showing potential as a versatile platform to produce a vast assortment of high value-added compounds. Several compounds can be produced from 3HP such as 1,3-propanediol, acrylic acid, acrylamide, propiolactone, malonic acid, among others (Matsakas et al., 2018), which in turn have a wide range of applications in manufacturing adhesives, polymers, fibres, cleaning agents and resins. Efforts for 3HP production rely mainly on glycerol and glucose, with the first one being the most straightforward approach by only requiring the sole activities of glycerol dehydratase and aldehyde dehydrogenase. Stemming on this glycerol-based approach, several microorganisms have been used as hosts for 3HP production, from where *E. coli* (Chu et al., 2015) and *Klebsiella pneumoniae* stand out as the more promising in this field, with the latter having the highest titre reported (83.8 g/L; Li et al., 2016). Regarding its production from glucose, Chen et al. (2017a) reported the highest titre to date (62.6 g/L) using a recombinant *C. glutamicum*. There are many different pathways described to produce 3HP from glucose, with research studies on *S. cerevisiae* 3HP production focusing mainly on malonyl-CoA or β-alanine pathways (Fig. 1) (Chen and Nielsen, 2016). The malonyl-CoA pathway is based on the expression of heterologous malonyl-CoA reductase (*MCR*). Chen et al. (2014) increased acetyl-CoA accumulation by blocking its consumption and increased NADPH formation by multiple gene overexpression and *MLS1* deletion, simultaneously with the overexpression of *ACC1* catalysing acetyl-CoA to malonyl-CoA, which led to the production of 463 mg/L of 3HP from glucose. Another study focused on the downregulation of lipid synthesis combined with the study of inositol and choline effect, resulted in 477 mg/L of 3HP (Chen et al., 2017b). Intracellular malonyl-CoA pool is critical for the production of 3HP via the malonyl-CoA pathway, as shown in a study that reports that acetyl-CoA carboxylase, crucial for malonyl-CoA synthesis, can be upregulated by eliminating phosphorylation inhibition, which improved 3HP production by 2.2-fold (Shi et al., 2014). Kildegaard et al. (2016) showed that integration of multiple copies of *MCR* and phosphorylation-deficient acetyl-CoA carboxylase $ACC1^{S659A,S1157A}$ genes improved 3HP titre by 5-fold, when compared with single integration, despite a reduced growth rate of the strains

expressing the malonyl-CoA route. After optimization of acetyl-CoA supply and NADPH formation, the resultant strain was able to produce 9.8 g/L of 3HP in fed-batch cultivation at pH 5.

The influence of gene expression levels has also been a subject of study. Based on a hierarchical dynamic control system around malonyl-CoA, a novel malonyl-CoA biosensor was used to activate expression of a heterologous malonyl-CoA pathway from *Chloroflexus aurantiacus.* This strategy led to a 10-fold increase in 3HP production (David et al., 2016). The effect of different promoters induced in glucose-limiting conditions was also assessed, where *ILC1* promoter was found to improve the titre of 3HP by 70% in comparison to *PGK1* promoter (Maury et al., 2018).

Regarding the β-alanine pathway, the (over)expression of several native and heterologous genes coupled with the production of a novel β-alanine-pyruvate aminotransferase discovered in *Bacillus cereus*, resulted in 13.7 g/L of 3HP in fed-batch conditions under low pH (Borodina et al., 2015). This strain was later on used in a couple of different studies focusing on the (1) development of a malonyl-CoA biosensor to control its intracellular concentration, which increased 3HP titre from glucose by 120% (Li et al., 2015, 2) process optimization in small-scale chemostat cultivation, yielding 15.9% C-mol and 0.45 g/g of cell dry weight under carbon-limiting conditions, using also glucose as substrate (Lis et al., 2019).

Production of 3HP from xylose was also reported, either through malonyl-CoA or β-alanine pathways (using two different approaches, a NADH-dependent β-alanine pathway and a NADPH-dependent β-alanine pathway). By introducing the three pathways separately into a previously engineered xylose-consuming *S. cerevisiae* strain, it was observed that the malonyl-CoA pathway was the better choice, when using glucose as substrate. Conversely, the NADPH-dependent β-alanine pathway yielded highest 3HP production from xylose, resulting in 7.37 g/L of 3HP in 120h under fed-batch cultivation using xylose as sole carbon source (Kildegaard et al., 2015). Despite being relatively low when compared with the overall panorama of 3HP production, these values are very promising in the biorefinery context, as glucose and xylose are two of the major sugars found in lignocellulosic biomass.

A study showed a highly efficient method based on the CRISPR/Cas9 technology where an industrial diploid *S. cerevisiae* strain was engineered for expression of *MCR* and $ACC1^{S659A,S1157A}$ coupled to the overexpression of the cytoplasmic pyruvate dehydrogenase (PDH) complex, leading to an improvement of 23% in 3HP production over the strain without the insertion of the PDH complex (Jessop-Fabre et al., 2016). Stemmed on the ability of dCas9-based strategies for fine-regulation of gene expression, Ferreira et al. (2019) reported novel fine-tuning setups that improved endogenous metabolic fluxes toward increasing levels of acetyl-CoA and malonyl-CoA. The highest boost in 3HP production was attained by targeting the gene encoding adenylate kinase 1, *ADK1*, resulting in an improvement of 36% when compared to the control strain where no guide RNA was added. Even though the already reported approaches led to titres above 10 g/L of 3HP in yeast, this is still distant from the over 100 g/L believed to be commercially viable (Kumar et al., 2013). Nevertheless, ongoing optimization of the known metabolic pathways allied to the arisen of novel and more efficient separation techniques can aid to increase 3HP titres (Ji et al., 2018), making its microbial-based production feasible at an industrial scale in a near-future.

2.1.4. Levulinic acid

Levulinic acid is a linear five-carbon keto acid, being considered a valuable commodity chemical. Levulinic acid-derived chemicals are used in a wide range of industries like solvents, electronics, textiles and pharmaceutical products, among many others (Signoretto et al., 2019). Global market size of levulinic acid is around $26.3 million as of 2018, expecting to reach $34.5 million by 2024, representing a CAGR of 4.9% in the forecast period between 2019 and 2024 (Kumar et al., 2020b). The main worldwide producer of levulinic acid is GF Biochemicals Ltd., which developed a novel technology for production, recovery and purification of this acid (Kumar et al., 2020b). Levulinic acid can be produced by chemical conversion processes from diverse renewable raw materials, like starch-rich waste and lignocellulosic biomass, or precursors such as HMF and furfural (Morone et al., 2015). The most common approach is the dehydration of biomass or carbohydrates through an acid treatment (Cha and Um, 2020). So far, to the best of our knowledge, no studies have reported biological production of levulinic acid. The same does not apply to its derivative 5-aminolevulinic acid (5-ALA), an industrial fine chemical with important physiological functions in humans and other organisms, such as acting as a substrate for heme biosynthesis (Hara et al., 2019). 5-ALA can be chemically synthesized, but is also produced biologically in animal cells, photosynthetic bacteria or algae, among others. 5-ALA can then be converted into levulinic acid by deamination (Morone et al., 2015). Its production has been conveyed since several decades ago in a vast range of microorganisms like *Chlorella sp*. (Beale, 1970), *Rhodobacter sphaeroides* (Sasaki et al., 2002) or recombinant *E. coli* (van der Werf and Zeikus, 1996), among others. More recently, Hara et al. (2019) have published the production of 5-ALA using an engineered *S. cerevisiae* strain, by overexpressing *HEM1* and *ACO2,* encoding for 5-ALA synthetase and aconitase, respectively, resulting in the production of 1.36 mg/L of 5-ALA. To date, the only report on levulinic acid production via metabolic engineering is a patented six-step alternative single pathway, from C5- or C6-sugars through pyruvate (Fig. 1), with potential application in a wide range of microorganisms such as *Pseudomonas sp., Escherichia coli, Saccharomyces sp*., *Pichia sp*. or *Bacillus sp*., in addition to cell-free systems (Zanghellini, 2012). Altogether, these findings may help to establish *S. cerevisiae* as a viable host for an environmental-friendly process for levulinic acid production.

