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Metabolic engineering of *Saccharomyces cerevisiae* for ethanol and butanol biofuel production

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Abstract— *The production of biofuels through biological processes has garnered increasing attention due to their potential benefits over conventional fuels, including lower greenhouse gas emissions, higher energy output, and reduced-price fluctuations. However, the metabolic processes of primitive microorganisms used in biofuel production are not compatible with those of fossil fuels. To address this, scholars have employed metabolic engineering techniques to modify the metabolisms of various microorganisms, including S. cerevisiae, for enhanced biofuel production. Specifically, overexpression of enzymes involved in bioethanol and biobutanol production, knockouts of competing pathways, improvements in carbon flux and tolerance have been applied to maximize the potential of S. cerevisiae for bioethanol and biobutanol production. This review focuses on the current state of metabolic engineering of S. cerevisiae for the production of bioethanol from lignocellulose and biobutanol from all kind of substrates, along with the potential use of cell surface technology in this field.*

Keywords— ***strain robustness, biofuel, biomass, bio-butanol, knocked in, bio-ethanol, knocked out.***

I. INTRODUCTION

The organic substance butanol has the chemical formula C4H9OH and is a member of the hydrocarbon family. The four isomers of butanol are 2-butanol, 1-butanol, tert-butyl alcohol and isobutanol. The most naturally produced butanol by microorganisms is isobutanol, which is also known as biobutanol, along with 1-butanol. Moreover, Bioethanol (also known as ethyl alcohol), chemical formula for ethanol is C2H5OH, made by microbial fermentation. Since the combustion of alcohol produces heat energy. The majority of biofuel which come from lignocellulosic biomass or the sugars or starches produced by plants like corn, sugarcane, sweet sorghum, or sugarcane, and have been utilized as a fuel [1]. The possibility to replace fossil fuels with biofuels like bioethanol and biobutanol and reduce greenhouse gas emissions when used directly or blended with gasoline in vehicles [2].

The interest in fuel synthesis from biomass has increased as a result of the rising costs of fossil fuels. Biofuel or bio-renewable fuel are the terms used to describe this fuel. The three main categories of biofuel feedstocks are as follows: (1) biomass made up of lignocellulosic materials like grasses, straw, and wood. (2) feedstocks that contain sucrose, such as sweet sorghum, sugar beet, sugarcane, and fruits. (3) Starchy foods, such as sweet potatoes, corn, potatoes, rice, wheat, and milo [3]. The usage of biofuel

blends in automobiles can greatly cut down on the consumption of petroleum and emissions of greenhouse gases. Microorganisms are used in the fermentation process to make biofuels. In order to convert polysaccharides into monomeric form, the process's feedstocks must also undergo pretreatment because microorganisms are unable to consume sugars in complex forms [4]. In the US, Brazil, and a few EU member states, there are programs in place that support the large-scale production of biofuels. After the United States, Brazil is the greatest producer of bioethanol in the world and its largest exporter.

According to recent World Health Organization research, air pollution causes 3 million deaths annually. Scientists' interest in finding alternate forms of energy has increased as a result of the environment's negative effects and the diminishing supply of fossil fuels [5]. In this regard, a variety of alternative energy sources, such as wind, hydro, geothermal, and solar, are accessible. The solar energy sector is one of them. It is expanding quickly and is thought to be a good solution to prevent environmental and energy issues [6]. The production of lignocellulosic-based biofuels at the biorefinery is another factor that qualifies it as a source of sustainable energy [7]. Bioethanol and biobutanol have gained popularity over time, surpassing other biofuels, and are now acknowledged as reliable sources of renewable energy [8]. By replacing fossil fuels with these biofuels, issues including environmental damage, price volatility, and the depletion of fossil fuel reservoirs would be less of a concern. However, because these biofuel operations are currently only dependent on food materials, a conflict between the food and fuel industries has resulted, which is indirectly a factor that has raised the cost of food ingredients [8].

Nitrogen oxide (NOx), sulfur oxide (SOx) and carbon monoxide (CO) emissions are reduced and absent in biofuels, making it a more affordable option [9]. Bioethanol is distinguished by having a high octane number (102), expanded flammability thresholds, a quick spread of flame, and a high heat of vaporization. It lowers particulate matter (PM10) emissions from tailpipes, which lowers pollution in general. The most popular ratio for blending bioethanol with gasoline is E10, sometimes referred to as "gasohol," which is 10% bioethanol to 90% gasoline.[10].

