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MINI-REVIEW

Molecular and physiological basis of *Saccharomyces cerevisiae* tolerance to adverse lignocellulose-based process conditions

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Abstract

Lignocellulose-based biorefineries have been gaining increasing attention to substitute current petroleum-based refineries. Biomass processing requires a pretreatment step to break lignocellulosic biomass recalcitrant structure, which results in the release of a broad range of microbial inhibitors, mainly weak acids, furans, and phenolic compounds. *Saccharomyces cerevisiae* is the most commonly used organism for ethanol production; however, it can be severely distressed by these lignocellulose-derived inhibitors, in addition to other challenging conditions, such as pentose sugar utilization and the high temperatures required for an efficient simultaneous saccharification and fermentation step. Therefore, a better understanding of the yeast response and adaptation towards the presence of these multiple stresses is of crucial importance to design strategies to improve yeast robustness and bioconversion capacity from lignocellulosic biomass. This review includes an overview of the main inhibitors derived from diverse raw material resultants from different biomass pretreatments, and describes the main mechanisms of yeast response to their presence, as well as to the presence of stresses imposed by xylose utilization and high-temperature conditions, with a special emphasis on the synergistic effect of multiple inhibitors/stressors. Furthermore, successful cases of tolerance improvement of *S. cerevisiae* are highlighted, in particular those associated with other process-related physiologically relevant conditions. Decoding the overall yeast response mechanisms will pave the way for the integrated development of sustainable yeast cell–based biorefineries.

Keywords Lignocellulosic biomass · Inhibitory compounds · Stress response mechanisms · *S. cerevisiae* · Metabolic engineering

Introduction

Depletion of fossil resources and environmental concerns related to their exploitation promote the transition of petroleum-based refinery towards a bio-based economy. The bioeconomy aims at the manufacturing of products and fuels from renewable materials using efficient biotechnologies, contributing to the creation of new jobs and industries.

Lignocellulosic biomass is the most available renewable resource on earth and may be used for the production of liquid biofuels, such as bioethanol. Nevertheless, the large-scale production of lignocellulosic bioethanol is not extensively implemented due to the elevated initial investment and operational costs related to the process. For instance, a physicochemical pretreatment is required to break down the recalcitrant and complex structure of lignocellulosic biomass to obtain fermentable sugars, significantly increasing the complexity and length of the process. Several strategies have been considered to reduce capital costs such as the use of whole slurry (liquid and solid phases) or slurries after pretreatment, to eliminate unnecessary washing steps, and operating at high solid loading, to reduce distillation costs (Romaní et al. 2014). Moreover, the use of all sugars present in the hemicellulosic fraction is also required for cost-effective lignocellulosic ethanol production. Xylose is the most abundant sugar in the hemicellulosic fraction; however, *Saccharomyces cerevisiae*, the preferred microorganism for bioethanol production, is not naturally capable of metabolizing this sugar. Considering this, several efforts have been applied in the last years for the development of *S. cerevisiae* strains capable of xylose consumption through the expression of heterologous pathways, such as

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xylose reductase and xylitol dehydrogenase (XR/XDH) from *Pichia stipitis*, or of xylose isomerases (XIs) from different bacterial and fungal species (Moysés et al. 2016). Both pathways convert xylose into xylulose, which is subsequently phosphorylated into xylulose-5P and then further metabolized in the pentose phosphate pathway (PPP).

Nevertheless, the hemicellulosic fraction (liquid phase) also contains toxic compounds, in concentrations dependent of pretreatment severity, which are inhibitors of the subsequent saccharification and fermentation steps (Modenbach and Nokes 2012). These inhibitory compounds are weak acids, furans, and phenolic compounds. Acetic acid is the most abundant weak acid in lignocellulosic hydrolysates and is present due to the deacetylation of acetyl groups linked to the main chain of hemicelluloses. Other weak acids, such as formic and levulinic acids, can also be present in hydrolysates resulting from furan compound degradation. Furfural and hydroxymethylfurfural (HMF) are produced by dehydration of pentoses and hexoses, respectively. On the other hand, phenolic compounds (such as syringic acid, vanillin, ferulic acid, vanillic acid, and coumaric acid) are produced by depolymerization of lignin. The amount of inhibitory compounds in the lignocellulosic hydrolysates is dependent on lignocellulosic source (e.g., agricultural residues, hardwoods, or softwoods), the selected pretreatment (hydrothermal treatment, diluted acid treatment, alkali treatment), and operational conditions (solid loading of lignocellulosic biomass, temperature, time, percentage of catalyst) (Modenbach and Nokes 2012; Ko et al. 2015; Dominguez et al. 2017). Additionally, the concentration of hemicellulose-derived sugars (xylose and xylooligosaccharides) can also vary depending on raw material and pretreatment selected for the processing of lignocellulosic biomass (Table 1). As seen in Table 1, the increase of temperature (from 210 to 220 °C) in steam explosion treatment increased the acetic acid concentration, furfural, and HMF in wheat straw hydrolysate (Alvira et al. 2011). Moreover, the acid-diluted treatment yields a higher concentration of hemicellulose-derived compounds as monomers (xylose, furfural, HMF, or acetic acid) than autohydrolysis treatment (using only water as reaction medium), since part of hemicellulose-derived compounds is solubilized as oligomers (xylooligosaccharides or acetyl groups) in autohydrolysis treatments (Yáñez et al. 2009; Jesus et al. 2017). On the other hand, the total inhibitory load in hardwood (e.g., eucalyptus) hydrolysates is superior to the inhibitory load in hydrolysates from agricultural residues (corn cob and wheat straw) (Costa et al. 2017). HMF concentration is higher than furfural in softwood hydrolysates as hemicellulose is composed mainly by hexoses (Table 1), while furfural is the predominant furan compound in hardwood and agricultural residue hydrolysates (Westman et al. 2012; Dominguez et al. 2017). Phenolic compounds are generally present in hydrolysates at lower concentration (Table 1), and their inhibitory effect is more described for enzymes in cellulose conversion (Ko et al. 2015). Under acid conditions, the formation of phenolic compounds can differ, depending of lignocellulosic source and treatment conditions (Ko et al. 2015). These compounds are generally considered inhibitory for *S. cerevisiae* growth, affecting its fermentative performance by increasing the fermentation lag phase and decreasing ethanol yield and productivity (Guo and Olsson 2014; Larsson et al. 2000; Liu et al. 2004). Furthermore, besides the presence of more than one inhibitory compound in the hydrolysate, for an efficient conversion of cellulose to glucose, higher fermentation temperatures are desirable in order to facilitate simultaneous saccharification and fermentation (SSF) processes, which can increase the yield of lignocellulosic ethanol (Kelbert et al. 2016), representing an additional stress factor. To partially overcome these physiological hurdles, proper nutrient supplementation together with an adequate yeast genetic background has shown to increase process efficiency (Kelbert et al. 2015).