2.2. Sugar alcohols: sorbitol and xylitol

2.2.1. Sorbitol

Sorbitol, also known as D-glucitol, is the sugar alcohol with the largest volume market (estimated to be 1.8 million tonnes/year) (De Jong et al., 2020). Due to the sweetness profile similar to sucrose and low caloric content, it is mainly used as a sweetener in a wide range of food products, being also used in pharmaceutical and nutraceutical formulations (Isikgor and Becer, 2015; Zhang et al., 2013). Additionally, sorbitol can be used for the industrial production of ascorbic acid (vitamin C) and for the synthesis of functional derivatives, such as glycerol, propylene glycol, ethylene glycol, ethanol and methanol, which can be further used in the manufacture of other attractive compounds (Isikgor and Becer, 2015). Sorbitol is currently manufactured on a large scale by several companies through the chemical hydrogenation of glucose obtained from the enzymatic hydrolysis of food crops (corn, cassava and wheat) (Rosales-Calderon and Arantes, 2019). Although the chemical process is well established and fully implemented, the biotechnological production of sorbitol has also been investigated. Some studies focusing on *Zymomonas mobilis* and *Lactobacillus plantarum* reported the production of sorbitol from fructose and glucose, respectively (Silveira and Jonas, 2002; Ladero et al., 2007). In *S. cerevisiae*, the native *SOR1* gene encodes a sorbitol dehydrogenase responsible for the conversion of sorbitol to fructose (using NAD^+ as cofactor) that also catalyses the reverse reaction (Sarthy et al., 1994; Jain et al., 2011). Duvnjak et al. (1991a) reported the production of sorbitol and ethanol in a fructose medium using a mutant of *S. cerevisiae* (ATCC 36859) lacking hexokinase activity. Taking into account that Jerusalem artichokes contain a large amount of fructose, the authors used this substrate to produce sorbitol. The fermentation of Jerusalem artichokes juice supplemented with yeast extract resulted in 2.19 g/L of sorbitol (Duvnjak et al., 1991b). An intergeneric protoplast fusion method was also proposed to produce sorbitol directly from Jerusalem artichokes. The stable fusant between *Kluyveromyces* sp. Y-85 (with high activity of inulinase that enables the use of inulin from Jerusalem artichokes) and *S. cerevisiae* E-15 (reported as a sorbitol high-producing strain) produced

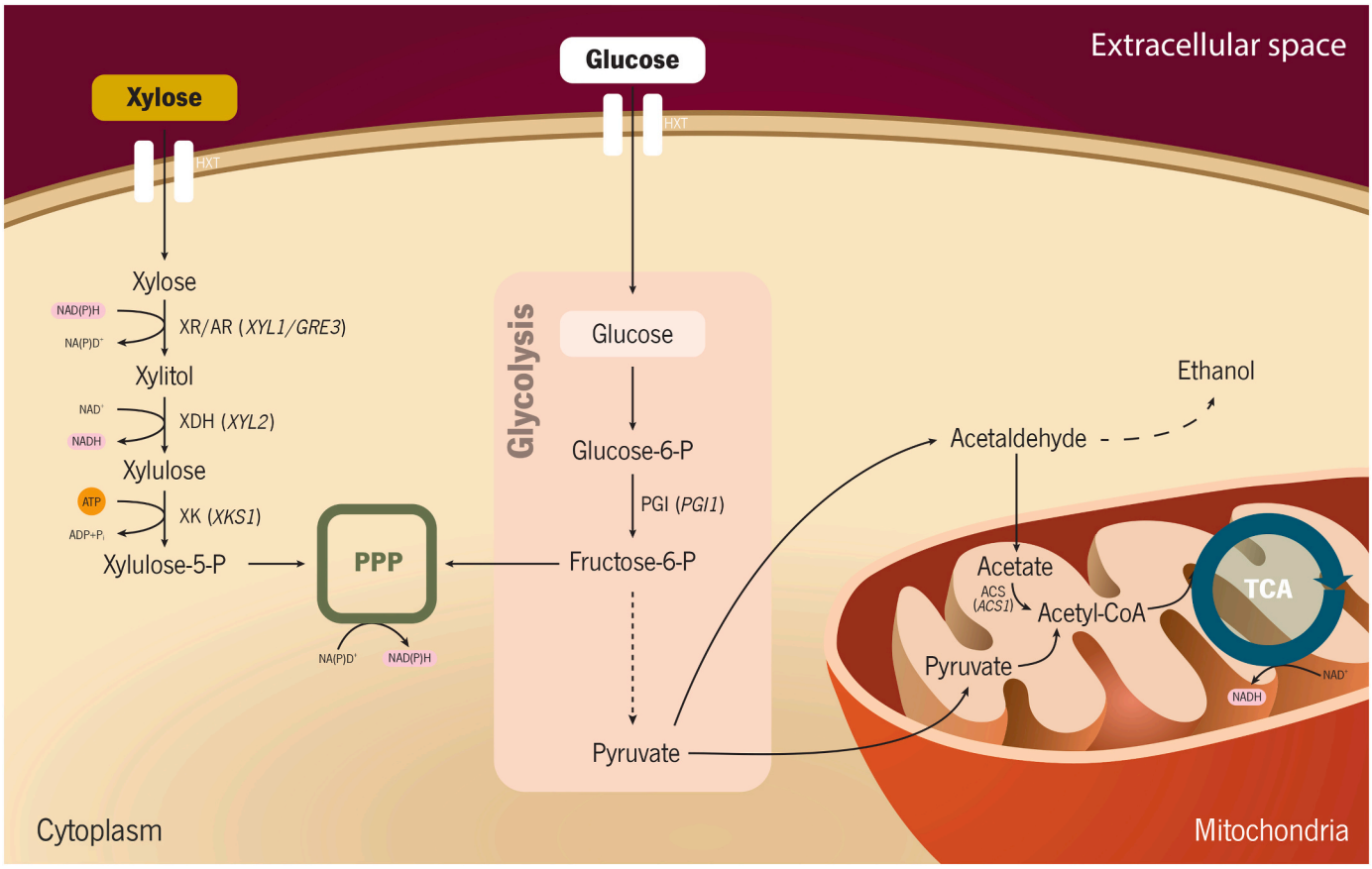

Fig. 2. Metabolic pathways involved in xylitol production by yeast and introduced genetic modifications. Dashed arrows indicate multiple step reactions. Abbreviations: XR, xylose reductase; AR, aldose reductase; XDH, xylitol dehydrogenase; XK, xylose kinase; PPP, pentose phosphate pathway; PGI, phosphoglucose isomerase.

48.7 g/L of sorbitol under optimal fermentation conditions (Wei et al., 2001). In a different approach, aiming to eliminate glycerol as a fermentation by-product and replace it with other metabolites, the production of sorbitol (4.39 g/L) was achieved by expression of the *E. coli srlD* gene (encoding for a sorbitol-6-phosphate dehydrogenase) in a *S. cerevisiae* strain unable to produce glycerol (*gpd1*Δ*gpd2*Δ double mutant) (Jain et al., 2011).

Presently, few biotechnological processes have been proposed for sorbitol production. In fact, the fermentative routes for the production of sorbitol cannot yet compete with the current catalytic hydrogenation process, which efficiently yields sorbitol (~99% reaction yields) and fulfils the requirements of the food industry. However, with the introduction of the concept of sustainable production, research efforts in biomass conversion and design of microorganisms capable to efficiently ferment streams with multiple compounds are being pursued, which may lead to greening opportunities in sorbitol production.

2.2.2. Xylitol

Xylitol is a five-carbon sugar alcohol widely used as a low-calorie sweetener in food and beverage products (bakery, confectionery, dairy), as well as in functional foods for diabetes management. The growing concerns about adverse health impacts related with excessive sugar consumption are increasing the demand for sugar substitutes. Consequently, xylitol market is expanding and is expected to reach $1.37 thousand million by 2025 with a price of $4000-5000 per tonne (Hernandéz-Peréz et al., 2019). In addition, xylitol has received special attention due to its anticariogenic properties, being therefore predominantly used in chewing gum and dental care products (Janakiram et al., 2017; Salli et al., 2019). It is also used in the chemical industry as intermediate for the synthesis of ethylene and propylene glycol, lactic acid, glycerol, xylaric and xylonic acids and polymers (Hernandéz-Peréz et al., 2019; Isikgor and Becer, 2015; Werpy and Petersen, 2004). This platform chemical can be obtained from xylose in a single-step bioconversion and represents an example of a promising value-added chemical produced from lignocellulosic biomass. In fact, the use of xylose extracted from corn cob feedstocks for xylitol production is already industrially implemented (De Jong et al., 2020). The production process involves the acid pretreatment of lignocellulosic biomass for xylan decomposition into monomeric xylose. The resulting xylose-enriched hydrolysates are purified using exchange chromatography and activated carbon prior to the catalytic hydrogenation of xylose to xylitol, since the chemical conversion requires pure xylose (Dasgupta et al., 2017). This production process, in spite of using a renewable raw material, is far from being environmentally friendly and requires considerable energy and materials consumption (Rosales-Calderon and Arantes, 2019). Bioconversion approaches based on whole-cell biocatalysts aim to reduce the chemical inputs and have the inherent advantage of directly using hemicellulose hydrolysates, since microbial conversion does not require isolated and purified xylose, eliminating the need for the costly purification steps prior to xylose reduction (Bozell and Petersen, 2010).

