



Resistance mechanisms and reprogramming of microorganisms for efficient biorefinery under multiple environmental stresses



Ke Xu^{a,b,1}, Yun Seo Lee^{a,1}, Jun Li^{a,**}, Chun Li^{a,*}

^a Institute for Synthetic Biosystem/Department of Biochemical Engineering, School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing 100081, PR China

^b Key Lab for Industrial Biocatalysis, Ministry of Education, Department of Chemical Engineering, Tsinghua University, Beijing 100084, PR China

ARTICLE INFO

Keywords:

Biorefinery
 Environmental stresses
 Microbial resistance mechanism
 Synthetic biology
 Bioprocess integration

ABSTRACT

In the fermentation process of biorefinery, industrial strains are normally subjected to adverse environmental stresses, which leads to their slow growth, yield decline, a substantial increase in energy consumption, and other negative consequences, which ultimately seriously hamper the development of biorefinery. How to minimize the impact of stress on microorganisms is of great significance. This review not only reveals the damaging effects of different environmental stresses on microbial strains but also introduces commonly used strategies to improve microbial tolerance, including adaptive evolution, reprogramming of the industrial host based on genetic circuits, global transcription machinery engineering (gTME) and bioprocess integration. Furthermore, by integrating the advantages of these strategies and reducing the cost of system operation, the tolerance of industrial strains, combined with production efficiency and process stability, will be greatly improved, and the development prospects of biorefinery will be more widespread.

1. Introduction

With the increasingly serious energy crisis and environmental issues worldwide, the traditional chemical industry based on petroleum refining faced upgrading to meet the requirements of green and sustainable development [1]. The biorefinery industry emerges at an historic moment. Major scientific and political agencies have been promoting the phenomenon of “biorefinery” as a solution for sustainable development [2,3].

The IEA (International Energy Agency) defines biorefinery as a sustainable production process which, through using biomass as raw materials, generates a series of biobased products and bioenergy [4]. In biorefinery, renewable feedstocks, which are raw materials used in biorefinery (biomass or food waste), are refined to yield fuels and commodity chemicals by means of chemical and biological conversion technologies. Biorefinery can provide the necessary energy and chemicals for production and living [2,5] (Fig. 1).

Biorefinery is often compared to a traditional petroleum refinery, which converts fossil crude oil into higher value products [6]. The biggest differences between the two processes are that the raw

materials of a petroleum refinery are nonrenewable and characterized by low oxygen content, which will lead to increased carbon emissions, thereby causing environmental problems [7]. Biorefinery uses a wide range of technologies. In particular, these concepts can be applied to biomass resources including bioproducts, biofuels, and chemicals [8]. The raw materials of biorefinery are renewable biomass, including organic crop waste, wood, and straw, which contain a high proportion of oxygen molecules and can enable carbon dioxide recycling by the petroleum refining industry [9,10]. The NREL (National Renewable Energy Laboratory) divided biorefinery into biomass precursors, basic structures, secondary chemicals, intermediates and final products based on the center compounds. With renewable biomass as raw material, biorefinery can produce various chemical compounds and can enable the upgrading of several industries [11]. Various technological processes of biorefinery are being applied jointly to produce different biological resources [8,12].

However, the development of the biorefinery industry is seriously restricted by the relatively high production cost required for maintaining the optimum fermentation conditions and the relatively low productivity of industrial strains, which are affected by different kinds

Peer review under responsibility of KeAi Communications Co., Ltd.

* Corresponding author.

** Corresponding author.

E-mail addresses: junliBIOTECH@bit.edu.cn (J. Li), lichun@bit.edu.cn (C. Li).

¹ Ke Xu and Yun Seo Lee contributed equally to this work.

<https://doi.org/10.1016/j.synbio.2019.02.003>

Received 19 December 2018; Received in revised form 1 February 2019; Accepted 20 February 2019

2405-805X/ © 2019 Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

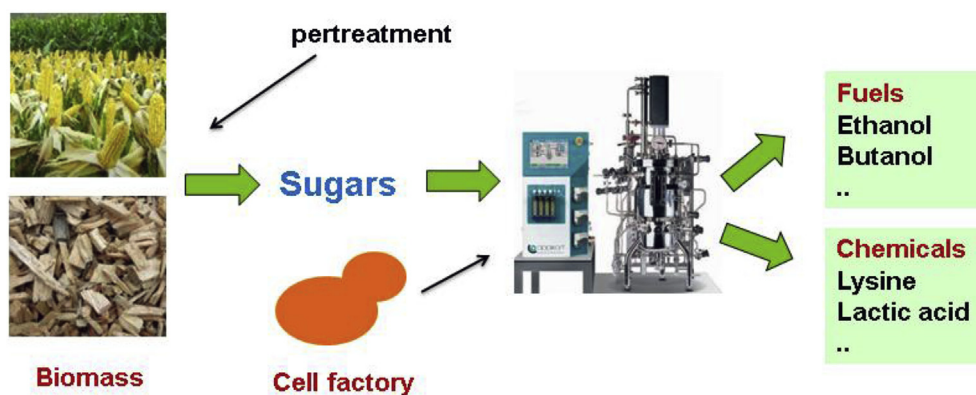


Fig. 1. The applications of biorefinery for bio-based products and bio-energy.

of stresses. For example, during fermentation, industrial strains encounter multiple stresses, including high temperature, low pH, organic solvents, toxic byproducts and mechanical damage [13,14]. If microorganisms are exposed to novel environments, they may mount erratic nonspecific responses, which may result in cell death [15]. For example, the production of bioethanol using lignocellulose as raw material is an important part of renewable energy. Compared with corn ethanol, cellulosic ethanol fermentation faces more stress factors (mainly from lignocellulose degradation inhibitors). Lignocellulose hydrolysate contains a variety of yeast inhibitors, including weak acids (formic acid, acetic acid, etc.), furan compounds (furfural, hydroxymethyl furfural, etc.) and phenols (vanillin, etc.). The undissociated weak acid can enter the cytoplasm, causing a decrease in intracellular pH and increasing the delay time of the yeast. Furfural, hydroxymethyl furfural and phenols increase ROS and inhibit cell growth through the toxicity of their aldehyde group. The combination fermentation environment of lignocellulose, which is inhibited with high temperature and high concentrations of ethanol, is a great challenge to the stress resistance of yeast. At present, the highest production of cellulosic ethanol reported is only 86 g/L, and it is very difficult to further improve the production of cellulosic ethanol due to the lack of multi-tolerant yeast [16–18]. Thus, we can expect that adaptation to novel environments will require the complete reprogramming of cellular functions; otherwise, strains exposed to these stress conditions will develop a decreased growth rate, a decline in production, and a higher energy consumption [19,20].