The adaptive response mechanism of yeast cells towards the presence of a single inhibitor such as acetic acid (Dong et al. 2017; Giannattasio et al. 2013; Guerreiro et al. 2016; Lindberg et al. 2013; Mira et al. 2010), formic acid (Henriques et al. 2017), furfural (Allen et al. 2010; Gorsich et al. 2006), HMF (Ma and Liu 2010), and vanillin (Nguyen et al. 2014a, b; Wang et al. 2016) has been extensively described and studied. Nevertheless, yeast response towards the synergistic effect of multiple inhibitors is generally less approached (Pereira et al. 2011a, 2014b). Furthermore, aforementioned conditions required for cost-efficient production of ethanol (high temperature and xylose co-consumption) together with the presence of inhibitory compounds will further increase the negative effects on lignocellulosic fermentation performance. Recently, the focus on more robust or tolerant yeast is emerging as a desirable strategy for the fermentation of lignocellulosic hydrolysates, with the previous phenotypic selection of stress-tolerant strains being an essential step (Jin et al. 2013; Wimalasena et al. 2014; Romaní et al. 2015). In this sense, this review aims to describe the negative effects caused by the presence of multiple lignocellulose-derived inhibitors linked to required process conditions (high temperature and xylose co-consumption), as well as the mechanisms of yeast response and tolerance towards the simultaneous presence of all these fermentation constrains (Fig. 1). Different from other reviews covering yeast lignocellulosic tolerance, this work will focus on the overall effect caused by the mixture of the main lignocellulose-derived inhibitors and not in the detached individual effects. In addition, special attention is given to the heterogeneous composition of different hydrolysates, which is considered of major importance to the yeast tolerance response. Furthermore, rational metabolic engineering strategies successfully applied to yeast under industrial-like conditions are discussed.

Table 1 Main hemicellulose-derived compounds (monosaccharide and oligosaccharide) and degradation compounds (weak acids, furans, and phenolic compounds) derived from the pretreatment of lignocellulosic biomass

Raw material	Pretreatment (operational conditions)	Sugar[a]	Main inhibitor compounds derived from lignocellulosic pretreatment			Reference
			Weak acid[b]	Furan compounds[c]	Phenolic compounds	
Agricultural residues						
Wheat straw	Steam explosion (220 °C for 2.5 min)	4.4 g/L of X; 19.2 g/L of XOS	6.4 g/L of AA; 2.6 g/L of FA	1.8 g/L of F; 0.4 g/L of HMF	NA	Alvira et al. (2011)
	Steam explosion (210 °C for 2.5 min)	2.7 g/L of X; 20.7 g/L of XOS	4.0 g/L of AA; 1.7 g/L of FA	0.7 g/L of F; 0.2 g/L of HMF		
Wheat straw	Autohydrolysis treatment (S_0 = 3.92); acid post-hydrolysis (165 °C, 0.5% H_2SO_4, 168 min)	16 g/L of X	2.6 g/L of AA	0.13 g/L of HMF; 0.35 g/L of F	NA	Costa et al. (2017)
Sweet sorghum hybrid CSSH 45	Steam process (200 °C for 1.5 min)	6.7% of X	0.1% of GA; 0.2% of FA; 1.1% of AA; 0.1% of LeA	0.4% of F; 0.1% of HMF	3.4 g GAE/L	Damay et al. (2018)
	Steam process (215 °C for 1.5 min)	12.8% of X	0.2% of GA; 0.6% of FA; 2.6% of AA; 0.1% of LeA	1.2% of F; 0.1% of HMF	NA	
	Steam process (215 °C for 6 min)	5.2% of X	0.4% of GA; 0.9% of FA; 2.9% of AA; 0.4% of LeA	2.0% of F; 0.1% of HMF	5.3 g GAE/L	
Vine pruning residue	Autohydrolysis treatment (180 °C for 60 min)	1.99 g/L of X; 17.22 g/L of XOS	2.66 g/L of AA; 6.10 g/L of AG	0.36 g/L of F; 0.66 g/L of HMF	2.35 g GAE/L; 47.54 mg/L of catechin; 34.70 mg/L of chlorogenic acid	Jesus et al. (2017)
Corn cob	Autohydrolysis treatment (S_0); acid post-hydrolysis (165 °C, 0.5% H_2SO_4, 168 min)	26 g/L of X	4 g/L of AA	0.2 g/L of HMF; 0.4 g/L of F	NA	Costa et al. (2017)
Corn cob	Wet oxidation treatment (195 °C for 15 min) with Na_2CO_3	1.3% of X	2.1 g/L of GA; 3.3 g/L of FA; 4.2 g/L of AA	NA	54 mg/L of 4-hydroxybenzaldehyde; 57 mg/L of vanillin	Varga et al. (2004)
	Wet oxidation treatment (195 °C for 15 min) with H_2SO_4	9% of X	1.8 g/L of GA; 2.9 g/L of FA; 2.8 g/L of AA	NA	102 mg/L of 4-hydroxybenzaldehyde; 74 mg/L of vanillin	
Sugarcane bagasse	Microwave-assisted pretreatment (0.5% H_2SO_4, 140 °C for 5 min)	20.1%	0.05 g/L of FA; 1.69 g/L of AA	0.028 g/L of HMF; 0.64 g/L of F	NA	Yu et al. (2018)
Hardwood						
Eucalyptus globulus wood	Autohydrolysis treatment (S_0 = 4.08); acid post-hydrolysis (165 °C, 0.5% H_2SO_4, 168 min)	16 g/L of X	6.2 g/L of AA	0.33 g/L of HMF; 1.66 g/L of F	2.01 g GAE/L	Costa et al. (2017)
Paulownia tomentosa	Autohydrolysis treatment (S_0 = 4.19); acid post-hydrolysis (165 °C, 0.5% H_2SO_4, 168 min)	14 g/L of X	5.84 g/L of AA	0.72 g/L of HMF; 1.96 g/L of F	NA	Cunha et al. (2018)
Acacia dealbata	Non-isothermal autohydrolysis treatment at 217 °C	1.29% of X; 10% of XOS	0.89% of AA; 4.7% of AG	0.40% of F; 0.17% of HMF	NA	Yáñez et al. (2009)
Softwood						
Spruce	SO_2-impregnated steam explosion	10.09 g/L of X	4.79 g/L of AA	1.57 g/L of F; 1.09 g/L of HMF	NA	Demeke et al. (2013a)
Lodgepole pine wood	SPORL pretreatment (165 °C for 75 min)	13.9% of Ga; 14.5% of M; 9.7% of X	0.76% of AA	1.7 g/L of HMF; 1.1 g/L of F	NA	Zhou et al. (2013)
Spruce chips	SO_2 steam explosion (pH 2; 18 bar for 5–7 min)		3.52 g/L of AA	0.30 g/L of F, 1.26 g/L of HMF	0.05 mg/L of catechol; 0.128 mg/L of vanillin	Westman et al. (2012)

Table 1 (continued)

Raw material	Pretreatment (operational conditions)	Sugar[a]	Main inhibitor compounds derived from lignocellulosic pretreatment			Reference
			Weak acid[b]	Furan compounds[c]	Phenolic compounds	
Pine wood biomass	SO_2 steam explosion (215 °C for 5 min)	9.2 g/L of G; 12.5 g/L of M; 2.5 g/L of Ga; 5.2 g/L of X NA	0.43 g/L of FA; 0.10 g/L of LA; 2.15 g/L of AA; 0.03 g/L of SA; 0.41 g/L of LeA	2.15 g/L of HMF; 1.18 g/L of F; 0.02 g/L of FuA	0.003 mg/L of 3,4-DHBA; 0.005 mg/L of 3-HBA; 0.050 mg/L of vanillic acid; 0.022 mg/L of vanillin; 0.015 mg/L of benzoic acid	Hawkins and Doran-Peterson (2011)