Considerable research efforts have focused on native xylose-utilizing yeasts (*Candida, Debaromyces, Kluveromyces, Pichia* sp). Xylose assimilation capacity is conferred by the oxidoreductase pathway in which two enzymes, xylose reductase (XR) and xylitol dehydrogenase (XDH), are used to convert D-xylose to D-xylulose (Fig. 2). Xylitol is the first intermediate of this pathway and its accumulation is caused by the cofactor imbalance between xylose reductase (XR) and xylose

Table 2
Metabolic engineering strategies for xylitol production in *S. cerevisiae*

Strain	Genetic modification	Substrate/ cultivation strategy	Xylitol			Reference
			Titre g/L	Productivity g/L·h	Yield g/g	
GPY55-15Bα + pMA91	Expression of *XYL1* (coding for xylose reductase) from *Scheffersomyces stipitis*	Xylose + glucose, Batch	19	NR	> 0.95	Hallborn et al., 1991
H475		Xylose + glucose, ethanol, acetate and glycerol, Batch	NR	NR	≈ 1	Hallborn et al., 1994
EH13.15:pY2XR		Xylose + glucose, Fed-batch	105	1.69	> 0.95	Lee et al., 2000
BJ3505/δXR		Xylose + glucose, Fed-batch	78	1.1	0.9	Chung et al., 2002
		Xylose + glucose, Fed-batch and cell-recycling fermentation	116	2.34	0.9	Bae et al., 2004
Y294:pRG16	Expression of *XYL1* from *Candida shehatae*	Xylose + glucose, Batch	15	NR	0.86	Govinden et al., 2001
DXXA	*XYL1* gene from *Scheffersomyces stipitis*; *AraE* (arabinose: H^+ symporter) from *Bacillus subtilis*	Xylose + glucose, Fed-batch	178	2.47	≈ 1	Kim et al., 2017
D-10-BT	*XYL1* (xylose reductase) from *Scheffersomyces Stipitis; CDT-1* (cellodextrin transporter); *GDH1-1* (ß-glucosidase) from the *Neurospora crassa*	Xylose + cellobiose, Fed-batch	93	1.50	0.98	Oh et al., 2013
SR8#22	Evolved strain mutated in *GLK1* (glucokinase), *HXK1* (hexokinase 1) and *HXK2* (hexokinase 2) genes, expressing *XYL* (xylose reductase)		21	NR	≈ 1	Lane et al., 2018
PE-2-GRE3	*GRE3* (endogenous aldose reductase)	Xylose + glucose, Fed-batch	148.5	1.16	0.95	Baptista et al., 2018
		Corn cob whole slurry, Simultaneous Saccharification and fermentation	30	0.54	0.95	
		Hardwood xylan, Simultaneous Saccharification and Fermentation	24	0.38	≈ 1	Romaní et al., 2020
ScpGT	*GRE3* (endogenous aldose reductase) and *SUT1* (xylose specific transporter)	Xylose + glucose, Fed-batch	21	0.34	≈ 1	Kogje and Ghosalkar, 2016
		Corn cob hydrolysate + glucose, Fed-batch	22	NR	≈ 1	
DWM-ZWF1-ACS1	*XYL1* (xylose reductase, NADPH-dependent) from *Scheffersomyces stipitis; ΔXYL* (mutant xylose reductase, NADH-preferring); *ZWF1* (glucose-6-phosphate dehydrogenase); *ACS1* (acetyl-CoA synthetase)	Xylose + glucose, Fed-batch	196	4.27	≈ 1	Jo et al., 2015
D-10-BT	*XYL1* (xylose reductase) from *Scheffersomyces stipitis; CDT1* (cellodextrin transporter); *GDH11*(ß-glucosidase) from the *Neurospora crassa*	Xylose + cellobiose, Fed-batch	93	1.50	0.98	Oh et al., 2013
XP-RTK	*XYL1* (xylose reductase) from *Scheffersomyces stipitis, Candida Tropicalis, Neurospora crassa* and *SUT1* (xylose specific transporter)	Glycerol, corn cob hydrolysate Fed-batch	47	0.37	≈ 1	Kogje and Ghosalkar, 2017
YPH499-XR-BGL-XYL-XYN	*XYL1 (xylose reductase) from Scheffersomyces stipitis, BGL (β-glucosidase) from Aspergillus aculeatus, XylA (β-xylosidase) from Aspergillus oryzae and XYN (endoxylanase II) from Trichoderma reesei*	Rice straw hydrolysate, CBP	6	NR	≈ 1	Guirimand et al., 2016
		Rice straw hydrolysate (membrane-filtrated), CBP	38	NR		
YPH499-XR-BGL-XYLsss-XYNsss	*XYL1* (xylose reductase) from *Scheffersomyces stipitis, BGL* (β-glucosidase) from *Aspergillus aculeatus, XylA* (β-xylosidase) from *Aspergillus oryzae* and *XYN* (endoxylanase II) from *Trichoderma reesei, SED1* promoter, secretion signal and *anchoring domain*	Rice straw hydrolysate, CBP	7	NR	≈ 1	Guirimand et al., 2019a
		Kraft pulp, CBP	4	NR		

dehydrogenase (XDH) enzymes. XR presents dual cofactor dependence but uses NADPH over NADH, whereas XDH is NAD^+ dependent. The differences in cofactor specificity in the XR and XDH reactions and the generation of NADPH by the pentose phosphate pathway (PPP) limit the availability of NAD^+ for the oxidation of xylitol to xylulose, resulting in xylitol secretion (Kötter and Ciriacy, 1993; Quehenberger et al., 2019). Among different xylose-utilizing yeasts, *Candida* species present the best xylitol production capacity (Barbosa et al., 1988; Dasgupta et al., 2017) and some process and metabolic engineering approaches have already proven to be successful for enhancing overall titres and productivities (Kim et al., 2004; Ko et al., 2006; Kwon et al., 2006). However, xylitol yields are limited by the use of xylose as carbon source for cell growth and maintenance energy. Given this context, the use of *S. cerevisiae*, which is naturally incapable of metabolizing xylose, is an appealing approach to enhance xylitol yields. The expression of genes coding for enzymes with XR activity allows the direction of xylose flux only for bioconversion and the xylitol produced is not further oxidized to xylulose and metabolized by this yeast. Consequently, engineered strains require a co-substrate for cell growth and metabolism but also to regenerate cofactors, essential for the NAD(P)H-dependent XR enzyme catalysis.

Initial attempts to produce xylitol in *S. cerevisiae* relied on the

expression of *XYL1* gene from the xylose-fermenting *Scheffersomyces stipitis* (formerly known as *Pichia stipitis*) coding for XR, which increased the yields near to the theoretical maximum (1 g of xylitol per gram of xylose) (Hallborn et al., 1991). The recombinant *S. cerevisiae* expressing *XYL1* gene was also evaluated for xylitol production using different co-substrates (glucose, ethanol, acetic acid and glycerol) and only glucose and ethanol were efficiently used (Hallborn et al., 1994). Similarly, the use of glucose improved the xylitol production by the recombinant *S. cerevisiae* expressing the *XYL1* gene from *Candida shahatae*, in comparison with the results obtained using galactose and maltose as co-substrate (Govinden et al., 2001). Nevertheless, the use of glucose as a co-substrate inhibits the transport of xylose into the cells (Subtil and Boles, 2012) decreasing xylitol productivity. A commonly used strategy for improving the xylose uptake in the presence of glucose is using a high molar ratio of xylose to glucose during the bioconversion phase. This glucose-limited fed-batch fermentation strategy has already proven to be successful for cofactor regeneration and to generate maintenance energy without glucose repression, resulting in high productivities and yields of xylitol (Table 2) (Bae et al., 2004; Chung et al., 2002; Lee et al., 2000). Another approach to bypass glucose repression and improve xylose transport relied on the expression of the *B. subtilis araE* gene coding for an arabinose:H^+ symporter, which has been previously proven to enhance xylose transport capacity in *S. cerevisiae* (Wang et al., 2013). The expression of *araE* together with the expression of *XYL1* gene from *S. stipitis*, increased the xylitol productivity to 2.47 g/L·h (Kim et al., 2017). Alternatively, Oh et al. (2013) efficiently produced xylitol without glucose repression through the utilization of cellobiose, a dimer of glucose. For this, a recombinant *S. cerevisiae* strain expressing a xylose reductase from *S. stipitis* was further engineered for cellobiose utilization by the expression of the *CDT-1* and *GH1-1* genes (from the filamentous fungus *Neurospora crassa*) coding for a cellodextrin transporter and intracellular ß-glucosidase, respectively. The resulting strain showed cellobiose and xylose co-consumption and higher xylitol productivity, compared to sequential utilization of glucose and xylose (Oh et al., 2013). More recently, adaptive evolution followed by genome sequencing of the evolved strains coupled with reverse engineering strategies showed that reduced glucose phosphorylation rates led to simultaneous glucose and xylose utilization, improving the xylitol production (Lane et al., 2018).