Butanol is manufactured annually to the tune of 15 to 17 billion dollars worldwide. The butanol derivatives with the highest economic value include butyl glycol ether, butyl acetate, and plasticizers. It has numerous uses in the pharmaceutical industry as a solvent and as a diluent in brake fluid compositions. Butanol is less hydroscopic and corrosive than bio-ethanol because It is less volatile, has more energy, and is somewhat miscible with water [11]. A gasoline engine may run directly on butanol without any alterations or replacements because its properties are comparable to those of gasoline [12], With a 22% oxygen concentration, it is a cleaner-burning, fuel extender [13].

Researchers' efforts to increase bio-butanol and bio-ethanol production through by-product accumulation reduction, usage of a cheaper substrate, and enhancement of butanol producers' robustness to butanol and oxygen by using engineered *Saccharomyces cerevisiae* have been diverted by metabolic engineering. However, much work remains to be done in order to make the biological production of bio-butanol and bio-ethanol more compatible with the one from petrochemical processes. Herein, the authors concluded the metabolic tuning of *S. cerevisiae* for enhanced bioethanol and butanol production.

II. METABOLIC ENGINEERING OF *S. CEREVISIAE* FOR BIOETHANOL PRODUCTION

Since *Saccharomyces cerevisiae* can withstand high bioethanol concentrations and inhibitors produced during the fermentation process, it is the ideal microbe for the fermentation of lignocellulosic biomass to bioethanol [14]. The cellular system of *S. cerevisiae* has undergone substantial engineering in order to attain the full use of all possible carbon sources in the lignocellulosic biomass in order to convert lignocellulosic feedstocks into bioethanol cheaply. Recent improvements in metabolic engineering and synthetic biology have enhanced the productivity and output of lignocellulosic bioethanol produced by *S. cerevisiae* [15]. The main goals of metabolic engineering strategies are either detoxification of inhibitors or modulation of stress responses. The former goal is mostly accomplished by expressing enzymes that transform inhibitors into less harmful chemicals and/or by establishing a redox balance between the detoxification pathways and the oxidoreductase xylose consumption pathways [16].

Lignocellulosic biomass primarily comprises cellulose-hemicellulose complexes that are enclosed within a lignin matrix. The cellulose-hemicellulose fraction of this biomass is deemed to be a highly effective source for bioethanol production [17]. Cellulose represents a significant homopolysaccharide component within plant cell wall, exclusively composed of glucose monomers, a vital substrate in the generation of biofuels [18]. The co-expression of multiple cellulases has proven valuable in developing strains proficient of growth and bioethanol production from a variety of cellulosic feedstocks. In this study, three cellobiohydrolase (CBH) enzymes, specifically CBH1 (from *Aspergillus aculeatus*), and CBH2 (from *Trichoderma reesei*) were combined with beta-glucosidase

(from *A. aculeatus*) and endoglucanase (from *T. reesei*) in a sequential integration approach within the yeast genome via the integration method. The resulting strain, which secreted all three CBH enzymes in addition to BGL and EGL, demonstrated the highest bioethanol concentration of 28 g/L from corncob. These findings underscore the importance of CBH enzyme diversity in efficiently hydrolyzing complex biomass [19]. In recombinant expression systems, the optimization of surface display of heterologous enzymes can be achieved by controlling the copy number of integrated genes, utilizing marker-less integration design, or by modifiying the enzyme ratio for process optimization. A method called "cocktail delta integration" has been established to engineer yeast displaying multi-enzyme components. This technique involves the frequent transformation of equimolar concentration of cellulase expression cassettes, which are integrated in yeast chromosomes at the delta sites simultaneously. Transformants with the optimal cellulose-degrading activity can then be screened easily. This strategy was employed to engineer *S. cerevisiae* for the co-display of three cellulases: exoglucanase and endoglucanase from *T. reesei* while beta-glucosidase from *A. aculeatus*, with the aim of achieving higher PASC (phosphoric acid-swollen cellulose) degradation activity compared to conventional integration methods [20]. In order to enhance the efficiency of cellulose degradation, another study implemented delta integration to boost cellulase expression in haploid yeast strains. These strains were subsequently bred to create a diploid strain with increased cellulase expression. The findings revealed that the engineered diploid strain displayed a six-fold improvement in PASC degradation activity and produced 7.6 g/L of bioethanol, compared to its haploid strain. Notably, the diploid strain also demonstrated the ability to directly produce bioethanol from pre-treated rice straw without the use of any exogenous enzymes [21]. In recent scientific research, combinations of cellulases were displayed on *S. cerevisiae* to produce bioethanol directly from lignocellulosic biomass pre-treated with ionic liquid. The efficacy of this method was tested on bagasse treated with 1-butyl-3methylimidazolium acetate [Bmim][OAc], resulting in 0.8 g/L bioethanol production within 96 hours, comparable to its theoretical maximum (73.4%). However, the efficiency of the engineered yeast was found to be low towards hardwoods, as only 21.2% and 18.3% of the theoretical bioethanol yield were obtained after 72 hours of fermentation from cedar biomass and [Bmim][OAc]-treated eucalyptus, respectively [22]. Yang, Zhang, et al. improved *S. cerevisiae's* ability to use lignocellulosic biomass (crushed orange peel) by incorporating a promoter for the control of the glyceraldehyde-3-phosphate dehydrogenase gene into the organism's genome. The mutant strain's bioethanol conversion rate was 37.7 times greater than that of the wild-type strain [23].