The hardness of hydrothermal treatments can be expressed in terms of "severity" (S_0), defined as the logarithm of the severity factor R_0 (Lavoie et al. 2010). Total phenolic compounds were measured as gallic acid equivalent (GAE) using the Folin-Ciocalteu method (Jesus et al. 2017); % g of component per 100 g of oven-dried raw material

[a] Sugar: xylose (X), xylooligomers (XOS), galactose (Ga), glucose (G), and mannose (M)

[b] Weak acids: acetic acid (AA), formic acid (FA), levulinic acid (LeA), succinic acid (SA), lactic acid (LA), acetyl groups (AG), and glycolic acid (GA)

[c] Furan compounds: furfural (F), hydroxymethylfurfural (HMF), and furoic acid (FuA)

Inhibitory effects on yeast during lignocellulosic fermentation

The overall metabolic and structural effects behind the negative effects of inhibitory compounds/process conditions on yeast growth and fermentation are listed on Table 2 and are further discussed below. Nevertheless, it should be taken into consideration that the specific effects of some of these inhibitors remain unknown or not well understood.

Intracellular acidification and ATP depletion

The effects of weak acids, mainly of acetic acid, on *S. cerevisiae* physiology and performance have been studied and recently reviewed (Palma et al. 2018). In the acidic pH conditions required for ethanol production from lignocellulosic biomass, weak acids enter the yeast cell in their protonated form (–COOH) and dissociate in the nearly pH-neutral cytoplasm, releasing hydrogen ions (H^+) and leading to intracellular acidification (Ullah et al. 2012; Fig. 1❶). To maintain intracellular pH homeostasis, this acidification is counteracted by the activity of the H^+-ATPase, which exports H^+ at the expense of ATP consumption (Fig. 1❷). Furthermore, the anionic form of the acid is presumably exported by several multidrug resistance (MDR) transporters also contributing to ATP depletion in the yeast cell (Palma et al. 2018). In turn, ATP depletion will further limit the activity of ATPases, causing the dissipation of the transmembrane electrochemical gradient of protons, compromising secondary solute transport systems and the maintenance of ion homeostasis in the yeast cell (Serrano 1984). In addition, weak acids are also known to inhibit glycolytic enzymes, preventing ATP regeneration (Pampulha and Loureiro-Dias 1990) and leading to an energy drain.

Reactive oxygen species accumulation/oxidative stress

Weak acids are also known to cause oxidative stress, being the accumulation of reactive oxygen species (ROS; Fig. 1❸) caused both by the increase of H^+ in the cytosol and by the decrease of the ROS scavenger reduced glutathione (GSH) (Guo and Olsson 2014). The rate of ROS production is also known to be significantly increased at high temperatures as a consequence of heat stress (Morano et al. 2012). In yeast, ROS are neutralized by non-enzymatic and enzymatic processes, with these last requiring NADPH as a source of reduction equivalents (Herrero et al. 2008). To compensate NADPH oxidation, yeast gradually increases the influx through pentose phosphate and acetic acid pathways (Celton et al. 2012). Thereby, an increase in acetic acid production is stimulated at high temperatures, representing a synergistic effect that leads to the decrease of growth and ethanol production rate

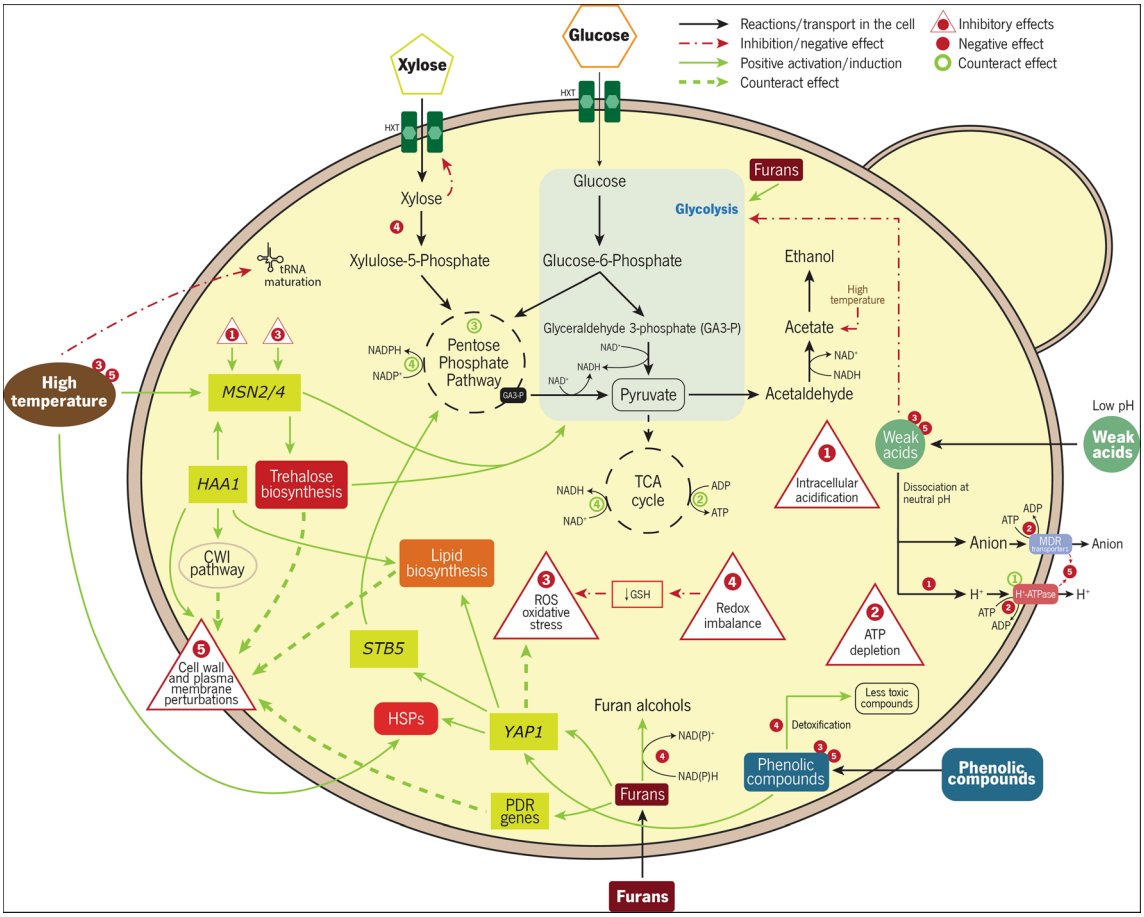

Fig. 1 Main mechanisms of the *S. cerevisiae* response towards the presence of lignocellulose-derived inhibitors. Main negative stressor effects are identified by numbered triangles: (1) intracellular acidification, (2) ATP depletion, (3) ROS oxidative stress, (4) redox imbalance, and (5) cell wall and plasma membrane perturbations. Superscript numbered red circles nearby stressors indicate its main negative effects. Superscript numbered green circles correspond to the counteract effects on the corresponding main negative effect. Black arrows represent transport of compounds and metabolic reactions; red arrows indicate negative effects; full green arrows represent positive activation/induction; dashed green arrows indicate counteract effects on the correspondent main negative stressor effect

(Woo et al. 2014). In a similar manner, furan aldehydes such as furfural and HMF also potentiate ROS generation by acting as thiol-reactive electrophiles and depleting GSH levels (Allen et al. 2010; Kim and Hahn 2013). Some phenolic compounds have also been reported to cause oxidative stress (Nguyen et al. 2014b); however, the mechanism behind their involvement in ROS accumulation is not yet understood. ROS accumulation in the yeast will ultimately result in damage at the mitochondrial and vacuolar membranes, the nuclear chromatin, and the actin cytoskeleton (Allen et al. 2010).