In addition, some research efforts aimed to improve the availability of reduced cofactors for XR activity, which is an important controlling factor for xylitol production. To understand the role of different enzymes and cofactors preference, an industrial *S. cerevisiae* was engineered to produce a wild-type XR (NADPH-preferable) and a mutant XR (NADH-preferable) from *S. stipitis,* and also to overproduce the endogenous aldose reductase (NADPH-dependent) encoded by the *GRE3* gene. The strains producing enzymes with NADPH preference showed an improved xylitol production using glucose as co-substrate, and the *GRE3*-overexpressing strain was able to produce 148.5 g/L of xylitol with a 0.95 g/g yield (Baptista et al., 2018). Also, the overexpression of the *GRE3* gene resulted in higher xylitol titre and productivity over the expression of the XR encoding gene from *S. stipitis, C. tropicalis* or *N. crassa* (Kogje and Ghosalkar, 2016). As aforementioned, XR enzymes show a preference for NADPH over NADH and the main source of NADPH in yeast cells is the oxidative PPP. To overcome the preference for NADPH and allow the simultaneous utilization of NADPH and NADH cofactors, the co-expression of wild and mutant *S. stipitis XYL1* genes enhanced the xylitol yield and productivity. This strain was further engineered to increase the intracellular concentrations of NADPH and NADH cofactors by the overexpression of both *ZWF1* and *ACS1* genes encoding for glucose-6-phosphate dehydrogenase (G6PDH) and acetyl-CoA synthetase. The overproduction of the G6PDH increases the flux through PPP, responsible for NADPH production, and acetyl-CoA synthetase can contribute for cofactor regeneration since it is responsible for the conversion of acetate to acetyl-CoA, which is further metabolized in the TCA cycle, generating NADH. This engineered strain produced 196.2 g/L of xylitol with remarkable productivity of 4.27 g/L·h (Jo et al., 2015). Another metabolic engineering approach to further increase the NADPH availability focused on the redirection of the carbon flux to the PPP, limiting the carbon flux into glycolysis. The downregulation of the *PGI1* gene, coding for phosphoglucose isomerase (PGI) reduces the conversion of glucose-6-phosphate to fructose-6-phosphate in glycolysis first step, resulting in glucose-6-phosphate accumulation, which can be used by G6PDH in the PPP. The reduction of PGI activity alone was not successful, except with the simultaneous overexpression of the *ZWF1* gene that improved the xylitol productivity by 1.9-fold when compared with the parental strain expressing only the *XYL1* gene (Oh et al., 2007).

Much progress has been made in the production of xylitol from lignocellulosic-derived xylose. Kogje and Ghosalkar (2016) engineered *S. cerevisiae* to produce xylitol from a non-detoxified corn cob hydrolysate supplemented with synthetic glucose in fed-batch mode. The recombinant ScpGT strain expressing the *SUT1* gene, coding for a specific xylose transporter and overexpressing the *GRE3* gene produced 22.4 g/L of xylitol. The dilution of the hemicellulosic hydrolysate decreased the xylose concentration from 65 g/L to 40 g/L enabling also the mitigation of negative effects caused by the presence of lignocellulose-derived inhibitors (Kogje and Ghosalkar, 2016). The same authors expressed both *GRE3* and *SUT1* genes in an industrial strain to produce xylitol from a detoxified corn cob hydrolysate, using glycerol as co-substrate. The recombinant *S. cerevisiae* XP-RTK efficiently produced 47 g/L of xylitol with a maximal productivity of 0.37 g/L·h (Kogje and Ghosalkar, 2017). In fact, the bioconversion process might profit from the replacement of glucose by glycerol, which prevents catabolite repression and ethanol fermentation, favouring the biomass production. More recently, Baptista et al. (2018) developed an integrated process to produce xylitol through the valorisation of both cellulose and hemicellulose fractions of corn cob. The industrial *S. cerevisiae* PE-2 strain, naturally prone to xylitol accumulation (Romaní et al., 2015) and presenting a suitable background to cope with the presence of lignocellulosic-derived inhibitors (Pereira et al., 2014) was engineered by overexpression of the *GRE3* gene. The corn cob whole-slurry, liquid (hemicellulosic hydrolysate) and solid fractions obtained from the corn cob autohydrolysis pretreatment, were used in a Simultaneous Saccharification and Fermentation (SSF) process. The glucan-enriched solid phase was efficiently hydrolysed by an enzymatic cocktail, providing glucose for cell growth and metabolism during the bioconversion of xylose (from the non-detoxified hydrolysate) into xylitol. This sustainability-based approach resulted in 29.6 g/L of xylitol with a maximal productivity of 0.54 g/L·h and demonstrated the feasibility of using whole slurry corn cob for xylitol production (Baptista et al., 2018). In addition, xylitol production through SSF was optimized using different enzyme and substrate loadings, achieving a maximum concentration of 47 g/L (Baptista et al., 2020). The same robust recombinant yeast strain was exploited for SSF of hardwood xylan into xylitol using aqueous solutions of deep eutectic systems as reaction media, attaining 23.7 g/L xylitol in an one-step process (Romaní et al., 2020). Cell surface engineering of *S. cerevisiae* has been proposed to produce xylitol directly from pretreated lignocellulosic biomass. Guirimand et al. (2016) engineered a *S. cerevisiae* strain to express *XYL1* from *S. stipitis* and co-display three different hydrolases on its cell surface: β-glucosidase (from *Aspergillus aculeatus*), xylosidase (from *Aspergillus oryzae*) and xylanase (from *Trichoderma reesei*). For the cell surface attachment, the target proteins were fused to the anchoring domain of yeast cell wall proteins, SED1 or SAG1. The recombinant strain produced 5.8 g/L of xylitol directly from the xylooligosaccharides-enriched liquid fraction of pretreated rice straw, representing 79.5% of the theoretical yield from the xylose contained in the hydrolysate. To improve xylitol titre, the rice straw hydrolysate was submitted to a membrane separation step (nanofiltration) to increase xylose concentration that also removed fermentation inhibitors, resulting in the production of 37.9 g/L of xylitol. Nevertheless, both rice straw hydrolysates (unfiltered and membrane separated) showed an incomplete xylose conversion. In this sense, the recombinant strain was further improved