2. Environmental stresses in biorefinery

2.1. Heat stress

Heat stress is one of the most important factors affecting the growth of microorganisms [21]. Most of the products of biorefinery are produced by medium temperature fermentation; during fermentation, a large amount of heat energy is released due to cellular metabolism and mechanical agitation, resulting in the continuous rise of the fermentation temperature [22]. When the fermentation temperature exceeds a certain range, the cell is in a heat stress environment.

The harmfulness of heat stress on morphology can be explained by high temperatures resulting in the aggregation of proteins and the imbalance of protein homeostasis. In addition to the incorrect folding of single proteins, high temperatures can also destroy the internal structure of the cell [23]. Moderate heat shock can lead to actin reorganization of stress fibers, and severe heat shock even can lead to the collapse of the actin and microtubule network [24,25]. With the disruption of the cytoskeleton, the correct location and transport function of the organelle is destroyed [26], Golgi systems and the endoplasmic reticulum become fragmented into small pieces [27], and the numbers of mitochondria and lysosomes are decreased [28]. At the same time,

the ATP level decreases sharply. Heat stress also affects nucleus function. For example, the ribosome assembly site will be swollen under heat shock and accompanied by a large number of granular errors, RNA deposition, and ribosomal protein aggregation [29]. Heat stress can also cause a serious impact on the cell membrane, including changing morphology and fluidity [30] and enhancing permeability, which leads to a change in cytoplasmic osmotic pressure and pH [31] (Fig. 2A).

2.2. Oxidative stress

When the mitochondrial electron transport system is damaged by environmental stresses, it produces the main toxic byproduct intracellular reactive oxygen species (ROS) [32]. Finally, it causes oxidative stress. ROS are mainly comprised of peroxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl free radical ($\cdot\text{OH}$) and other components. All of these components are based on a molecule or group containing one or more nonpaired electrons and are derived from the molecular state O_2 [33]. High levels of ROS may change DNA structure, modify proteins and lipids, activate several stress-induced transcription factors, and even produce pro-inflammatory and anti-inflammatory cytokines [34]. ROS can also damage biological macromolecules at different levels, such as gene duplication and transcription, protein expression and complex metabolism. Together, these processes have a negative effect on the whole biological function of the production strain. As a result, cell viability and fermentation capacity are greatly damaged, which can ultimately result in cell death [35] (Fig. 2B).

2.3. Acid stress

The unpredictable fluctuation of pH in fermentation is usually complex. The activity of intracellular enzymes can be tremendously influenced by environmental pH, and eventually, fermentation effectiveness will decrease [36]. Acid stress also has a negative effect on the host cell, and it may advance calcification and oxidative stress, worsening inflammation and causing anaerobic metabolism [37]. During organic acid fermentation, the accumulation of products causes acid stress to the strains. The main reason for acid stress is that most of the organic acids exist in a nondissociated form at low pH conditions, and they can directly diffuse into the cytoplasm, quickly releasing protons and reducing the intracellular pH [38]. The continuous acidification of the intracellular environment damages some acid sensitive DNA, denatures proteins, and ultimately affects cell growth. Anderson et al. [39] found that when the cell produces succinic acid in *E. coli*, with the accumulation of intracellular nadide acid, cell viability and biomass were obviously decreased. Roa et al. [40] used *Rhizopus oryzae*, which produces fumaric acid. The production reached 30.21 g/L at pH = 5, and when the pH decreased to 3, the yield was only 9.36 g/L (Fig. 2C).

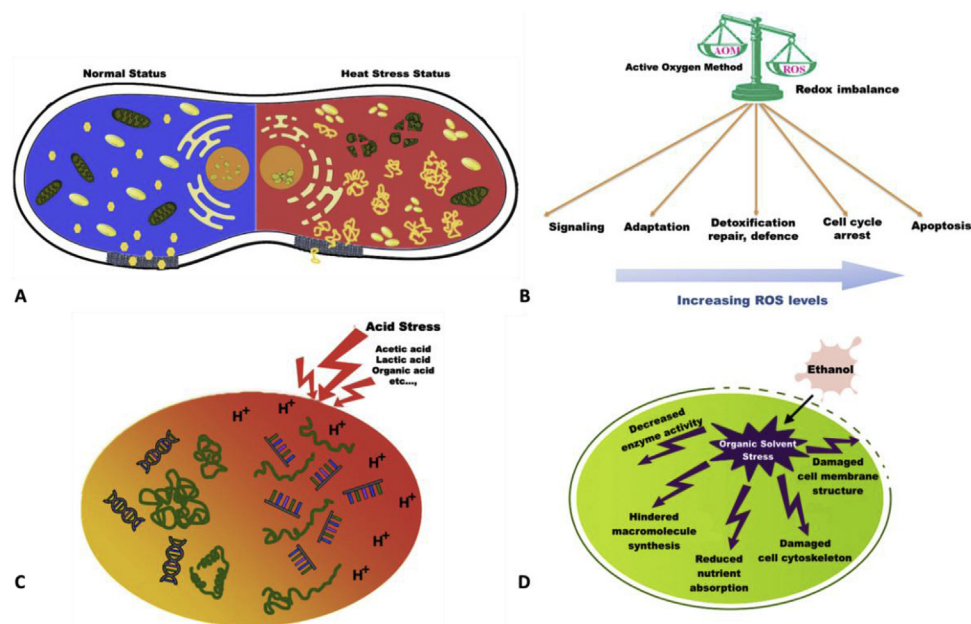


Fig. 2. A. Heat Stress (the left side is a normal status, and the right side is heat resistant status, as the mitochondria and Golgi complex of cell were destroyed) B. Oxidative Stress and the unpleasant effects on cells C. Acid Stress (when the cell were exposed to acid stress, intracellular pH was decreased, DNA was mutated and intracellular protein also was mutated) D. Organic Solvent Stress (when the cell were exposed to organic solvent, the membrane was destroyed, and organic solvent decreased enzyme activity, hindered macromolecule synthesis and reduced nutrient absorption ...).