Redox imbalance

In the yeast cell, furans are converted into their corresponding less toxic alcohols, through reactions mediated by NAD(P)H-dependent oxidoreductases, which will ultimately lead to redox imbalance (Ask et al. 2013; Fig. 1④). This decrease/drop in the reduction potential of the yeast intracellular compartment also contributes to oxidative stress, as NADPH is required for the reduction of oxidized glutathione, thus also decreasing GSH levels. The yeast *S. cerevisiae* also has the capacity to detoxify phenolic compounds, converting them into less toxic derivatives, either through a series of decarboxylations and oxidations (Adeboye et al. 2015; Adeboye et al. 2017) or by NADPH-dependent reductions (Nguyen et al. 2014a; Wang et al. 2016), which may also contribute to a redox imbalance depending on the prevalence of phenolic compounds derived from the lignocellulose pretreatment.

Structural defects

In addition to these metabolic effects, the lignocellulose-derived inhibitors also cause structural changes in the yeast cell (Fig. 1⑤), mainly in its cellular envelope. The presence of these compounds is known to lead to the reduction of plasma membrane stability and its selective permeability, by reducing its ergosterol content (Godinho et al. 2018) or by changing its protein-to-lipid ratio (Campos et al. 2009).

Table 2 Main metabolic and structural effects of the presence of lignocellulose-derived inhibitors in the yeast cell

Effects	Stress (reference)
Metabolic	
Redox imbalance	Furans (Ask et al. 2013)
	Phenolics (Adeboye et al. 2014, 2017; Nguyen et al. 2014a; Wang et al. 2016)
ROS accumulation	Furans (Kim and Hahn 2013)
	Weak acids (Guo and Olsson 2014; Woo et al. 2014)
	Phenolics (Nguyen et al. 2014b)
	High temperature (Woo et al. 2014)
Intracellular acidification (ATP depletion)	Weak acids (Ullah et al. 2012)
Inhibition of glycolytic enzymes (ATP depletion)	Weak acids (Pampulha and Loureiro-Dias 1990; Pearce et al. 2001)
	Sugar co-fermentation (Subtil and Boles 2012)
Structural	
Membrane and cell wall integrity	Weak acids (Godinho et al. 2018)
	Phenolics (Campos et al. 2009)
	High temperature (Verghese et al. 2012)
Organelle integrity	Weak acids (Pereira et al. 2010a; Verghese et al. 2012)
Macromolecule production and/or stability	Furans (Iwaki et al. 2013a)
	Phenolics (Iwaki et al. 2013b)
	High temperature (Foretek et al. 2016; Verghese et al. 2012)

Furthermore, the integrity and organization of the cell wall is also compromised by these inhibitors; e.g., weak acids are capable of increasing cell wall porosity and decreasing its robustness (Simões et al. 2006). The decrease on the integrity of the yeast cellular envelope significantly facilitates and increases the entry of inhibitory compounds into the yeast cell, synergistically contributing to their toxic effect. In fact, Ding and collaborators (2011) have observed that the severe effects of acetic acid on the yeast cell were potentiated by the presence of phenol and furfural, due to the loss of membrane integrity and metabolism inhibition. In fact, the synergistic effect between weak acids, furans, and phenolic compounds has been for long recognized as the main cause of the high toxicity of lignocellulosic hydrolysates, as the cumulative effect of the inhibitors present in a hydrolysate is far beyond that of the sum of their individual toxic effects (Ding et al. 2011; Keating et al. 2006; Klinke et al. 2003; Palmqvist et al. 1999).

Effects of high temperature

High temperature is one of the conditions required for simultaneous saccharification and fermentation from lignocellulosic biomass, and it can significantly affect yeast. Heat stress is known to disturb protein stability, cell membrane, and cytoskeleton structures, which leads to protein dysfunction, metabolic imbalances (Verghese et al. 2012), loss of metabolic activity (Woo et al. 2014), and defects in transfer RNA (tRNA) maturation by the accumulation of aberrant tRNA processing intermediates upon shift of cells to high-temperature conditions (Foretek et al. 2016). Heat shock response is a fundamental cytoprotective pathway that enables yeast to cope with high-temperature stress, by activation of heat shock protein (HSP) synthesis (Verghese et al. 2012).

Contribution of modifications for xylose consumption to the inhibitory effects

Xylose consumption, by expression of heterologous pathways, on *S. cerevisiae* presents another hurdle on the production of second-generation bioethanol, as it has been described to increase yeast susceptibility to the inhibitory effects of the compounds present in lignocellulosic hydrolysates (Bellissimi et al. 2009). In fact, the genetic modifications used for xylose consumption can disturb the metabolic homeostasis of the yeast cell, decreasing its tolerance. For instance, it is known that expression of the *P. stipitis* XR/XDH pathway results in a redox imbalance caused by the co-factor difference between XR and XDH (while XR mainly uses NADPH, and XDH co-factor is NAD^+) (Zhang et al. 2012), which may interfere with the metabolic effects of the inhibitory compounds, in addition to the undesirable accumulation of the by-product xylitol. Xylose uptake is mediated by hexose transporters in yeast, which are unspecific for pentose sugars (Subtil and Boles 2012). In low concentrations, glucose improves xylose uptake by activating these transporters; however, in higher concentrations, it outcompetes xylose, with high-glucose phosphorylation rates

repressing sugar co-consumption (Lane et al. 2018). Hexose and pentose catabolism converges at the level of phosphofructokinase, and glucose limits glycolytic enzyme activity at this level (Subtil and Boles 2012). Also, after glucose depletion in a medium containing glucose and xylose (which occurs in lignocellulosic fermentations), cell growth and xylose consumption rate decrease sharply to values even lower than those in media containing xylose as the sole carbon source, and cells cease to respond to residual xylose, entering a new lag phase, named post-glucose effect lag phase (Wei et al. 2018). Intracellular xylose can, in fact, trigger a signal similar to carbon limitation in yeast cells actively metabolizing xylose, which causes low assimilation rates (Osiro et al. 2018). Xylose metabolism can also lead to downregulation of genes encoding gluconeogenic enzymes (Salusjärvi et al. 2006) and cause upregulation of genes involved in response to stress, starvation, DNA damage, and lipid metabolism by being forced to metabolize unconventional substrates (Gopinarayanan and Nair 2018).

End-product inhibition

On top of these stress factors inherent to efficient processing of lignocellulosic materials, the target product itself will, in most of the cases, affect negatively yeast cell metabolism. The most well-described end-product inhibition is ethanol, having pleiotropic effects on yeast cell (Deparis et al. 2017) affecting cell growth and viability (Pereira et al. 2011b) mainly by distressing cell wall and membrane integrity. An adequate medium supplementation partly counteracts the ethanol negative effects (Pereira et al. 2010b).