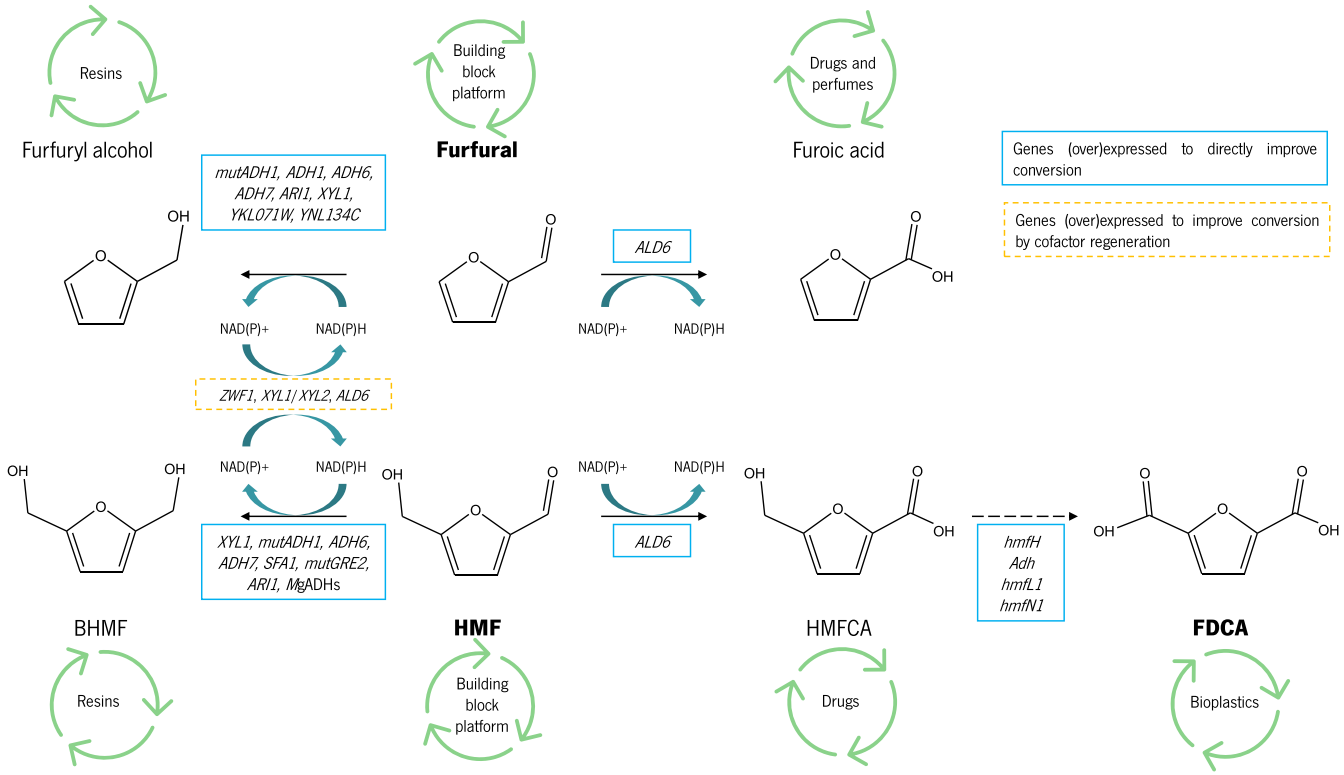

Fig. 3. Genetic strategies to improve conversion of furfural and HMF in Saccharomyces cerevisiae, and applications of the furan derivatives. XYL1 and XYL2 are from Pichia stipitis; hmfH and Adh are from Cupriavidus basilensis HMF14; hmfL1and hmfN1 from Penicillium brasilianum; MgADHs are from Meyerozyma guilliermondii SC1103; the other genes represented are overexpressions from S. cerevisiae. hmfH, Adh, hmfL1, hmfN1 are expressed to promote conversion of HMFCA/HMF into FDCA.

in terms of promoters, secretion signal and anchoring domain sequences, achieving maximal xylitol concentrations of 6.97 g/L and 4.2 g/L from rice straw hydrolysate and Kraft pulp residue, respectively (Guirimand et al., 2019a). Furthermore, this cell surface strategy was combined with the expression of different sugar transports to improve the xylose uptake, and the expression of *MAL11* gene, encoding for a maltose transporter, resulted in a 30% increase in xylitol production (Guirimand et al., 2019b).

A considerable number of metabolic engineering strategies have been successfully applied to convert *S. cerevisiae* into an efficient xylitol producer. The recent research development has greatly expanded the understanding of mechanisms involved in xylitol synthesis by yeast, which is essential to develop sustainable xylitol production systems based on renewable raw material. However, the full potential of the technology for production at large scale, needs to be improved to reach industrial applications.

2.3. *Furans: furandicarboxylic acid and other hydroxymethylfurfural/ furfural-derivatives*

Furfural and hydroxymethylfurfural (HMF) are valuable building blocks, given their versatile composition: an aromatic furan ring and reactive functional groups (aldehyde group in furfural; aldehyde and alcohol groups in HMF). This makes them attractive building block platforms, as they can be transformed into higher value derivative compounds, having applications in several areas such as plastic, pharmaceutical, fragrance and textile industries (Fig. 3). Accordingly, the global market of furfural and HMF are expected to reach $700 million and $61 million by 2024, respectively (Markets and Markets, 2019; Market Study Report, 2019). Furfural and HMF are commonly present in lignocellulosic hydrolysates and are usually regarded as microbial inhibitors. They derive from the dehydration of pentoses and hexoses, respectively, and their accumulation depends on the biomass used, and on the type and severity of pretreatment and hydrolysis applied. As their value has been receiving growing attention, and while their microbial production has not yet been reported, several studies focus on optimizing pretreatment methods to increase furfural and HMF accumulation from lignocellulosic biomasses by using different catalysts and/or reaction media (e.g. ionic liquids, Lewis acids, Brönsted acids, solid acid catalysts, salts) as well as alternative heating methods (e.g. microwave) (Chen et al., 2020; Luo et al., 2019; Morais et al., 2020; Peleteiro et al., 2016; Steinbach et al., 2017; Sweygers et al., 2018; Zhang and Zhao, 2010).

At the same time, increased importance has been given to furfural and HMF as substrates for biotransformation into higher value furan-derivatives. Some microorganisms can use furfural and/or HMF as sole carbon sources (Crigler et al., 2020; Igeño et al., 2018; Koopman et al., 2010), being promising bioprospecting tools for identification of enzymes for production of furan-derivatives in more suitable hosts. *S. cerevisiae* is incapable of using furfural or HMF as carbon source but can convert them into less toxic compounds. In fact, this yeast has long been known to perform a NAD(P)H-dependent reduction of furfural and HMF into their corresponding alcohols: furfuryl alcohol and 2,5-bis-hydroxymethylfuran (BHMF), respectively (Liu et al., 2008). Nevertheless, this capacity has been mainly studied from the point of view of fermentation media detoxification to improve second-generation bioethanol production instead of the possible production of these furan alcohols. In fact, only a few studies focus on the production of furfuryl alcohol using *S. cerevisiae* (Diaz De Villegas et al., 1992; Villa et al., 1992; Liu et al., 2005; Mandalika et al., 2014; Yan et al., 2019). Even without strain improvement, high yields of furfuryl alcohol were obtained from high levels of furfural: 96% yield from 30 g/L of furfural in

Table 3
Genes (over)expressed to improve *Saccharomyces cerevisiae* capacity to convert furfural and HMF into their derivatives. The listed genes are from *S. cerevisiae* unless otherwise stated. Mut-GRE2 is a mutant constructed by direct enzyme evolution with improved reductase activity towards furfural and HMF using the cofactor NADPH (Moon and Liu, 2012). mut-*ADH1* is an *ADH1* from the *S. cerevisiae* strain TMB300 with mutations that result in an unusual NADH-dependent HMF reductase activity (Laadan et al., 2008).

Substrate/Product	Genes	Reference
(1) coding for NAD(P)H-dependent reductases/alcohol dehydrogenases		
Furfural/Furfuryl alcohol	*ADH1*	Hasunuma et al., 2014; Ishii et al., 2013
	ORF *YKL071W*	Heer et al., 2009
	ORF *YNL134C*	Zhao et al., 2015
HMF/BHMF	mut-*GRE2*	Moon and Liu, 2012
	SFA1	Petersson et al., 2006
	ADH genes from *Meyerozyma guilliermondii* SC1103	Xia et al., 2020
Furfural/Furfuryl alcohol	mut-*ADH1*	Almeida et al., 2008a; Laadan et al., 2008; Ishii et al., 2013
and	*ADH6*	Petersson et al., 2006; Almeida et al., 2008a; Ishii et al., 2013
HMF/BHMF	*ADH7*	Heer et al., 2009
	ARI1	Divate et al., 2017; Liu and Moon, 2009
	XYL1 from *Scheffersomyces stipitis*	Almeida et al., 2008b
(2) involved in the regeneration of NAD(P)H		
$NADP^+$/NADPH	*ZWF1*	Gorsich et al., 2006
	ALD6	Park et al., 2011
$NAD(P)^+$/NAD(P)H	*XYL1/XYL2* from *S. stipitis*	Cunha et al., 2019b
(3) overexpression of genes coding for NADPH-dependent aldehyde dehydrogenases		
Furfural/Furoic acid	*ALD6*	Park et al., 2011
and		
HMF/HMFCA		

fed-batch (Villa et al., 1992) and 93% from 25 g/L in batch (Mandalika et al., 2014). This shows the potential of *S. cerevisiae* as a whole-cell biocatalyst for production of furan-derivatives. Regarding BHMF, its production by this yeast is even less explored (Liu et al., 2005). The first study where BHMF was confirmed as the reduction product of HMF by *S. cerevisiae* dates from 2004 (Liu et al., 2004). Despite that, a recent study focused on improving BHMF production from HMF used recombinant *S. cerevisiae* strains harbouring different alcohol dehydrogenases (ADHs) from *Meyerozyma guilliermondii* SC1103 and obtained 345 mM of BHMF with a selectivity higher than 99% (Xia et al., 2020). These reductions of furfural and HMF by *S. cerevisiae* occur in anaerobic conditions, while aerobic conditions favour the oxidation of these compounds into furoic acid and 5-hydroxymethyl-2-furancarboxylic acid (HMFCA), respectively (Taherzadeh et al., 2000; Taherzadeh et al., 1999).