Due to *S. cerevisiae's* inability to use hemicellulose sugars naturally due to the absence of pentose fermentation enzymes, xylose catabolizing genes from other microorganisms have been expressed heterologous [24]. In Sakamoto et al. study, endogenous xylulokinase (XKS), xylose reductase (XR), and xylitol dehydrogenase (XDH) from *Scheffersomyces stipitis* were expressed in the recombinant strain of *S. cerevisiae* to carry out simultaneous saccharification and fermentation (SSF) of rice straw hydrolysate made up of several hemicelluloses. Moreover, surface displays of the same strain were made with hemicellulose-degrading enzymes from *Aspergillus oryzae*, *Trichoderma reesei*, and *Aspergillus aculeatus*. After 72 hours of fermentation, the final modified strain generated an bioethanol titer of 8.2 g/L [25]. *S. cerevisiae* was genetically altered to use xylan by co-expressing a number of xylan-degrading and xylose-accumulating enzymes. To facilitate full conversion of xylan into xylose, the recombinant strain was specifically designed to express xylosidase (from *A. niger*) and endoxylanase (from *T. reesei*). The production of new xylulose kinase (xyl3) from *S. stipitis* and xylose isomerase (xylA) from Bacteroides thetaiotaomicron, which circumvented the cofactor requirement of the alternative xylose reductase (XR)-xylitol dehydrogenase (XDH) pathway, further accelerated the accumulation of xylose. The natural aldose-reductase gene (GRE3) was removed to reduce xylitol buildup. The new yeast strain produced more enzymes and thrived in an aerobic setting. When grown solely on xylose while oxygen was scarce, it generated 9 g/L of bioethanol [26].

Thermostable and inhibitor resistant strain of *S. cerevisiae* was modified to display hemicellulolytic enzymes on its surface and engineered to optimize xylose utilization pathways for hemicellulose degradation. The developed strain was able to convert hemicellulose hydrolysate from hydrothermally treated maize-cob feedstock into 11.1 g/L bioethanol [27]. In a study, metabolic engineering and adaptive evolution were combined to modify *S. cerevisiae* to consume xylose and arabinose alongside glucose simultaneously. The resulting strain was able to utilize 24% extra pentose sugar after 120 hours of fermentation on a mixed sugar medium [28]. Through a combination of CRISPR-Cas9-mediated rational and evolutionary engineering, a highly efficient strain of *S. cerevisiae* capable of fermenting xylose was created. That strain, called XUSE, used an isomerase-based process to convert xylose into bioethanol with a yield of 0.43 g/g, and was able to simultaneously ferment glucose and xylose without significant glucose inhibition [29]. To further improve bioethanol yield, the high osmolarity glycerol pathway was

also engineered [30]. Although a mutant SFA1 in *S.* cerevisiae has achieved the highest bioethanol yield from lignocellulosic hydrolysates at 0.492 g/g total sugars [31]. The fermentation rates of xylose remain suboptimal, typically 20-35% smaller than those of glucose, which restricts overall bioethanol productivity in lignocellulosic fermentation. Additionally, as the hydrolysates become more concentrated to reach economically feasible bioethanol production with titers of 40-50 g/L, inhibitor concentration also increases, further impeding the functioning of the modified strains. Thus, the challenge remains to develop a robust *S. cerevisiae* platform capable of efficiently producing lignocellulosic bioethanol while tolerating high inhibitor concentrations [15]

Table 1: Metabolic engineering of Saccharomyces cerevisiae to produce bioethanol from lignocellulosic biomass