2.4. Organic Solvent Stress

The stress of organic solvents in industrial fermentation is mainly due to alcohol products. For example, in ethanol fermentation, the accumulation of ethanol may reach up to 17.5% [41]. High-concentration mash fermentation technology can increase the yield and reduce the costs of downstream operations. As ethanol production strains, yeast can resist up to 15% (v/v) of ethanol, but in fermentation, when the ethanol concentration reached 8% (v/v), the growth rate of yeast showed a significant reduction [42] (Fig. 2D).

The stress of organic solvents on cells is mainly due to two aspects, namely, the effect on cell morphology and the decrease in cell physiological activity. High concentrations of organic solvents can damage the cytoskeleton, damage cell membrane structure, significantly reduce the absorption of nutrients, hinder the synthesis of biological macromolecules, and decrease the activity of enzymes associated with glycolysis [43,44]. At present, researchers can improve the ethanol-tolerance of yeast by changing the culture medium components or optimizing the culture conditions, but the mechanisms of these processes are not clear [13].

2.5. Ionic liquid stress

Ionic liquids (ILs) are superior solvents for numerous industrial applications, including in the manufacturing of paint additives, and they are also used in pharmaceutical intermediaries [45]. For example, the inherent recalcitrance of biomass requires an initial pretreatment step to render polysaccharides free from lignin for subsequent enzymatic or chemical hydrolysis into fermentable sugars. To solubilize lignocellulosic biomass, certain hydrophilic ILs are highly effective and environmentally friendly pretreatment agents that generate relatively low amounts of biomass-derived inhibitors compared with other conventional pretreatment methods [46–48].

IL toxicity is closely connected to the cation families containing imidazolium, pyridinium, piperidinium, and quaternary ammonium cations. In particular, increasing the length of the alkyl chain results in cations having a more linear structure, which increases the toxicity of ILs. The lipophilic nature of ILs is also connected to their toxicity. This property makes ILs permeate the phospholipid bilayer of the cell, thereby disrupting cell membrane structure [49,50]. A major disadvantage of ILs is their intrinsic microbial toxicity, which impairs the

growth of typical biofuel-producing hosts such as *E. coli* and *S. cerevisiae* [51,52]. In addition, the inhibition of biofuel synthetic enzymes by ILs can severely reduce the yield of the final product. The mechanism of microbial tolerance to ILs has not been elucidated. Researchers preliminarily found that ionic liquid resistance is strongly related to the cationic substituted side chain. However, the toxic mechanisms of ILs on various types of organisms remain poorly understood, as the period of genotoxicity, extent of DNA damage, and bioaccumulation of ILs are unknown.

2.6. Toxicity byproducts stress

During fermentation, some toxicity byproducts also exert great stress on industrial strains. For example, the pretreatment process of cellulose is based on the premise that, in the industrial production of bioethanol, with the help of cellulase, cellulose will be converted into sugar [53]. At the same time, the process will produce a large number of inhibitors, and the major compound of these inhibitors is furan aldehyde (mainly furfural and HMF). These inhibitors may delay the growth of yeast and reduce the production of ethanol. Inhibitors of high concentration may even cause a large amount of cell death [54].

The possible inhibition mechanisms of furfural aldehyde compounds on yeast include 1) directly inhibiting alcohol dehydrogenase, aldehyde dehydrogenase, pyruvate dehydrogenase, hexokinase and glyceraldehyde-3-Phosphate dehydrogenase, resulting in a decreased cell production capacity and prolonged stagnation, 2) inhibiting intracellular aldehyde-oxidizing enzymes, leading to increased ROS content and 3) the fact that yeast can utilize NAD (P) H, which participates in the reduction reaction and converts furfural and HMF to their corresponding alcohol compounds; however, the conversion process leads to a large amount of coenzyme consumption, resulting in the imbalance of intracellular coenzyme levels [55,56]. Some antioxidant proteins are also inactivated when the coenzyme is reduced, making the yeast cells susceptible to oxidative damage.

2.7. Mechanical damage stress

Mechanical damage stress also seriously affects biorefinery. One of the traditional beliefs of the brewing industry is that mechanical agitation during fermentation damages the yeast cell. The damage mainly consists of fluid mechanical stress due to agitation and bursting bubbles