Several studies described genetic modifications to improve *S. cerevisiae* capacity to convert furfural and HMF into their derivatives (Fig. 3). These mainly rely on three different strategies: (1) (over) expression of genes coding for NAD(P)H-dependent reductases/alcohol dehydrogenases directly involved in the reduction of these compounds, (2) (over)expression of genes involved in the regeneration of NAD(P)H, to potentiate the activity of the reduction enzymes and (3) overexpression of genes coding for NADPH-dependent aldehyde dehydrogenases directly involved in the oxidation of furfural and HMF (Fig. 3, Table 3). While mainly analysed from a detoxification perspective, these strategies present valid tools to improve the production of furfural- and HMF-derivatives in *S. cerevisiae* through genetic engineering, which is currently an underexploited area.

Besides the ones mentioned, other high-value derivatives may be obtained from furfural and HMF. Among these, furandicarboxylic acid (FDCA), an HMF-derivative, has also been identified as one of the top products to be obtained from biomass and its worldwide market is expected to reach $850 million by 2025 (Acumen Research and Consulting, 2019). Its main application is the polymerization with ethylene glycol for the production of PEF (polyethylene furanoate) to substitute the petroleum-derived PET (polyethylene terephthalate) plastic. In fact, a consortium of eleven companies named "PEFerence", was funded by the Europen Union's Horizon 2020 to establish an innovative biobased production of FDCA and PEF (https://peference.eu/). FDCA is obtained by oxidation of HMF, and some microorganisms such as *Acinetobacter oleivorans, Aspergillus flavus* or *Burkholderia cepacian* (Godan et al., 2019; Rajesh et al., 2019; Yang and Huang, 2016) have the innate capacity to produce it. Nevertheless, most industries depend on chemical processes for its production, with Corbion being a pioneer in using microbial biocatalyst to produce FDCA from HMF. In a recent patented study, a laboratory *S. cerevisiae* was engineered to produce FDCA (de Bont et al., 2018). In a first attempt HMF-oxidase (*hmfH*) and HMF/FFCA dehydrogenase (*Adh*) from *Cupriavidus basilensis* HMF14 were expressed but resulted in the production of only 0.033 g/L of FDCA from ~0.5 g/L of HMF. Subsequently, the expression of alcohol dehydrogenase (*hmfL1*) and aldehyde dehydrogenase (*hmfN1*) from *Penicillium brasilianum* resulted in the production of 0.47 g/L of FDCA from approximately the same HMF concentration. In similar conditions, the control wild-type *S. cerevisiae* strain produced no FDCA but accumulated 0.41 g/L of HMFCA. Despite the low values obtained, *S. cerevisiae* presents as a promising host for FDCA production, due to its high tolerance towards HMF and innate capacity to produce the intermediate HMFCA (Fig. 3). The development of more efficient FDCA-producing *S. cerevisiae* strains still requires further research in terms of genetic engineering approaches (e.g. following approaches already used in other microorganisms or expressing enzymes with reported activity for conversion of HMF into FDCA), but also in terms of optimization of process conditions (such as oxygen availability, temperature and pH).

2.4. Biohydrocarbons: isoprene

Hydrocarbons are valuable products due to their high energy density, both on a mass and volume basis (Peralta-Yahya and Keasling, 2010). Isoprene (C_5H_8), also identified as 2-methyl-1,3-butadiene, is a volatile hydrophobic terpenoid hydrocarbon (Hong et al., 2012) with several industrial applications, from the production of synthetic rubber for tires and coatings to potential fuel additive for gasoline or diesel (Bentley et al., 2014). The worldwide isoprene market is approximately $3 thousand million, with a projected CAGR of over 7%, attaining a predictable value of near $4 thousand million by 2025 (Market Research Future, 2020). Nowadays, its production relies almost entirely on petrochemical sources, being commonly obtained by dehydrogenation of C5 isoalkanes and isoalkenes or via direct isolation from C5 cracking fractions (Weissermel and Arpe, 1992). Besides its dependence on the C5 supply, which is gradually diminishing due to the employment of new technologies in petrochemical chemistry, its chemical synthesis is

Table 4
S. cerevisiae metabolic engineering strategies for the production of organic acids: (lactic, succinic and 3-hydroxypropionic) and biohydrocarbons (isoprene)

Reference	Strategy	Main results	Substrate
Hong et al., 2012	Multiple copies of codon optimized isoprene synthase gene (*IspS*) from *P. montana*	0.5 mg/L of isoprene	Galactose
Lv et al., 2014	Engineering of the native acetyl-CoA and mevalonic acid pathways (BY4741, þ2 copies of codon optimized *ispS* from *P. alba, þtHMG1, IDI1, ACS2 ERG10* from *S. cerevisiae*, down-regulation of *ERG20* by promoter replacement)	37 mg/L of isoprene (about 782-fold increase compared to the parental strain); yield 0.025 g/g	Sucrose and glycerol
Lv et al., 2016	Dual metabolic engineering of cytoplasmic and mitochondrial acetyl-CoA utilization; hibridization of strains	2527 mg/L of isoprene	Sucrose
Wang et al., 2017	Development of PGAL1-controlled ISPS by overexpression of *GAL4*; Δ*gal1/7/10* promoters; disruption of *GAL80* and insertion of an *ISPSM4* mutant from direct evolution	640 mg/L and 3.7 g/L of isoprene in aerobic batch (from sucrose) and fed-batch (from glucose) fermentations, respectively	Sucrose and glucose
Yao et al., 2018	Hybridization of BY4741-M-08-HIS and YXM54, followed by transformation with high-copy plasmid pESC-URA-ISPSLN-MISPSLN	1.044 g/L of isoprene in batch conditions; 11.9 g/L isoprene in fed-batch	Glucose

energy-consuming and environmentally unfriendly, and its yields might be insufficient for future demand (Ye et al., 2016). Unlike other biobased chemicals, and due to its low boiling point, of 34 °C, and low solubility in water, isoprene can be continuously recovered as a gas in fermentation processes, which has some potential benefits such as reduction of product feedback inhibition and efficient recovery and purification (Ye et al., 2016).

Isoprene is produced by both eukaryotes and prokaryotes, including humans, plants, yeast, and bacteria (Kuzuyama, 2002), being the most produced biogenic volatile organic compound in the planet with atmospheric emissions of ca. 500 Tg(C) per year (Murrell et al., 2020). Plants are the major annual isoprene producer among all organisms, reaching around 600 million tonnes per year (Guenther et al., 2006). Even though plants have a high isoprene yield, its economical exploitation in a commercial perspective is not viable, due to the difficulty of collecting this volatile compound from the vast canopy of leafy plants (Ye et al., 2016). Despite the small global contribution, microbial production of isoprene is also ubiquitous in nature. The common soil bacterium *B. subtilis* is found to be the best natural bacterial producer of isoprene (Kuzma et al., 1995), but *E. coli* has been the most broadly explored microorganism as recombinant cell factory, yielding up to 24 g/L of isoprene production (Yao et al., 2018). Over the last decade, some studies have focused on the production of isoprene using *S. cerevisiae* as host in multiple substrates (Table 4). Hong et al. (2012) engineered a strain where multiple copies of codon-optimized isoprene synthase (ISPS) gene from *Pueraria montana* were expressed, which led to a production of 0.5 mg/L of isoprene from galactose. A different study stemmed on the engineering of the native cytoplasmatic acetyl-CoA and mevalonic acid pathways to enhance the metabolic flux towards isoprene synthesis, achieving a production of 37 mg/L of isoprene using sucrose and glycerol as carbon sources (Lv et al., 2014). Then, dual metabolic engineering of both cytoplasmic and mitochondrial acetyl-CoA utilization boosted isoprene synthesis, yielding 2.527 g/L of isoprene from sucrose (Lv et al., 2016). More recently, an approach on directed evolution of ISPS, coupled with Gal4p-mediated expression enhancement, led to a production up to 3.7 g/L in fed-batch conditions from glucose (Wang et al., 2017). Yao et al. (2018) have reported a strategy for improved isoprene biosynthesis by simultaneous strengthening of precursor supply and conversion via a combination of pathway compartmentation and protein engineering. Initially, a superior isoprene synthase mutant ISPSLN was created by saturation mutagenesis, which resulted in a near 4-fold improvement in isoprene production. Subsequent introduction of ISPSLN into strains with strengthened precursor supply in either cytoplasm or mitochondria led to a metabolic imbalance between the upstream and downstream flux, solved by expressing additional copies of diphosphomevalonate decarboxylase gene (*MVD1*) and isopentenyl-diphosphate delta-isomerase gene (*IDI1*) into the cytoplasmic and mitochondrial engineered strains. Lastly, by hybridization of these two haploid strains, the resultant diploid strain yielded 11.9 g/L in fed-batch with glucose as carbon source, the highest concentration reported to date in eukaryotic cells. Nonetheless, achieving maximal yield of isoprene is still an upcoming challenge to tackle, either through direct evolution or finding novel enzymes and/or metabolic engineering strategies to maximize carbon flux into this target compound.