Purpose	Modifications	Productivity	References
Improving xylose catabolism	△PHO13, TAL1	About 3.4 times rise in xylose utilization rate	[32]
Acetate utilization	gndA	13% higher bioethanol yield on glucose	[33]
Improving bioethanol production capacity	PHO4	About 4 times higher bioethanol yield	[34]
S. cerevisiae	Δssk1Δsmp1	6% higher bioethanol yield	[30]
Improving inhibitor tolerances	RTC3, ANB1	10% higher bioethanol yield from xylose	[35]
Carbon loss minimization	cfxP1, XKS1, XYL1, mXYL1 and XYL2	1.33 times higher bioethanol yield	[36]
Acetate utilization	*Adh*E	6% higher bioethanol yield from xylose	[37]
S. cerevisiae	SeACS, adhE	14.8% higher bioethanol	[38]
Extracellular secretion of cellulases	Integration of cellulases from different sources within the yeast genome	bioethanol concentration of 28 g/L	[19]
Improve expression of cellulases	integration of sestc expression cassette	37.7 times higher (7.53 g/L) bioethanol	[23]
Surface display	hemicellulose-degrading enzymes from different sources	8.2 g/L of Bioethanol	[25]
Improving xylan degradation	Co-expression of xylan-degrading and xylose-accumulating enzymes	9 g/L of bioethanol	[26]
hemicellulose degradation	Display of hemicellulolytic enzymes on surface and optimization of xylose assimilation	11.1 g/L bioethanol	[27]

III. METABOLIC ENGINEERING OF *S.CEREVISIAE* FOR BIO BUTANOL PRODUCTION

S. cerevisiae, a type of yeast that possesses a natural ability to use the 2-ketoisovalerate synthesis pathway, sometimes referred to as the valine pathway, is a method for producing isobutanol from glucose. This process results in the creation of 2-ketoisovalerate, an intermediary substance in the biosynthesis of valine. Aldehyde dehydrogenase is used in the Ehrlich pathway, however, to transform 2-ketoisovalerate, a by-product of valine biosynthesis, into isobutanol. In essence, the Ehrlich route in *S. cerevisiae* uses 2-ketoisovalerate as a precursor molecule to produce isobutanol [39, 40]. Despite the possibility of producing 1-butanol using *S. cerevisiae*, little has been done to completely understand its cellular metabolism. However, two potential routes for 1-butanol biosynthesis in *S. cerevisiae* have been discovered: the first includes using the amino acid absorption pathway, while the second involves

either separately or in combination with the first, expressing heterologous clostridial 1-butanol biosynthetic pathways. [41, 42].

S. cerevisiae is considered to be the most extensively studied eukaryotic organism, and is recognized as a highly proficient producer of bio-butanol[43]. The levels of butanol production achieved through yeast fermentation are significantly lower compared to those achieved through the fermentation processes of *Clostridium* and *E. coli* bacteria. There are various advantages to using S. cerevisiae to manufacture butanol, including its ability to withstand low pH levels and inhibitors [44]. Additionally, *S. cerevisiae* is widely regarded as the most resilient microorganism when it comes to tolerance to butanol[45]. *S. cerevisiae* is the most suitable cellular organism for integration into existing industrial infrastructure as a cell factory. Numerous investigations necessitate experimentation on yeast to generate isomers of butanol, namely isobutanol and 1-butanol, that can be compared to those produced by *E. coli* and *Clostridium* species. As a result, researchers have attempted metabolic engineering of *S. cerevisiae* to make it a viable contender for industrial-level butanol synthesis. Similar to *E. coli* and *Clostridium species*, *S. cerevisiae* may synthesize butanol. various metabolic approaches have been applied to *S. cerevisiae* for production of two main isomers of butanol such as Iso-butanol and 1-butanol [40].