Mechanisms of yeast response to the presence of multiple inhibitors/hydrolysates

S. cerevisiae has developed several mechanisms to cope with the presence of lignocellulose-derived inhibitors and their effects (Fig. 1). Additionally, the yeast also exhibits responses towards the hurdles typical of lignocellulosic processes, such as high temperatures and xylose co-consumption (Fig. 1). As lignocellulosic materials are a platform to obtain several different compounds, the end-product inhibition response will not be addressed in here.

Oxidative stress response

One of the most toxic effects of the presence of lignocellulose-derived inhibitors on the yeast cell is oxidative stress, an imbalance between ROS generation and antioxidant response. The *YAP1* gene encodes a transcription factor, activated by the presence of both furans (Kim and Hahn 2013) and some phenolic compounds (Nguyen et al. 2014b), and is the major regulon in oxidative stress response (Herrero et al. 2008). It induces expression of genes involved in the detoxification of superoxide anions (*SOD1*), reduction of hydrogen peroxide (*GPX2*, *CTT1*, *TSA1*), and thiol reduction (*TRX2*, *TRR1*), as well as expression of genes involved in the glutathione system (*GSH1*, *GSH2*, *GLR1*, *GRX1*, *YCF1*) (Hélène et al. 2000). YAP1 also regulates the expression of other genes involved in response to several stressful conditions, such as MDR proteins (*FLR1*, *ATR1*) (Sundström et al. 2010) and HSPs (*SSA1*) (Maeta et al. 2004).

Furthermore, *YAP1* is known to induce *STB5* (Ouyang et al. 2011), a transcription factor that regulates most genes of the PPP, being a key player for NADPH regeneration required for oxidative stress response (Larochelle et al. 2006), but also for the detoxification of inhibitory compounds (Gorsich et al. 2006; Nguyen et al. 2014b). As already mentioned, in the presence of furfural and HMF, the yeast cell responds with the activity of NAD(P)H-dependent oxidoreductases to convert them into the less toxic furfuryl alcohol and furan dimethanol, respectively (Heer et al. 2009; Liu et al. 2008; Xianxian et al. 2015). In addition, being PPP the primary pathway for xylose metabolism, *STB5* regulation is important not only for tolerance towards inhibitory compounds but also for the consumption of alternative carbon sources present in lignocellulosic biomass (Kim et al. 2015). The detoxification of some phenolic compounds, involving genes such as *ALD5*, *PAD1*, *ATF1*, and *ATF2* (Adeboye et al. 2017), and several decarboxylation and oxidation reactions (Adeboye et al. 2015) could hypothetically counteract the redox imbalance created by the reduction of furan compounds. Nevertheless, the detoxification of other phenolic compounds, such as vanillin, involves NADPH-dependent reductases, and in this sense, the effects of the phenolic compounds in the redox homeostasis of the yeast will strongly depend on their chemical nature (Adeboye et al. 2014). Additionally, *S. cerevisiae* induces the synthesis of diverse molecules with antioxidant activity against heat-induced oxidative stress (Morano et al. 2012), with several molecules being identified as important for yeast response to heat stress, such as HSPs, H^+-ATPases, ubiquitin, and antioxidant enzymes (Gao et al. 2016).

Structural response: cell membrane

Accumulation of trehalose is another defense mechanism activated by oxidative stress, where it plays an important protective role in the maintenance of the integrity of the cell membrane (Alvarez-Peral et al. 2002), probably by stabilization of membrane proteins (Jain and Roy 2009). In this sense, trehalose accumulation has been described to be activated in response to membrane-disrupting stresses, such as high temperatures (Mensonides et al. 2014) and exposure to weak acids (Guo and Olsson 2014). Accordingly, the genes involved in trehalose synthesis have been found to be regulated by the MSN2/4

transcription factors, which are activated upon oxidative stress (Gasch et al. 2000; Hasan et al. 2002), but also by stressors such as high temperature and low pH (Causton et al. 2001).

Another factor that has been identified as a determinant for yeast tolerance is its capacity to largely rearrange the lipid composition of the plasma membrane (e.g., sphingolipids and sterols) (Lindberg et al. 2013). Sphingolipid content was found to be increased in response to acetic acid stress (Lindberg et al. 2013), and the upregulation of sphingolipid biosynthesis was described to be mediated by the TORC2-Ypk1 signaling complex (Roelants et al. 2011), which is activated not only by acetic acid (Guerreiro et al. 2016) but also by heat stress (Sun et al. 2012). Ergosterol, a major constituent of the yeast plasma membrane, is another molecule required to maintain membrane integrity. In fact, a possible interaction has been suggested between ergosterol biosynthesis and the oxidative stress response (Higgins et al. 2003). Furthermore, several genes from the ergosterol biosynthetic pathway were upregulated in response to acetic acid stress, as well as *PDR18*, which was found to have a physiological role in ergosterol transport and proper incorporation into the plasma membrane, increasing its lipid order and decreasing the non-specific membrane permeability (Godinho et al. 2018). *PDR16* (positively regulated by YAP1) and *PDR17* have also been described to be important for lipid biosynthesis (ergosterol and phospholipids, respectively), not only playing an important role on plasma membrane integrity but also controlling lipid content in various compartments of the cell, providing mechanisms for multidrug resistance (van den Hazel et al. 1999). In fact, the expression of genes of the pleiotropic drug resistance (PDR) family was found to be enhanced in response to the presence of furfural and HMF (Liu et al. 2008; Ma and Liu 2010). The PDR family mainly consists of membrane- and transport-related proteins, such as the ATP-binding cassette (ABC) transporters, including the weak acid–inducible PDR12 which contributes for the efflux of anions (Ullah et al. 2012). In fact, *PDR12* is regulated by WAR1, a transcription factor that is activated by phosphorylation in the presence of weak acids (Frohner et al. 2010; Gregori et al. 2008; Kren et al. 2003). Nevertheless, Pdr12 role in response to weak acid stress is not common to weak acids in general, as its absence leads to high susceptibility to the more lipophilic weak acids but seems to be advantageous for tolerance to shorter acids, such as acetic and formic (Nygård et al. 2014). In fact, *TPO2* and *TPO3*, encoding MDR transporters of the major facilitator superfamily, have been found to confer resistance to acetic, propionic, benzoic, and octanoic acids (with a slightly more evident effect for the more hydrophilic acids), presumably through the active export of the counter ions (Fernandes et al. 2005). More recently, *TRK1*, encoding for a high-affinity potassium transporter, has been found to have a detrimental effect in the yeast response to formic acid, presumably by contributing to the influx of this acid into the cell (Henriques et al. 2017). The fact that *TRK1* is a determinant of yeast tolerance towards acetic acid is another example of how diverse weak acids may activate different response mechanisms. Accordingly, it has been proposed that dissimilar weak acids may activate unique tolerance mechanisms: while less lipophilic acids (acetate and propionate) were found to mainly regulate membrane-associated transport processes, the transcriptional response to more strongly lipophilic acids (benzoate and sorbate) mainly regulates genes related to the cell wall (Abbott et al. 2007).