2.5. Glycerol and derivatives

Glycerol ($C_3H_8O_3$) is a polyol with a wide range of commercial applications. It has been widely used in the manufacture of skin care products, medicines and toothpastes to increase humidity, viscosity and smoothness, and included in food products as solvent, preservative and softening agent (Tan et al., 2013). In addition, glycerol shows great potential on becoming a primary building block for biorefineries (Bozell and Petersen, 2010). It can be produced by chemical synthesis from propylene, by microbial fermentation or can be recovered as by-product resulting from the transesterification of fats and oils in biodiesel manufacturing (Wang et al., 2001). Currently, the demand for renewable fuels is expanding the biodiesel industry, leading to crude glycerol overproduction. This biodiesel-derived crude glycerol contains process-related impurities (salts, proteins, methanol, soap, triglycerides, fatty acids and metals) and its utilization requires purification and refining processes that are not economically viable (Kumar et al., 2019).

Considering the growing demand for non-petroleum derived products and the safety concerns regarding chemical manufacturing procedures, research has been carried out to develop efficient biotechnological processes for glycerol production, focusing mainly on yeasts. *S. cerevisiae* naturally produces glycerol as a by-product during alcoholic fermentation. It is formed from the glycolytic intermediate dihydroxyacetone phosphate (DHAP) through the action of two isoforms of glycerol-3-phosphate dehydrogenase (GPDH) and glycerol-3-phosphatase (GPP), encoded by *GPD1/GPD2* and *GPP1/GPP2* homologous genes, respectively (Fig. 4) (Gancedo et al., 1968; Wang et al., 2001). Several genetic engineering strategies have been explored to improve glycerol synthesis in *S. cerevisiae.* Some of the strategies included the expression of genes directly involved in glycerol formation. For example the overexpression of the *GPD1* resulted in a 4-fold enhanced glycerol production (Michnick et al., 1997; Remize et al., 2001). Other approaches aimed at redirecting carbon flux from ethanol towards glycerol production by reducing the activity of enzymes involved in alcoholic fermentation (Cordier et al., 2007; Drewke et al., 1990; Nevoigt and Stahl, 1996). Alternatively, the deletion of *TPI1* gene, coding for the triose phosphatase isomerase responsible for the conversion of DHAP to glyceraldehyde-3-phosphate (Fig. 4), resulted in an improved glycerol yield and productivity (Compagno et al., 1996). However, the inactivation of this glycolytic enzyme led to an

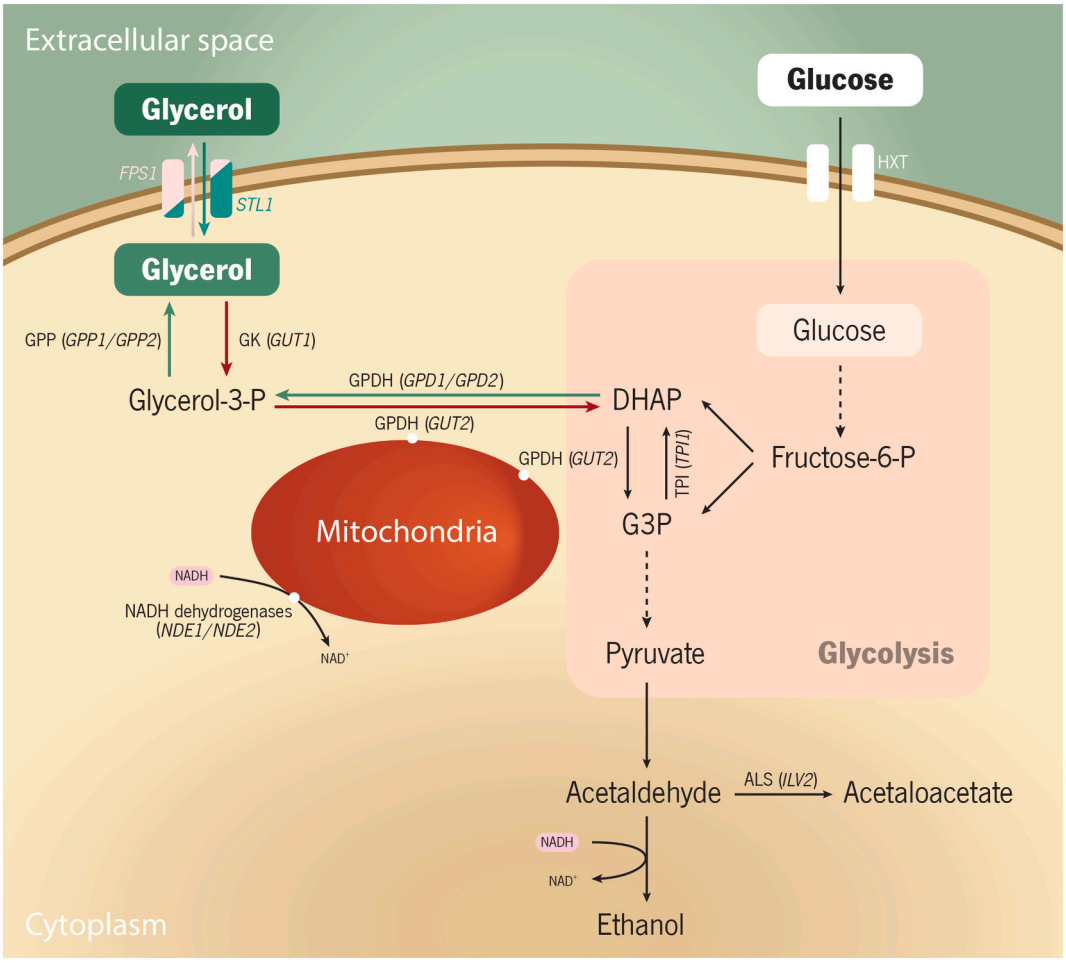

Fig. 4. Metabolic pathways for glycerol catabolism (red arrows) and anabolism (green arrows) in yeast. Dashed arrows indicate multiple step reactions. Abbreviations: G3P, glyceraldehyde-3-phosphate; GPDH, glyceraldehyde-3-phosphate dehydrogenase; GPP, glycerol phosphatase; GK, glycerol kinase; TPI, triose phosphate isomerase; ALS, acetolactate synthase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

accumulation of DHAP, which affected negatively the growth on glucose as sole carbon source. This glucose growth defect was partially circumvented by the deletion of *GUT2* gene and *NDE1* and *NDE2* isogenes. *GUT2* encodes for a mitochondrial respiratory-chain-linked GPDH that catalyses the reversible conversion of glycerol-3-phosphate (G3P) to DHAP while *NDE1/NDE2* isogenes encode for NADH dehydrogenases enzymes responsible for the mitochondrial reoxidation of cytosolic NADH (Fig. 4). Considering the requirement of NADH for the conversion of DHAP to glycerol, the inactivation of these NADH dehydrogenases increased the availability of cytosolic NADH, reducing the accumulation of DHAP. Additionally, evolutionary engineering of this recombinant *S. cerevisiae* strain (*tpi1*Δ *nde1*Δ *nde2*Δ *gut2*Δ quadruple mutant) improved the specific growth rates in high-glucose and yielded 200 g/L of glycerol (Overkamp et al., 2002).