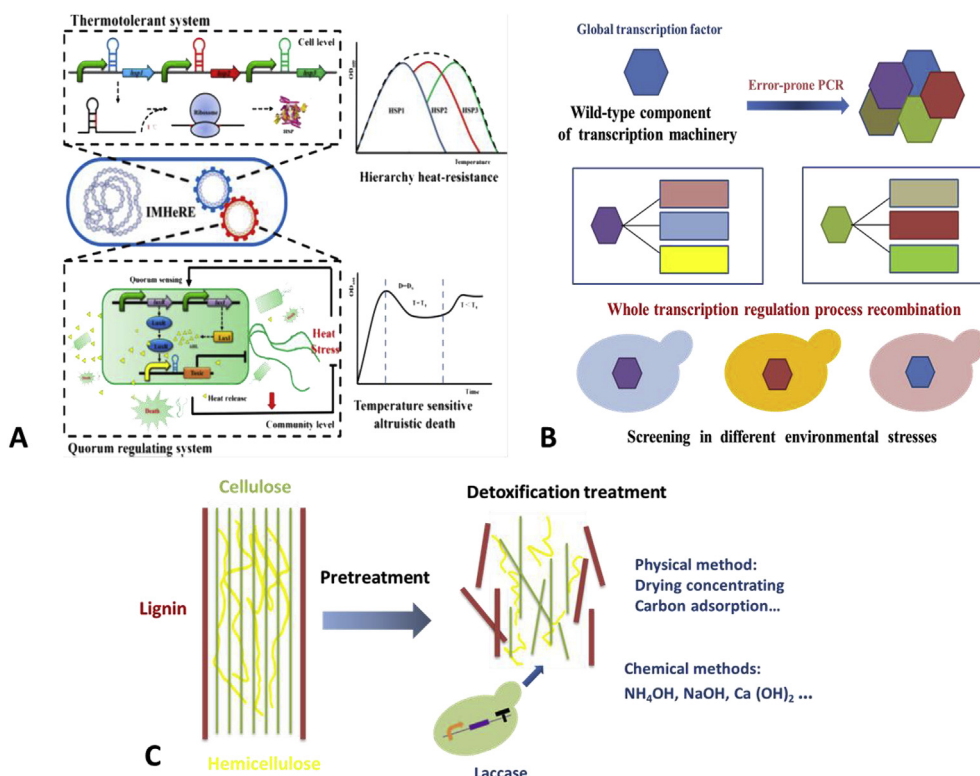


Fig. 3. A. Thermo-robustness mechanism of IMHeRE (including thermo-tolerant system and quorum-regulating system) B. global transcription machinery engineering project (gTME) C. Bioprocess integration and optimization in the pretreatment process of the cellulose.

[57]. Frequently, this process is referred to as “shear damage” to explain the detrimental changes in bioprocessing when mechanical agitation and aeration are introduced into a bioreactor. Because the fluid mechanical stress, which is associated with bubbles bursting at the surface of the media, has local specific energy dissipation rates, i.e., eT (W/kg), two to three orders of magnitude higher than those found under typical agitation conditions, the stress arising can damage cells [58,59].

3. Strategies for improving the tolerance of industrial strains

In the past decades, researchers have obtained some laboratory strains with different tolerant characteristics through various biological technologies.

3.1. Adaptation evolution

Adaptive evolution, also known as laboratory evolution or adaptive laboratory evolution (ALE), is an effective method to study the evolution of microorganisms under specific environmental conditions. It occurs through the long-term domestication of microorganisms under certain environmental pressures to obtain mutant strains with specific physiological functions [60].

Adaptive evolution has been widely used in the research of microbial evolutionary mechanisms. It is used to screen microorganisms resistant to environmental stresses [61,62]. Nielsen et al. [63] obtained high yield ethanol yeast strains with adaptive evolution under culture conditions $\geq 40^\circ\text{C}$. Genome sequencing and metabolic flux analysis showed that the composition of sterols was significantly changed compared with the original strain. To enhance acid-tolerance, Zhang et al. [64] used adaptation evolution and obtained a *Lactobacillus casei* strain with good growth performance, a high lactic acid yield, a biomass 60% higher than the original strain, and a growth rate 10% higher than the original strain. The new strain's tolerance to hydrochloric acid

was increased by 3.5 times, and its tolerance to lactic acid was increased by 638 times.

Using adaptive evolution to improve the tolerance of microbial strains has made some progress. However, the limitation of the tolerance mechanisms and current research methods limit the effects of the process. In addition, the long breeding cycle, poor passage stability, and the inability to control strain modification seriously obstruct its further development. How to improve the microbial hosts' intelligent response to environmental stress changes is still the key problem.

3.2. Reprogramming industrial host based on synthetic biology

With the development of synthetic biology, the construction of an artificial synthesis system on a molecular level has attracted considerable attention [65]. By the continuous exploration of various functional gene devices, a large number of new artificial synthetic biological systems have been built. With the continuous analysis of microbial tolerant mechanisms, breeding technology has gradually changed from the nonrational to rational gene operation technology. These developments make the research on robust and intelligent microbial strains more desperately needed. Rational reconstruction focuses on either regulating endogenous transcriptional levels [66] or introducing heterogenous tolerant genes [67].

Extremophiles refer to microbial strains grown in an extreme environment (high temperature, low temperature, high salt, acid, and other environments). They represent the extreme of life and have a wealth of unknown biological processes and functions [68]. At the same time, they contain many functional proteins with unique properties, which are a rich treasure trove of special functional protein resources [69]. Extremophiles have good adaptability to harsh environments, and as a result, they have a great potential for biotechnology development. Liu et al. [67] have effectively improved the thermotolerance of *S. cerevisiae* by introducing heat shock protein from *Thermoanaerobacter tengcongensis*, and their results show that engineered *S. cerevisiae*

possesses a greatly improved growth ability, as well as production efficiency, under high temperatures. Xu et al. [70] developed an artificial antioxidant defense system and used it to improve the thermotolerance of yeast for the first time. The system showed great potential in balancing the host's oxidative-reductive homeostasis. Jia et al. [71] devised a gene network and named it intelligent microbial heat-regulating engine (IMHeRE) (Fig. 3A). IMHeRE was utilized to improve the thermobustness of *Escherichia coli* via the integration of a thermotolerant system and a quorum-regulating system. At the cell level, the thermotolerant system composed of different heat shock proteins and RNA thermometers hierarchically expands the optimum temperature by sensing heat changes. At the community level, the quorum-regulating system dynamically programs the altruistic sacrifice of individuals to reduce metabolic heat release by sensing the temperature and cell density.

Regulating endogenous transcriptional levels is also an effective way to improve strain tolerance. Si et al. [72] report an automated platform for multiplex genome-scale engineering. Standardized genetic parts encoding overexpression and knockdown mutations of $\geq 90\%$ of yeast genes are created in a single step from a full-length cDNA library. With the aid of CRISPR-Cas, they improved the acetic acid (HAc) tolerance of *Saccharomyces cerevisiae*. The Synthetic Yeast Genome Project (Sc2.0) is a trending topic in the field of synthetic biology in recent years. The goal of the Sc2.0 is the complete synthesis of a custom-designed genome for a eukaryotic model organism to serve as a platform for systematic studies of eukaryotic chromosomes [73]. SCRaMbLE (Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution) is a genome restructuring technique that can be used in synthetic genomes such as that of Sc2.0 and contains hundreds to thousands of strategically positioned loxP sites [74]. Shen et al. [75] used SCRaMbLE to induce rearrangements in yeast strains harboring one or more synthetic chromosomes, and the heterozygous diploid strains showed increased thermotolerance and caffeine tolerance.