Structural response: cell wall

The cell wall integrity (CWI) signaling pathway in *S. cerevisiae* is activated in response to several forms of cell wall stress and acts on cell wall remodeling (through control of wall biosynthetic enzymes), transcriptional regulation of cell wall–related genes, and organization of actin cytoskeleton (Levin 2005, 2011). CWI pathway has been found to play an important role in yeast tolerance towards major components of lignocellulosic hydrolysates, such as acetic acid (Nishida et al. 2014), furfural (Liu et al. 2018), and HMF (Liu et al. 2018; Zhou et al. 2014). Weak acid stress is also known to cause the activation of HAA1, a transcription factor responsible for yeast adaptation and tolerance to short-chain weak acids, such as acetic and formic acids (Fernandes et al. 2005; Henriques et al. 2017). HAA1 has been found to transcriptionally regulate cell wall proteins, such as SPI1 and YGP1; proteins from the plasma membrane, such as the MDR transporters TPO2 and TPO3; and proteins involved in the biosynthesis of lipids (contributing to the integrity of the plasma membrane) (Fernandes et al. 2005; Mira et al. 2010; Simões et al. 2006). More recently, HAA1 has been hypothesized to play a role in acetic acid tolerance through the activation of the CWI pathway (Cunha et al. 2018).

ATP and NADH regeneration

Another inhibitory effect occurring during lignocellulosic ethanol fermentation is ATP depletion, mainly caused by the activity of ATP-dependent pumps required to cope with the intracellular acidification caused by weak acids, in particular the plasma and vacuolar H^+-ATPases and multidrug efflux pumps. In this situation, the yeast cell adjusts its carbon flux distribution between respiratory and fermentative growth to achieve energy homeostasis through optimal ATP regeneration (Guo and Olsson 2014). Furthermore, the trehalose synthase (TPS1) has been found to be essential to maintain ATP levels during heat shock (Petitjean et al. 2015). MSN2/4 transcription factors, known to regulate trehalose biosynthesis genes, were also reported to induce glycolysis, increasing the levels of acetyl-CoA, an essential metabolite to generate ATP in the tricarboxylic acid (TCA) cycle and to promote yeast cell growth and proliferation (Kuang et al. 2017).

Additionally, the presence of furan compounds was found to result in the activation of glycolysis and TCA cycle, contributing to both ATP and NADH regenerations (Lin et al. 2009).

Successful cases of yeast robustness improvement for industrial-like conditions

Industrial-derived tolerant strains

The use of *S. cerevisiae* strains isolated from industrial harsh conditions (such as high sugar and ethanol concentrations, elevated temperatures, pH variations, and presence of toxic compounds) for the production of second-generation bioethanol has been receiving increased attention in the last years (Della-Bianca and Gombert 2013). These isolated strains have shown superior abilities than laboratory strains, with the differences in fermentation performance being related to metabolic activity, not only with sugar consumption and ethanol production (Pereira et al. 2010c) but also with furan conversion (Brandberg et al. 2004; Pereira et al. 2014a). Interestingly, the better fermentation performance of industrial isolates compared to laboratory strains in very high-gravity conditions was related with an increased accumulated content of sterols, glycogen, and trehalose in the industrial isolates (Pereira et al. 2011b). On the other hand, under second-generation inhibitory conditions, the *S. cerevisiae* ATCC96581 strain (isolated from spent sulfite liquor at Swedish pulp plant) converted almost completely the furfural of spruce hydrolysate, whereas the laboratory strain CBS 8066 only detoxified 25% (Brandberg et al. 2004). This fact could be explained by a higher activity of alcohol dehydrogenase responsible for the conversion of furfural into less toxic alcohols. Pereira and co-workers (2014a) also reported a faster bioconversion/detoxification of furfural and HMF in eucalyptus hydrolysate by two industrial strains, PE-2 and flocculating CCUG53310 isolated from first- and second-generation bioethanol industries, respectively. The authors concluded that the ability for detoxification of furan compounds is dependent on strain background, which is determinant for an efficient ethanol production (Pereira et al. 2014a). Moreover, the flocculant character of strains, which has well-known process–related advantages (Gomes et al. 2012), has been also related to inhibitor tolerance (Purwadi et al. 2007; Westman et al. 2014). The mechanism and robustness of the flocculating CCUG53310 strain have been investigated and compared with the laboratorial *S. cerevisiae* CBS 8066 (Westman et al. 2012). The flocculant strain showed higher tolerance to the inhibitors present in a spruce hydrolysate, even though it presented lower expression levels of *YAP1*, *ATR1*, and *FLR1* genes (known to confer resistance to lignocellulose-derived inhibitors) than the laboratorial strain, highlighting flocculation as a physiological trait determinant of yeast tolerance. The authors also hypothesized that the lower expression of *YAP1* (normally activated in response to oxidative stress) in the CCUG53310 strain indicated that flocculation may prevent ROS accumulation, through mechanisms that are still not elucidated but are likely related with a reduction of toxic concentrations around the cell and in the cell interior.

Therefore, the selection of robust yeast chassis for metabolic engineering purposes (such as xylose consumption) shows a further edge for the lignocellulose-to-ethanol fermentations (Costa et al. 2017). In fact, Romaní et al. (2015) expressed a xylose consumption pathway in three different *S. cerevisiae* strains: the laboratorial CEN.PK113-5D and two industrial isolates from first-generation bioethanol plants (PE-2 and CAT-1), and observed that the two industrial strains presented higher xylose consumption and ethanol production than the strain with laboratorial background, both in synthetic media and in a corn cob hydrolysate. Kim et al. (2017b) also evaluated the host strain background of a haploid derivative of the industrial strain *S. cerevisiae* ATCC 4124 and of the laboratory D452-2 strain by genetically engineering them for xylose consumption. They observed that the industrial-derived strain had a superior fermentative performance in a *Miscanthus* hydrolysate (superior efficiency of xylose fermentation and ethanol production) than the laboratorial strain containing the same genetic modification, highlighting the importance of selecting a naturally robust host strain. In addition, Costa et al. (2017) showed differences among metabolically engineered industrial strains for xylose consumption depending of the hemicellulosic hydrolysate used.

Moreover, these desirable traits for inhibitor tolerance of the industrial isolates can still be improved through metabolic engineering, mutagenesis, genome shuffling, or evolutionary engineering. The work developed by Liu et al. (2005, 2008, 2018) and Liu and Moon (2009) is a clear example of the development of new improved strains. The industrial *S. cerevisiae* NRRL Y-12632, isolated from the brewer's top yeast in Netherlands in 1925, was subjected to evolutionary engineering in HMF- and furfural-containing media, resulting in the reduction of lag phase, improvement of glucose consumption, and ethanol production in media containing these inhibitors. It was further described that these improved traits resulted from determinant yeast response mechanisms, such as enhanced expression of PDR gene family, increased NAD(P)H-dependent aldehyde reduction activities, increased expression of genes from glycolysis, and PPP for NAD(P)H regeneration and robust cell wall integrity pathway.

Rational metabolic engineering strategies to improve tolerance to lignocellulosic hydrolysates

The use of industrial strains as hosts for metabolic engineering is a promising approach for the feasibility of second-generation bioethanol industry. An extensive knowledge of the mechanisms required for the yeast response towards lignocellulose-

derived inhibitors has been guiding the use of several strategies to develop *S. cerevisiae* strains capable of withstanding acute stresses with improved growth/fermentative performances (Table 3).