More recently, the expression of the truncated version of *ILV2* gene resulted in a 4-fold increase in glycerol production, comparing with the wild-type strain. *ILV2* codes for a mitochondrial acetolactate synthase that catalyses the first step in isoleucine and valine biosynthesis, converting two pyruvate molecules into one acetolactate molecule. The truncated *ILV2* gene, lacking a mitochondria targeting sequence, encodes a cytosolic form of acetolactate synthase (Fig. 4). The retention of the acetolactate synthase on the cytosol resulted on the conversion of pyruvate to acetolactate, decreasing the available pyruvate for ethanol formation and limiting the acetaldehyde synthesis, thereby increasing NADH availability for glycerol production (Murashchenko et al., 2016). Semkiv et al. (2017) described a more efficient process for the production of glycerol as the primary product under anaerobic conditions. For this, the overexpression of the *ILV2* truncated gene and *FPS1* coding for aquaglyceporin (involved in glycerol efflux), combined with decreased expression of *TPI1* and overexpression of a fused gene coding for GPDH resulted in the production of 16 g/L of glycerol (Semkiv et al., 2017).

In addition to being sold for a variety of uses, glycerol can be converted into other valuable chemicals. Moreover, the need to implement valorisation routes for crude glycerol has drawn attention for the use of this waste stream as substrate for the production of high-value chemicals by microorganisms. The use of biotechnology, especially fermentation technologies, is a promising alternative to convert crude glycerol into higher value products since it requires less energy input (occurs at milder conditions), which may offset the disposal costs of crude glycerol (Kaur et al., 2012). In addition, glycerol presents an higher degree of reduction per carbon compared to the commonly used sugar substrates (glucose and xylose), which results in higher maximum theoretical yields of target reduced compounds (Clomburg and Gonzalez, 2013).

Some biotechnological processes using glycerol as feedstock have already been developed in *Clostridium* species (Gonzalez-Pajuelo et al., 2005). However, the utilization of glycerol by *S. cerevisiae* still poses a challenge, since wild-type strains cannot efficiently grow using glycerol as carbon source without nutrient supplementation (Swinnen et al., 2016). The native L-glycerol-3-phosphate pathway has been reported as the main route for glycerol catabolism in *S. cerevisiae*. This pathway, with glycerol-3-phosphate as intermediate, involves the action of two

Table 5
Development stages of high value compounds production in *Saccharomyces cerevisiae*.

Compounds	Production in *S. cerevisiae*	Genetic engineering to increase/allow production	Production using industrial/wild *S. cerevisiae*	Production from renewable carbons
Lactic acid	YES	YES	YES	YES
Succinic acid	YES	YES	NO	NO
3-HP	YES	NO	YES	NO
Levulinic acid	NO	NO	NO	NO
Sorbitol	YES	NO	NO	NO
Xylitol	YES	YES	YES	YES
FDCA	YES	YES	NO	NO
Other HMF-derivatives	YES	YES	YES	NO
Furfural-derivatives	YES	YES	YES	NO
Isoprene	YES	YES	NO	NO
Glycerol/derivatives	YES	YES	NO	NO

enzymes: a glycerol kinase (*GUT1*) and a GPDH (*GUT2*) for the conversion of glycerol into DHAP, which is further directed to the glycolysis and gluconeogenesis (Fig. 4) (Klein et al., 2017). DHAP can also be used as a metabolic precursor to produce 1,2-propanediol (propylene glycol), an important chemical that is applied as humectant and food preservative. Jung et al. (2011) successfully engineered *S. cerevisiae* for 1,2-propanediol production, using glycerol as carbon source. The overexpression of *GUP1* (involved in glycerol consumption and metabolism) and glycerol-3-phosphate pathway genes (*GUT1* and *GUT2*) together with the expression of the heterologous glycerol dehydrogenase gene (*gdh*) from *Pichia angusta* improved the glycerol uptake rate. In addition, the introduction of *E. coli* methylglyoxal pathway genes (*mgsA* and *gldA*, coding for methylglyoxal synthase and glycerol dehydrogenase) responsible for the conversion of DHAP to 1,2-propanediol, resulted in 2.19 g/L of 1,2-propanediol (Jung et al., 2011). For the exploitation of the abovementioned glycerol reducing power, the electrons derived from glycerol oxidation must be saved in the form of cytosolic NAD(P)H. However, the electrons resulting from glycerol oxidation through the L-glycerol-3-phosphate pathway are transferred via $FADH_2$ to the mitochondrial respiratory chain (Klein et al., 2016). Therefore, the L-glycerol-3-phosphate native pathway was replaced by a synthetic NAD^+-dependent DHA pathway. This strategy involved the *GUT1* gene deletion and the expression of *gdh* gene from the yeast *Ogataea parapolymorpha*, coding for NAD^+-dependent glycerol dehydrogenase for oxidation of glycerol to dihydroxyacetone (DHA), alongside the overexpression of the native *DAK1* gene, coding for glycerol kinase, involved in the subsequent phosphorylation of DHA (Klein et al., 2016). This improved strain was used to introduce the heterologous methylglyoxal pathway and to increase precursor (DHAP) supply by reducing triosephosphate isomerase (TPI) activity, resulting in the highest titre of 1,2-propanediol obtained in yeast (> 4 g/L) (Islam et al., 2017).

Progress has been made in metabolic engineering of *S. cerevisiae* for glycerol biosynthesis and also to generate strains with superior capacity to use glycerol as carbon source for the production of glycerol derivatives. Considering that high production cost is the major drawback for the implementation of industrial processes for glycerol biosynthesis, the achieved biotechnological advancements need to be coupled with metabolic engineering approaches to improve yeast tolerance to lignocellulose-based process conditions, opening new opportunities for utilizing biomass-derived carbohydrate sources. A strong improvement is also expected regarding the production of useful chemicals from glycerol by *S. cerevisiae*. Although significant research advancements continue to be made, there is still a narrow range of derivative compounds produced from glycerol. Given the potential of glycerol as a substrate, novel bioconversions routes may help to expand the plethora of its derivatives.

3. Concluding remarks

The implementation of a sustainable biobased economy relies on the substitution of single product biorefineries for integrated versions producing biofuels combined with biobased products. An encouraging approach would be combining second-generation bioethanol manufacture with the production of key value-added chemicals: organic acids (lactic, succinic, levulinic and 3-hydroxypropionic acids), sugar alcohols (xylitol and sorbitol), furans and derivatives (hydroxymethylfurfural, furfural and furandicarboxylic acid), biohydrocarbons (isoprene), and glycerol and its derivatives (Bozell and Petersen, 2010). The extensive know-how of *S. cerevisiae* metabolism in inexpensive renewable feedstocks, such as lignocellulosic biomass, poses as an advantage to make this yeast a suitable production platform and substitute the chemical routes to produce target top value compounds as well as their higher value derivatives. Significant progress on metabolic pathway design and optimization, transport engineering and carbon partition strategies has been fuelling the development of *S. cerevisiae* for the production of these value-added chemicals. At this moment, there are reports of *S. cerevisiae* strain optimization to produce 8 out of the 11 top value chemicals here revised: FDCA, isoprene, 1,2-propanediol (glycerol derivatives), lactic, succinic and 3-hydroxypropionic acids, sorbitol and xylitol. Table 5 provides a comparison between research advances on *S. cerevisiae* for the production of the 11 products with identification of whether the production of the target compound has been reported in *S. cerevisiae* and in particular, on industrial strains, if there are reports on genetic engineering approaches for increased production and finally if the production from renewable carbons has been reported. Clearly, the development stage is quite differentiated among target products. Xylitol, followed by lactic acid, stand out as the most advanced with promising demonstrated lab-scale production processes from lignocellulosic biomasses. Furthermore, most of the top value compounds show potential as building blocks platforms, and higher value products may be obtained from them using *S. cerevisiae*, e.g. furfuryl alcohol, HMFCA, 1,2-propanediol. The efforts to construct strains for consolidated bioprocessing, containing lignocellulose-degrading enzymes for the direct conversion of biomass into bioethanol, allow their application for the production of value-added chemicals, such as xylitol, decreasing the overall cost of the process and solving problems like catabolic repression. Implementation of integrated biorefineries will ultimately rely on the development of metabolic routes for the production of value-added chemicals from renewable carbohydrates with high titres, yield and productivity in industrial strains of *S. cerevisiae*. Furthermore, the upsurge of disruptive tools like CRISPR/Cas9 and other alternative CRISPR systems is already making noticeable progress to the expansion of genome editing toolbox available for *S. cerevisiae* (Verwaal et al., 2018). This will progressively allow faster strain engineering and multiple simultaneous genome edits (independent of marker cassette integration), as well as optimal transcriptional regulation, resulting in more stable strains - a requirement for their use in industrial processes. In this sense, *S. cerevisiae* offers

unique advantages to develop microbial cell factories for these integrated biorefineries, coupling environmentally friendly and economically feasible production of ethanol and value-added compounds.

Declaration of Competing Interest

None

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