3.1 Biological isobutanol production by engineered *S.cerevisiae*

Isobutanol is a liquid having organic nature with no color that is combustible. Which is one of butanol isomers. Isobutanol have been considered and employed as a fuel alternative and solvent in certain industries [46]. In a pioneering study, the isobutanol synthesis in *S. cerevisiae* was verified by elevating the expression of enzymes named as *Ilv*5, Ilv2 and Ilv3, which encode acetohydroxyacid reductoisomerase, dihydroxy acid dehydratase, and acetolactate synthase, respectively) using glucose as a substrate, about 3.86 mg/l yield of isobutanol was achieved [47]. This study was the first to report on isobutanol production using *S. cerevisiae*. The results show that among various tested *Kiv*Ds and alcohol dehydrogenases, *Adh*6 along with *Kiv*Ds from *Lactococcus lactis* are the most effective enzymes in catalyzing α-ketoisovalerate to isobutanol. By increasing the expression of *L. lactis Kiv*D, *Adh*6 and *Ilv*2 in a *pdc*1 knocked out yeast strain, isobutanol titers of approximately 6.6 mg/l were achieved [48]. The *Pdc*1 coding pyruvate Decarboxylase, *Ilv*2, and Alcohol Dehydrogenase 6 (*Adh*6) enzymes controlling the conversion of pyruvate into acetaldehyde, acetolactate and NADPH-alcohol respectively. Subsequently, acetaldehyde is transformed into acetate via the action of *Ald*6 (Acetaldehyde Dehydrogenase 6) enzyme.

*L. lactis Kiv*Ds Overexpression in cytosol was evaluated for its potential to increase isobutanol production [47]. The in vitro specificity of *Kiv*D was found to be the highest and led to an increase in synthesis of isobutanol to 20.0 mg/L from 15.0 mg/L when co-overexpressed with *Ilv*5, *Ilv*3, and *Ilv*2 and exploiting glucose as a single carbon source [49]. Nevertheless, Brat and his team found that the presence of an active mitochondrial valine synthetic route prevented further increases in the isobutanol titer when all of the enzymes in the valine biosynthesis pathway were overexpressed in the cytosol [50]. The researchers successfully expressed *Ilv*5, *Ilv*3, and *Ilv*2 in the cytosol without the addition of N-terminal amino sequences. They further assessed the activity of several *L. lactis Kiv*D and Aro10 (ketoisovalerate decarboxylase) in a *Pdc*-minus strain. The maximum activity was observed with both Aro10 and *Adh*2. As a result, co-expression of the Aro10, *Adh*2, *Ilv*5, *Ilv*3, and *Ilv*2 pathways in an *Ilv*1 (threonine ammonia-lyase) deleted strain resulted in an isobutanol synthesis of 630 mg/L.

In order to create a cytosolic artificial isobutanol route in *S. cerevisiae*, the enzymes *Ilv*5p, *Ilv*3p, and *Ilv*2p were expressed along with *Adh* and *Kiv*D. This improved isobutanol production [50, 51]. To increase the isobutanol titer, different techniques were used, such as correcting cofactor imbalances and downregulating competing routes. In addition, a cytosolic route was established by upregulating natural mitochondrial valine pathway enzymes. To do this, shortened genes (*Ilv*5c, *Ilv*3c, and *Ilv*2c) lacking mitochondrial targeting regions were overexpressed. It was discovered that upregulating the transhydrogenase shunt and removing the *lpd*1 gene yielded a titer of up to 1.62 g/liter when the strain was overexpressing *kiv*D and *Adh*6. [52]. The overexpression of *Adh*6, Aro10, *Ilv*3, *Ilv*5, *Ilv*2, *Adh*2, and *L. lactis Kiv*D enzymes in their native compartment, in combination with the downregulation of enzymes such as Bat1, *pd*1 and *Ald*6, has been reported to frequently improve isobutanol production [52, 53]. All these approaches were applied to an *lpd*1 downregulated strain, JHY465, which resulted in a further overexpression of *Adh*2 and Aro10 in the mitochondrial through fusing COX4-MLS (N-terminal mitochondrial localization signal) [54]. The final isobutanol production as a result was 330.9 mg/L. After inactivating the enzymes acetaldehyde dehydrogenase (*Ald*6), *Adh*1, *Ilv*1, amino-acid aminotransferase (*Bat*1), and *leu*1 along with upregulating enzymes tangled in the valine biogenesis isobutanol pathway, the D452-2 strain yielded in isobutanol (662.0 mg/L) using glucose as source of carbon [55]. The deletion of (*bdh*1, *bdh*2 encoding NAD-dependent butanediol dehydrogenase), acetolactate synthase (*ilv*2), *leu*9 ketopantothenate hydroxymethyltransferase (ecm31),

*leu*4, *adh*1, acetaldehyde dehydrogenase (*ald*6), *gpd*1, *gpd*2, and *ilv*1 genes in a yeast strain named as CEN.PK113-7D increase its ability of isobutanol synthesis. CEN.PK113-7D accumulated about 2.1 g/L, when the competing metabolites synthesis pathway for 2,3-butanediol, leucine, isoleucine, pantothenate, ethanol, and glycerol from glucose has been knocked out [56].