Several of these strategies have focused on the detoxification of inhibitory compounds. Jayakody and collaborators (2018) improved the fermentation of a *Miscanthus* hydrolysate by overexpressing of *GRE2* (encoding a NADPH-dependent aldehyde reductase), increasing the yeast capacity to detoxify aldehyde inhibitors, such as vanillin and glycolaldehyde. The overexpression of *PRS3*, responsible for the synthesis of PRPP (a precursor of nucleotide and histidine biosynthesis), was found to improve yeast fermentation rates and productivities in different lignocellulosic hydrolysates, through a hypothesized increase in NADH regeneration which facilitates detoxification furans (Cunha et al. 2015). Nevertheless, it should be noted that this positive effect was dependent of the strain background and composition of the fermentation media, highlighting the importance of the selection of yeast chassis and fermentation conditions for effective metabolic engineering (Cunha et al. 2015). Detoxification of phenolic compounds has also been addressed to improve yeast tolerance: the expression of a laccase from the white rot fungus *Trametes versicolor* in a laboratorial *S. cerevisiae* strain has increased the yeast ability to convert coniferyl aldehyde into less toxic compounds, increasing yeast growth and ethanol production in a dilute acid spruce hydrolysate (Larsson et al. 2001). Wallace-Salinas and collaborators (2014) decreased the lag phase and improved the growth rate of an Ethanol Red strain (previously modified for xylose consumption) (Demeke et al. 2013a, b), in a spruce hydrolysate, by overexpressing *YAP1*, a transcription factor involved in oxidative stress response and tolerance. Furthermore, these authors also overexpressed *MCR1*, coding for the mitochondrial NADH-cytochrome b5 reductase, resulting in a faster furaldehyde reduction capacity with positive effects on yeast growth (similar to the ones resultant from *YAP1* overexpression). Nevertheless, no cumulative effect of the simultaneous overexpression of these two genes on yeast tolerance was observed (Wallace-Salinas et al. 2014).

Other studies have also attempted to improve yeast tolerance together with xylose consumption capacity. In fact, a haploid derivative of an industrial strain, isolated from a molasses distillery, was modified for xylose consumption with the XR/XDH pathway and for acetate consumption by expression of a NADH-dependent acetate reduction pathway (*adhE* gene from *Escherichia coli* coding for an acetylating acetaldehyde dehydrogenase) (Kim et al. 2017b). This later modification not only allowed the in situ detoxification of acetic acid but also increased intracellular NAD^+ levels, potentiating XDH activity and reducing xylitol accumulation, leading to a higher ethanol yield. Hasunuma et al. (2014) also improved ethanol production from wheat straw–derived xylose (in an industrial strain also expressing hemicellulolytic enzymes) through the overexpression of *TAL1* and *FDH1* and expression of a mutant NADH–dependent *ADH1*, which resulted in formate detoxification and faster detoxification of furfural, leading to a higher regeneration of NAD^+ co-factor, improving the XR/XDH consumption pathway. More recently, *HAA1* (encoding a transcription factor involved in adaptation and tolerance to weak acid stress) and *PRS3* (encoding a 5-phospho-ribosyl-1(alpha)-pyrophosphate synthetase that synthesizes PRPP, which is required for nucleotide, histidine, and tryptophan biosynthesis) have been expressed in a first-generation bioethanol strain (PE-2), previously modified for xylose consumption, improving its adaptation to a non-detoxified Paulownia hydrolysate (Cunha et al. 2018). Furthermore, the simultaneous overexpression of both genes had a cumulative positive effect on yeast growth, and expression of both *HAA1* and *PRS3* was found to play a role in yeast cell wall integrity.

These successful strategies show that a thorough knowledge of the mechanisms involved in yeast response towards the presence of inhibitory compounds is a determinant for the development of tolerant strains to attain an efficient and economical production of lignocellulosic bioethanol.

Final remarks and future perspectives

The lignocellulosic process–derived stress factors lead to negative effects in the yeast cell at molecular, metabolic, and structural levels, being the most noteworthy, intracellular acidification, ATP depletion, ROS-induced oxidative stress, redox imbalance, and cell wall and plasma membrane perturbations. In order to cope with these conditions, the cell falls back on several global mechanisms that counteract their synergistic negative effects. In spite of their complexity, some of these mechanisms are nowadays relatively well described and linked with successful cases of yeast engineering. Nonetheless, the majority of studies regarding this subject use laboratorial yeast strains and focus on the effect and response to a single inhibitor. As depicted in this review, in process-like conditions, the synergetic effect of the presence of several inhibitors is of major influence in the process and should always be considered and evaluated in order to efficiently develop lignocellulosic hydrolysate-tolerant strains. Furthermore, the selection of chassis' strains for metabolic engineering strategies should be regarded as a crucial step to attain more robust and efficient strains. In fact, industrial isolates has been receiving growing attention in this field, as they naturally present advantageous traits (such as higher capacity for inhibitor tolerance/detoxification, thermotolerance, faster sugar consumption) that could represent a leverage for the attainment of efficient second-generation bioethanol processes. However, metabolic engineering of these industrial strains still poses some constrains that are being overcome by the presently available molecular toolbox for *S. cerevisiae* (in constant evolution), which facilitates the development of highly engineered

Table 3 Successful cases of rational metabolic engineering of *S. cerevisiae* for improved tolerance towards lignocellulosic hydrolysates