Reprogramming industrial hosts based on synthetic biology mainly reforms a single or a few genes to enhance the tolerance of the strains. However, it is still necessary to explore the mechanism and identify all tolerant genes for a specific restriction factor. Moreover, overexpression of tolerant genes may excessively burden the host cell, resulting in the imbalance of whole cell metabolism or uncontrolled cell growth.

3.3. Overall improving the industrial microorganisms stress resistance ability

It is now generally accepted that most cellular phenotypes are affected by a group of interrelated genes. As a result, engineering a desired phenotype would be facilitated enormously by simultaneous multiple gene modification, yet the capacity to introduce these modifications is very limited. In recent years, with the gradual understanding of microbial regulation mechanisms, overall tolerance transformation methods have been of great concern, and global transcription machinery engineering (gTME) emerges at an historic moment [76] (Fig. 3B).

gTME is a new cell phenotype transformation method, which was proposed by Alper and Stephanopoulos from MIT in 2006 [43]. It uses genetic engineering methods for the transformation of global transcription factors, altering the whole transcription regulation process and thereby affecting the transcription and expression of a series of target genes. gTME can regulate the transcription level of several tens or even hundreds of genes by regulating the key components of the metabolic regulatory network to obtain specific tolerance. Compared with adaptive evolution, gTME has a short experimental period and a clear genetic background. Compared with the expression of tolerant genes, gTME does not depend on a single specific tolerance mechanism.

At present, global tolerant transformation is mainly realized in the following three ways: (1) the transformation of endogenous transcription mechanisms, (2) the introduction of exogenous regulatory factors,

and (3) the construction of artificial transcription factors [43,77]. Alper and Stephanopoulos first used gTME in *E. coli* and randomly mutated the rpoD gene, which encodes the sigma factor $\sigma 70$, as $\sigma 70$ can effect RNA polymerase binding with the promoter. They obtained mutant strains that improved ethanol tolerance and lycopene yield and obtained mutant strains that improved ethanol and SDS tolerance at the same time. The same strategy was used for the transformation of the global transcription factor TATA binding protein TBP (encoding SPT15) in yeast, and the mutant strain spt15-300 was successfully screened for improving both ethanol and glucose tolerance. The analysis showed that spt15-300 has hundreds of genes that are differentially expressed when compared to the original strain [78].

Tan et al. [77] demonstrate that the ethanol tolerance of *Zymomonas mobilis* can be greatly enhanced through the random mutagenesis of the global transcription factor RpoD ($\sigma 70$). All of these examples demonstrate that gTME can provide an alternative and useful approach for strain improvement for complex phenotypes.

3.4. Bioprocess integration and optimization for improving the efficiency of biorefinery

Today, bioprocess integration has been developed to optimize both the yield and the cost-effectiveness of production. Researchers aim at developing novel concepts for compact, clean and efficient biotechnological manufacturing processes. For example, because the pretreatment of cellulose would produce a large amount of inhibitor [79], an effective method of detoxification of pretreatment raw materials before fermentation would be of great use [80].

Detoxification includes the use of specific enzymes (e.g., laccase) or microorganisms' detoxification to hydrolysis solution. Laccase is a copper containing redox enzyme, which is specific to a single electron oxidated to the phenolic substrate molecule, and generates the corresponding active radical; the active intermediate then is converted to dimers, oligomers, and polymers [81]. In this process, small and medium molecular phenolic compounds are oxidized and polymerized, resulting in lower toxicity of high molecular weight compounds and also reduced toxicity of phenolic substances in lignin cellulose pretreatment solution.

Physical methods include vacuum drying concentrating, cooking, activated carbon adsorption, ion exchange adsorption, and solvent extraction. Vacuum concentration and cooking can reduce volatile inhibitors, and ion exchange and solvent extraction can effectively reduce the acetic acid, furfural and phenolic compounds [82,83].

Chemical methods, including the use of bases (NH_4OH , NaOH , $\text{Ca}(\text{OH})_2$, etc.) and the excess lime method, can be used for the treatment of hydrolysate. NH_4OH can effectively remove furan aldehydes, and excess lime treatment can improve the utilization rate of monosaccharides in the hydrolysate [84]. In addition, the washing of pretreatment raw materials is also a simple detoxification process [85]. The combination of two or more methods can achieve better detoxification (Fig. 3C).

Fermentation optimization can also reduce the effects of inhibitors on production strains. Yeast has a certain degree of inherent resistance to furan aldehydes and phenols. In aerobic and anaerobic conditions, furfural and HMF can be transformed into the corresponding lower toxicity alcohol by yeast. Once furfural and HMF have been transformed completely, the growth and ethanol production ability of yeast would be restored. Optimization of substrate feeding strategy is also an effective measure to alleviate this inhibition. By controlling the feeding rate of the substrate, we can ensure the concentration of inhibitor does not exceed the tolerance of yeast, and the fermentation process can be carried out smoothly.

4. Discussion

In biorefinery, industrial strains often suffer from different stresses

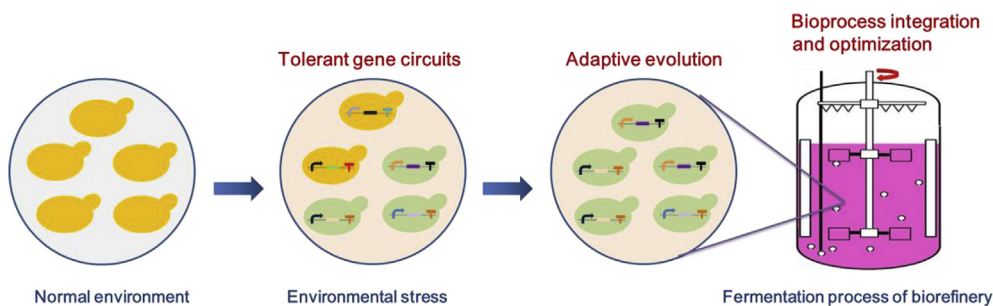


Fig. 4. Multiple strategies synergy in boosting multi-level tolerance.