Modification	*S. cerevisiae* strain	Lignocellulosic hydrolysate	Effect	Mechanism	Reference
Laboratory strains					
Expression of laccase from *Trametes versicolor* and overexpression of *SSO2*	INVSC1 (MATa his3-Δ1 leu2 trp1-289 ura3-52)	Dilute acid spruce hydrolysate in g/L: 2.8 acetic acid, 0.7 formic acid, 1.1 levulinic acid, 1.4 furfural, 2.3 HMF, and 2.9 phenolic compounds	Faster growth (μ_{max} increased from 0.000 to 0.012 h^{-1}) and ethanol formation (ethanol yield increased from 0.02 to 0.44 g/g of total sugars)	Improved detoxification of phenolic compounds	Larsson et al. (2001)
Overexpression of *YAP1*	INVSC1 (MATa his3-Δ1 leu2 trp1-289 ura3-52)	Diluted spruce hydrolysate	Improved ethanol productivity (0.17 g/L/h vs. 0.05 g/L/h of the control)	YAP1 plays an important role in the oxidative stress response	Alriksson et al. (2010)
Expression of the *adhE* gene from *E. coli* (coding for acetylating acetaldehyde dehydrogenase) and of the mutant *Salmonella ACS* gene (coding for a acetyl-CoA synthetase)	D452-2 (*MATα*, *leu2*, *his3*, *ura3*, and *can1*)	*Miscanthus* hydrolysate in g/L: 20 glucose, 50 xylose, 10 acetate, 1 HMF, and 2 furfural	Acetate consumption and faster xylose consumption Increased ethanol yield (18.4%) and decreased glycerol and xylitol yields (40.3%)	In situ detoxification of acetic acid Decrease of redox imbalance of xylose consumption pathway	Zhang et al. (2016)
Xylose consumption: *XYL1*, *XYL2*, and *XYL3*, evolutionary engineered in xylose-containing media and deletion of *PHO13* and *ALD6*					
Overexpression of *SPE3* and deletion of *TPO1* and *OAZ1*	D452-2 (*MATα*, *leu2*, *his3*, *ura3*, and *can1*)	Corn stover hydrolysate in g/L: 3.3 acetic acid, 0.8 HMF, and 0.4 furfural	Improved ethanol productivity (14% higher than the control strain)	Spermidine extends the lifespan of *S. cerevisiae* (Eisenberg et al. 2009)	Kim et al. (2017a)
Overexpression of *YAP1*, *STB5*, *WAR1*, *PDR8*, *CAT8*, *PUT3*, and *GZF3*, separately	*BY4741* (*MATa*, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*)	Sugarcane bagasse hydrolysate in g/L: 0.89 furfural, 0.11 HMF, 1.4 acetic acid, 0.03 formic acid, and 0.05 levulinic acid Spruce hydrolysate in g/L: 0.36 furfural, 0.03 HMF, 0.72 acetic acid, 0.27 formic acid, and 0.12 levulinic acid	Enhanced relative growth rates: 2.85- and 2.75-fold higher than those of the control strain in bagasse and spruce hydrolysate	Transcription factors involved in oxidative stress (*YAP1* and *STB5*), acid stress adaptation (*WAR1*), pleiotropic drug resistance (*PDR8*), carbon source responsiveness (*CAT1*), amino acid biosynthesis (*PUT3*), and nitrogen catabolism (*GZF3*)	Wu et al. (2017)
Overexpression of *GRE2* Xylose consumption: *XYL1*, *XYL2*, and *XYL3*, evolutionary engineered in xylose-containing media, and knockout *ALD6*	D452-2 (*MATα*, *leu2*, *his3*, *ura3*, and *can1*)	*Miscanthus* hydrolysate	Growth in the toxic hydrolysate at low inoculum (vs. no growth of the control strain) Improved xylose consumption and ethanol yields at higher inoculum	Possible detoxification of glycolaldehyde and other major inhibitory compounds GRE2 also converts vanillin into the less toxic vanillin alcohol through an NADPH-dependent reaction	Jayakody et al. (2018)
Overexpression of *TRX1* (coding for thioredoxin)	BY4742 (MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0)	Diluted bagasse hydrolysate in g/L: 44 glucose, 5.8 xylose, 4.1 acetic acid, 0.6 furfural, and 0.2 HMF	Higher ethanol titer (10.50 g/L vs. 8.61 g/L), yield (0.30 g/L vs. 0.23 g/L), and productivity (0.46 g/L/h vs. 0.30 g/L/h) than the control	Maintenance of energy and redox homeostasis and minimization of stress-induced cell damages Increased levels of trehalose, fatty acids, GABA, and putrescine provided additional defense against oxidative and redox stresses	Unrean et al. (2018)
Industrial strains					
				Detoxification of formate (*FDH1*)	

Table 3 (continued)

Modification	*S. cerevisiae* strain	Lignocellulosic hydrolysate	Effect	Mechanism	Reference
Overexpression of *FDH1* and *TAL1* and expression of a mutant NADH-dependent *ADH1* Xylose consumption: *Scheffersomyces stipitis Xyl1* and *Xyl2* and *S. cerevisiae XKS1* Hemicellulolytic enzymes: *Trichoderma reesei XYNII*, *Aspergillus oryzae XylA*, and *Aspergillus aculeatus BGL1*	Sun049, obtained from Suntory Limited (brewing and distilling company)	Rice straw hydrolysate in mM: 17.6 formate, 28.6 acetate, 0.3 vanillin, 12.6 furfural, and 0.9 5-HMF	2.7-fold higher ethanol titers and improved xylose consumption	Faster furfural detoxification, regenerating NAD^+ which improved ethanol yield from xylose (*ADH1*) *TAL1* overexpression improves PPP	Hasunuma et al. (2014)
Overexpression of *YAP1* and *MCR1*, separately Previous modifications: GSE16 (*XylA*; *XKS1*; *TAL1*; *TKL1*; *RPE1*; *RKI1*; *HXT7*; *AraT*; *AraA*; *AraB*; *AraD*; *TAL2*; *TKL2* + mutagenesis (*EMS*) + genome shuffling and evolutionary adaptation + backcrossing with a haploid segregant of Ethanol Red that is tolerant towards acetic acid)	Ethanol Red, a strain currently commonly used in industrial bioethanol fermentations	Spruce hydrolysate in g/L: 3.7 acetate, 0.96 HMF, and 0.78 furfural	Decreased lag phase (9 h to 5.3 h) and improved growth rate (around 60% higher)	Faster furaldehyde reduction capacity	Wallace-Salinas et al. (2014)
Overexpression of *PRS3*	PE2 and CCUG53310, isolated from the first- and second-generation bioethanol plants, respectively	*Eucalyptus globulus* wood hydrolysate in g/L: 2.1 acetic acid, 1.4 furfural, and 0.25 HMF Corn cob hydrolysate in g/L: 1.6 acetic acid, 1.6 furfural, and 0.12 HMF	Improved fermentation rate (up to 32%) and productivity (up to 48%) in the different hydrolysates	Possible increase in redox balance through NADH regeneration for furfural and HMF detoxification	Cunha et al. (2015)
Expression of the *adhE* gene from *E. coli* (coding for acetylating acetaldehyde dehydrogenase) Xylose consumption: expression of *XYL1*, *XYL2*, and *XYL3* from *S. stipitis* and deletion of *PHO13* and *ALD6*	Haploid derivative of ATCC 4124 strain, isolated from a molasses distillery	*Miscanthus* hydrolysate	Higher ethanol yield and lower by-product yield	In situ detoxification of acetic acid Decrease of redox imbalance of xylose consumption pathway	Kim et al. (2017b)
Overexpression of *HAA1* and/or *PRS3* Xylose consumption: expression of *XYL1* and *XYL2* from *S. stipitis*, overexpression of *TAL1* and *XKS*, and deletion of *GRE3*	PE-2, isolated from first-generation bioethanol plant	Paulownia hydrolysate in g/L: 5.84 acetic acid, 1.96 furfural, and 0.719 HMF	Improved yeast adaptation to non-detoxified hydrolysate with high acetic acid content, resulting in higher ethanol titers (≥ 12%)	Increased robustness of yeast cell wall when challenged with acetic acid stress, caused by a possible involvement of *HAA1* and/or *PRS3* in the modulation of the cell wall integrity pathway	Cunha et al. (2018)

yeast strains. Accordingly, more recent studies have been using industrial yeast and lignocellulosic hydrolysates to develop more tolerant strains. Nevertheless, there is a lack of fundamental understanding regarding the response mechanisms that confer higher tolerance and robustness to these industrial isolates, being a subject requiring further investigation. As the complexity of yeast cell response is unraveled, an increasing number of metabolic engineering strategies will become successful, feeding back the accumulated knowledge. Nowadays, works to improve yeast tolerance still mainly focus on only one part of the inhibitory effects (such as oxidative stress or specific inhibitor detoxification). Due to the complexity of the multifactorial yeast tolerance to stress and in order to be effective, metabolic engineering strategies should be rationally designed to simultaneously overcome all the stresses imposed by the lignocellulosic hydrolysates. Additionally, the heterogeneity of lignocellulosic hydrolysates (dependent on the raw material and pretreatments used) should also be taken into consideration, as possible synergetic and antagonistic effects may arise from different inhibitory compositions and trigger different yeast responses. Taken together, this knowledge can unlock a wide range of strategies to develop tailor-made *S. cerevisiae* strains through rational metabolic engineering approaches for industrial processes, ultimately resulting in improved robustness when challenged in lignocellulosic hydrolysates, greatly contributing to the development of sustainable growth based on a bioeconomy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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