caused by culture environment or self-metabolism, for which extra operations for process conditioning to industrial strain tolerance are economically and energetically prohibited. Strain tolerance induced by stress involves the interactions of many genes on the genome level, including genes associated with protein synthesis, material transport, energy metabolism, lipid metabolism, membrane and cell wall maintenance, transcription factors and signal transduction pathways, etc. Therefore, improving the robustness and tolerance of industrial strains will markedly enhance productivity and the economic efficiency of biorefinery. Now, the challenge facing the development of tolerant industrial strains is that current research is mostly carried out on laboratory model strains and composing medium, thereby making the results difficult to realize in industrial production. To date, no one has applied modern breeding technology to popularize industrial yeast in an effective way from the perspective of industrial application. Adaptive evolution or the introduction of tolerance genes has a limited effect on relieving multiple stresses. As a cell phenotype transformation method, gTME has shown its application potential for improving the multitolerance of strains. Similarly, bioprocess integration and optimization is a wonderful strategy for integrating the advantages of various technologies and reducing the cost of system operation.

With the advancement of technology in synthetic biology, many fully automatized robotic platforms have been established to facilitate high-throughput screening for engineered strains. With the help of high-throughput screening, the combination of genetic circuits, adaptive evolution and bioprocess integration and optimization demonstrated the superiority and necessity of boosting multilevel tolerance (unpublished data) (Fig. 4). At the same time, the tolerance mechanism of the engineered industrial strains was revealed through transcriptional genome-scale analysis. All these strategies will further improve the industrial microorganisms' resistance ability in biorefinery, expand the product space, and enhance its development prospects.

Acknowledgments

The authors acknowledge funding support from the National Natural Science Foundation of China (21736002, 21576027, 21425624).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.synbio.2019.02.003>.

References

- [1] Rodionova MV, Poudyal RS, Tiwari I, et al. Biofuel production: challenges and opportunities. *Int J Hydrog Energy* 2017;42(12):8450–61.
- [2] Sarma SJ, Ayadi M, Brar SK. *Biorefinery* [M]. 2016.
- [3] Maity SK. Opportunities, recent trends and challenges of integrated biorefinery: Part I. *Renew Sustain Energy Rev* 2015;43(1):1446–66.
- [4] Clark JH, Luque R, Matharu AS. Green chemistry, biofuels, and biorefinery. *Annu Rev Chem Biomol Eng* 2012;3:183–207.
- [5] Springer. *Biomass conversion and biorefinery* [D]. Springer Berlin Heidelberg; 2016.
- [6] Kumar RV, Pakshirajan K, Pugazhenti G. Petroleum versus biorefinery-based platform chemicals [M]. Elsevier Inc.; 2016.
- [7] Xuan G, Zhan YL, Chen CM, et al. Influence of packing material characteristics on the performance of microbial fuel cells using petroleum refinery wastewater as fuel. *Renew Energy* 2016;87:437–44.
- [8] Cherubini F, Ulgiati S. Crop residues as raw materials for biorefinery systems – a LCA case study. *Appl Energy* 2010;87(1):47–57.
- [9] Ramirez MM. Biomass: a feed for value-added fuels, chemicals and materials; proceedings of the Seminar. F; 2016.
- [10] Holladay JE, White JF, Bozell JJ, et al. Top value-added chemicals from biomass – volume II—results of screening for potential candidates from biorefinery lignin. *Biomass Fuels* 2007;2:263–75.
- [11] Fernando S, Adhikari S, Chandrapal C, et al. Biorefineries: current status, challenges, and future direction. *Energy Fuels* 2006;20(4):1727–37.
- [12] Cherubini F. The biorefinery concept: using biomass instead of oil for producing energy and chemicals. *Energy Convers Manag* 2010;51(7):1412–21.
- [13] Lam FH, Ghaderi A, Fink GR, et al. Engineering alcohol tolerance in yeast. *Science* 2014;346(6205):71.
- [14] Unrean P. Flux control-based design of furfural-resistance strains of *Saccharomyces cerevisiae* for lignocellulosic biorefinery. *Bioproc Biosyst Eng* 2017;40(4):611–23.
- [15] Auesukaree C. Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation. *J Biosci Bioeng* 2017;124(2):133.
- [16] Nguyen TY, Cai CM, Kumar R, et al. Overcoming factors limiting high-solids fermentation of lignocellulosic biomass to ethanol. *P Natl Acad Sci USA* 2017;114(44):11673.
- [17] J'Nsson LJ, Carlos M. Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour Technol* 2015;199:103–12.
- [18] Qin L, Liu L, Li WC, et al. Evaluation of soluble fraction and enzymatic residual fraction of dilute dry acid, ethylenediamine, and steam explosion pretreated corn stover on the enzymatic hydrolysis of cellulose. *Bioresour technology* 2016;209:172–9.
- [19] Rowlett VW, Vkps M, Karlstaedt A, et al. The impact of membrane phospholipid alterations in *Escherichia coli* on cellular function and bacterial stress adaptation. *J Bacteriol* 2017;199(13):00849–16.
- [20] Morano KA, Grant CM, Moye-Rowley WS. The response to heat shock and oxidative stress in *Saccharomyces cerevisiae*. *Genetics* 2012;190(4):1157–95.
- [21] Liu YN, Zhang TJ, Lu XX, et al. Membrane fluidity is involved in the regulation of heat stress induced secondary metabolism in *Ganoderma lucidum*. *Environ Microbiol* 2017;19(4).
- [22] Xu K, Lv B, Huo YX, et al. Toward the lowest energy consumption and emission in biofuel production: combination of ideal reactors and robust hosts. *Curr Opin Biotechnol* 2017;50:19–24.
- [23] Toivola DM, Strnad P, Habtezion A, et al. Intermediate filaments take the heat as stress proteins. *Trends Cell Biol* 2010;20(2):79.
- [24] Szalay MS, Kovács IA, Korcsmáros T, et al. Stress-induced rearrangements of cellular networks: consequences for protection and drug design. *FEBS Lett* 2007;581(19):3675–80.
- [25] Welch WJ, Suhan JP. Cellular and biochemical events in mammalian cells during and after recovery from physiological stress. *J Cell Biol* 1986;103(5):2035–52.
- [26] Weiss CV, Roop JJ, Hackley RK, et al. Genetic dissection of interspecific differences in yeast thermotolerance. *Nat Genet* 2018;50(11):1501.
- [27] Sun H, Jia H, Li J, et al. Rational synthetic combination genetic devices boosting high temperature ethanol fermentation. *Synth Syst Biotechnol* 2017;2(2):121–9.
- [28] Huang C-J, Lu M-Y, Chang Y-W, et al. Experimental evolution of yeast for high temperature tolerance. *Mol Biol Evol* 2018:msy077.
- [29] Boulon S, Westman BJ, Hutten S, et al. The nucleolus under stress. *Mol Cell* 2010;40(2):216–27.
- [30] Vigh L, Nakamoto H, Landry J, et al. Membrane regulation of the stress response from prokaryotic models to mammalian cells. *Ann NY Acad Sci* 2007;1113(1):40–51.
- [31] Piper PW, Millson SH, Mollapour M, et al. Sensitivity to Hsp90-targeting drugs can arise with mutation to the Hsp90 chaperone, cochaperones and plasma membrane ATP binding cassette transporters of yeast. *Eur J Biochem* 2003;270(23):4689–95.
- [32] Davidson JF, Schiestl RH. Mitochondrial respiratory electron carriers are involved in oxidative stress during heat stress in *Saccharomyces cerevisiae*. *Mol Cell Biol*

- 2001;21(24):8483–9.
- [33] Suzuki N, Katano K. Coordination between ROS regulatory systems and other pathways under heat stress and pathogen attack. *Front Plant Sci* 2018;9:490.
- [34] Liu Y, Wang Z, Xie W. Oxidative stress regulates mitogen-activated protein kinases and c-Jun activation involved in heat stress and lipopolysaccharide-induced intestinal epithelial cell apoptosis. *Mol Med Rep* 2017;16(3):2579–87.
- [35] Volpe CMO, Villardelfino PH, Anjos PMF, et al. Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell Death Dis* 2018;9(2):119.
- [36] H.Å.E., Gjuraälä K, Kos B, et al. Acid resistance and response to pH-induced stress in two *Lactobacillus plantarum* strains with probiotic potential. *Benef Microbes* 2016;6(3):369–79.
- [37] Djoko KY, Phan M-D, Peters KM, et al. Interplay between tolerance mechanisms to copper and acid stress in *Escherichia coli*. *Proc Natl Acad Sci Unit States Am* 2017. 201620232.
- [38] Liu Y, Lv C, Xu Q, et al. Enhanced acid tolerance of *Rhizopus oryzae* during fumaric acid production. *Bioproc Biosyst Eng* 2015;38(2):323–8.
- [39] Andersson C, Petrova E, Berglund K, et al. Maintaining high anaerobic succinic acid productivity by product removal. *Bioproc Biosyst Eng* 2010;33(6):711.
- [40] Engel CAR, Gulik WMV, Marang L, et al. Development of a low pH fermentation strategy for fumaric acid production by *Rhizopus oryzae*. *Enzym Microb Technol* 2011;48(1):39–47.
- [41] Auesukaree C. Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation. *J Biosci Bioeng* 2017;124(2):133–42.
- [42] Divate NR, Chen GH, Wang PM, et al. Engineering *Saccharomyces cerevisiae* for improvement in ethanol tolerance by accumulation of trehalose. *Bioeng Bugs* 2016;7(6):445–58.
- [43] Alper H, Stephanopoulos G. Global transcription machinery engineering: a new approach for improving cellular phenotype. *Metab Eng* 2007;9(3):258–67.
- [44] Snoek T, Verstrepen KJ, Voordeckers K. How do yeast cells become tolerant to high ethanol concentrations? *Curr Genet* 2016;62(3):475–80.
- [45] María PDD. Chapter 6 – ionic liquids, switchable solvents, and eutectic mixtures. *Appl Green Solv Sep Processes* 2017:139–54.
- [46] Mora-Pale M, Meli L, Doherty TV, et al. Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass. *Biotechnol Bioeng* 2011;108(6):1229–45.
- [47] Zakaria SM, Idris A, Alias Y. Lignin extraction from coconut shell using aprotic ionic liquids. *Bioresources* 2017;12(3):5749–74.
- [48] Mcmillan JD. Pretreatment of lignocellulosic biomass; proceedings of the ACS symposium series (USA), F. 2016. [C].
- [49] Reid JESJ, Prydderch H, Spulak M, et al. Green profiling of aprotic versus protic ionic liquids: synthesis and microbial toxicity of analogous structures. *Sustain Chem Pharm* 2018;7:17–26.
- [50] Xia Y, Liu D, Dong Y, et al. Effect of ionic liquids with different cations and anions on photosystem and cell structure of *Scenedesmus obliquus*. *Chemosphere* 2017;195:437.
- [51] Ouellet M, Datta S, Dibble DC, et al. Impact of ionic liquid pretreated plant biomass on *Saccharomyces cerevisiae* growth and biofuel production. *Green Chem* 2011;13(10):2743–9.
- [52] Park JI, Steen EJ, Burd H, et al. A thermophilic ionic liquid-tolerant cellulase cocktail for the production of cellulosic biofuels. *PLoS One* 2015;7(5):e37010.
- [53] Qin L, Li BZ, Xu H, et al. Optimization of ethylenediamine pretreatment and enzymatic hydrolysis to produce fermentable sugars from corn stover. *Ind Crops Prod* 2017;102:51–7.
- [54] Qin L, Li WC, Liu L, et al. Inhibition of lignin-derived phenolic compounds to cellulase. *Biotechnol Biofuels* 2016;9(1):70.
- [55] Mishra A, Sharma AK, Sharma S, et al. Lignocellulosic bioethanol production employing newly isolated inhibitor and thermotolerant *Saccharomyces cerevisiae* DBTIOC S24 strain in SSF and SHF. *Rsc Adv* 2016;6(29):24381–90.
- [56] QIN L, ZHAO X, LI W-C, et al. Process analysis and optimization of simultaneous saccharification and co-fermentation of ethylenediamine-pretreated corn stover for ethanol production. *Biotechnol Biofuels* 2018;11(1):118.
- [57] Durand A. Bioreactor designs for solid state fermentation. *Biochem Eng J* 2003;13(2–3):113–25.
- [58] Nienow AW. Reactor engineering in large scale animal cell culture. *Cytotechnology* 2006;50(1–3):9.
- [59] Freshney RI. Culture of animal cells: a manual of basic technique and specialized applications [M]. John Wiley & Sons; 2015.
- [60] Portnoy VA, Bezdán D, Zengler K. Adaptive laboratory evolution—harnessing the power of biology for metabolic engineering. *Curr Opin Biotechnol* 2011;22(4):590–4.
- [61] Caspeta L, Chen Y, Nielsen J. Thermotolerant yeasts selected by adaptive evolution express heat stress response at 30 C. *Sci Rep* 2016;6:27003.
- [62] Oide S, Gunji W, Moteki Y, et al. Thermal and solvent stress cross-tolerance conferred to *Corynebacterium glutamicum* by adaptive laboratory evolution. *Appl Environ Microbiol* 2015;81(7):2284–98.
- [63] Caspeta L, Chen Y, Ghiaci P, et al. Biofuels. Altered sterol composition renders yeast thermotolerant. *Science* 2014;346(6205):75.
- [64] Zhang J, Wu C, Du G, et al. Enhanced acid tolerance in *Lactobacillus casei* by adaptive evolution and compared stress response during acid stress. *Biotechnol Bioproc Eng* 2012;17(2):283–9.
- [65] Wrighton KH. Synthetic biology: multiplex genome engineering in eukaryotes. *Nat Rev Genet* 2017;19(1):6.
- [66] Teixeira MC, Godinho CP, Cabrito TR, et al. Increased expression of the yeast multidrug resistance ABC transporter Pdr18 leads to increased ethanol tolerance and ethanol production in high gravity alcoholic fermentation. *Microb Cell Fact* 2012;11:98.
- [67] Liu Y, Zhang G, Sun H, et al. Enhanced pathway efficiency of *Saccharomyces cerevisiae* by introducing thermo-tolerant devices. *Bioresour Technol* 2014;170:38–44.
- [68] Chen G-Q, Jiang X-R. Next generation industrial biotechnology based on extremophilic bacteria. *Curr Opin Biotechnol* 2018;50:94–100.
- [69] Chénard C, Lauro FM. Microbial ecology of extreme environments [M]. Springer; 2017.
- [70] Xu K, Gao L, Hassan JU, et al. Improving the thermo-tolerance of yeast base on the antioxidant defense system. *Chem Eng Sci* 2018;175:335–42.
- [71] Jia H, Sun X, Sun H, et al. Intelligent microbial heat regulating engine (IMHeRE) for improved thermo-robustness and efficiency of bioconversion. *ACS Synth Biol* 2016;5(4):312.
- [72] Si T, Chao R, Min Y, et al. Automated multiplex genome-scale engineering in yeast. *Nat Commun* 2017;8:15187.
- [73] Richardson SM, Mitchell LA, Stracquadiano G, et al. Design of a synthetic yeast genome. *Science* 2017;355(6329):1040–4.
- [74] Hoess RH, Wierzbicki A, Abremski K. The role of the loxP spacer region in PI site-specific recombination. *Nucleic Acids Res* 1986;14(5):2287–300.
- [75] Shen MJ, Wu Y, Yang K, et al. Heterozygous diploid and interspecies SCRaMbLEing. *Nat Commun* 2018;9(1):1934.
- [76] Swinnen S, Henriques SF, Shrestha R, et al. Improvement of yeast tolerance to acetic acid through Haa1 transcription factor engineering: towards the underlying mechanisms. *Microb Cell Factories* 2017;16(1):7.
- [77] Tan F, Wu B, Dai L, et al. Using global transcription machinery engineering (gTME) to improve ethanol tolerance of *Zymomonas mobilis*. *Microb Cell Factories* 2016;15(1):4.
- [78] Baerends RJ, Qiu J-L, Rasmussen S, et al. Impaired uptake and/or utilization of leucine by *Saccharomyces cerevisiae* is suppressed by the SPT15-300 allele of the TATA-binding protein gene. *Appl Environ Microbiol* 2009;75(19):6055–61.
- [79] Qin L, Liu ZH, Li BZ, et al. Mass balance and transformation of corn stover by pretreatment with different dilute organic acids. *Bioresour Technol* 2012;112(1):319–26.
- [80] Qin L, Liu ZH, Jin M, et al. High temperature aqueous ammonia pretreatment and post-washing enhance the high solids enzymatic hydrolysis of corn stover. *Bioresour Technol* 2013;146(10):504–11.
- [81] Larsson S, Cassland P, Jönsson LJ. Development of a *Saccharomyces cerevisiae* strain with enhanced resistance to phenolic fermentation inhibitors in lignocellulose hydrolysates by heterologous expression of laccase. *Appl Environ Microbiol* 2001;67(3):1163–70.
- [82] Palmqvist E, Hahn-Hägerdal B. Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. *Bioresour Technol* 2000;74(1):17–24.
- [83] Xiros C, Olsson L. Comparison of strategies to overcome the inhibitory effects in high-gravity fermentation of lignocellulosic hydrolysates. *Biomass and Bioenergy* 2014;65:79–90.
- [84] Freitas JV, Farinas CS. Sugarcane bagasse fly ash as a no-cost adsorbent for removal of phenolic inhibitors and improvement of biomass saccharification. *ACS Sustainable Chem Eng* 2017;5(12):11727–36.
- [85] Toquero C, Bolado S. Effect of four pretreatments on enzymatic hydrolysis and ethanol fermentation of wheat straw. Influence of inhibitors and washing. *Bioresour Technol* 2014;157:68–76.