Xylose was effectively converted into isobutanol in a groundbreaking study using yeast that had been metabolically altered. The yield was 0.16 mg/g and the concentration was 1.36 mg/L after overexpressing and upregulating the valine biosynthesis enzymes and the xylose isomerase enzyme from the yeast cytoplasm, both of which are found in *C. phytofermentans*. In terms of producing isobutanol industrially from renewable feedstocks, this constitutes a significant advancement [57]. Recently, a higher isobutanol titer of 3.10 g/L was attained by expressing the mitochondrial isobutanol pathways in yeast, uptake of xylose isomerase, and eradication of competitive isobutanol-producing routes by removing the enzymes held to account for their biogenesis, *Bat*1, *Ald*6, and Pho13 [58].

3.2 Biological production of 1-butanol by engineered *S.cerevisiae*

The clostridial 1-butanol route in *S. cerevisiae* produces acetyl-CoA, an intermediate. The transformation of acetyl-CoA to acetoacetyl-CoA is then catalyzed by ERG10, also known as native thiolase. Since many different biomolecules require acetoacetyl-CoA as a precursor, foreign genes must be expressed to create heterologous enzymes that can convert acetoacetyl-CoA into 1-butanol. [40]. In their first attempt, Steen and his team successfully produced 1-butanol in *S. cerevisiae* by expressing isoenzymes from multiple sources to construct the 1-butanol pathway. The ESY7 mutant strain that has natural thiolase (ERG10) and Hb-CoA dehydrogenase (*Hbd*) overexpressed on a high copy number produced the best yield. Galactose was converted into 1-butanol at a rate of 2.50 mg/L. [59]. The CEN.PK113-11C yeast strain containing a plasmid with *Adh*2, ERG10, *Acs*, *crt*, *hbd*, *Ald*6, and *ter* enzymes was metabolically modified to create a pathway for the biosynthesis of 1-butanol and boost carbon flux. Using glucose as the substrate, this resulted in the synthesis of 16.30 mg/l butanol [43]. This is a six and a half times increase than the previously generated butanol titer, which was 2.50 mg/L [59].

A recent discovery showed that a yeast strain W303-1A with a 1-butanol biosynthetic pathway produced butanol at a low rate when glucose was employed as the growth substrate [60]. Trans-enoyl CoA reductase enzyme was introduced to the butanol pathway to boost productivity after the Pdc1 and Pdc2 genes were deleted to decrease glycerol generation. After fermentation for 49 h, Butanol 2.0 mg/L was generated by the mutant strain. The highest butanol concentration reported 835.0 mg/L, resulting in a yield of about 42 mg/g, was by [61]. A genetically engineered yeast strain's clostridial aceto-acetyl-CoA pathway production of 1-butanol was negatively impacted by the lower cytosolic acetyl-CoA and Coenzyme-A levels [41]. The researchers noticed a substantial rise in the formation of 1-butanol up to 130 mg/L in an oxygen-free environment when they boosted the amounts of NADH, Coenzyme-A and acetyl-CoA. This was achieved by expressing adhE$^{A267T/E568K/R577S}$ genes, producing an improved form of acetyl-CoA that can acetylate both trans-2-enoyl-CoA reductase and acetaldehyde dehydrogenase in the presence of NAD+. Interestingly, The modified yeast strain produced the highest 1-butanol titer of 0.86 g/L under aerobic conditions due to the overexpression of pantothenate kinase and amine oxidase (Fms1) [41].

IV. CELL SURFACE TECHNOLOGY FOR BIOETHANOL PRODUCTION

In several bioethanol manufacturing processes, this technique has been used in place of the conventional procedure [71, 72]. Various membrane technologies, including membrane distillation (MD), pervaporation (PV), ultrafiltration (UF), nanofiltration (NF), and microfiltration (MF), were employed to produce bioethanol. Microalgae can be recovered by employing MF/UF. Pretreatment is required for second and third generation bioethanol in order to make the biomass's carbohydrates available for conversion. After pretreatment and before fermentation, the second potential membrane use is the purification and concentration of prehydrolyzates. Concentrating the sugar solution and removing fermentation inhibitors are capabilities of MD, NF, and RO. An NF process with UF has been investigated in conjunction with enzyme recovery and other value-added manufacturing. After fermentation, bioethanol with low concentration is delivered for pre-concentration and pervaporation. In order to execute continuous fermentation, pervaporation and fermentation have been merged. Yeast and fermentation inhibitors can be eliminated throughout the process by utilizing UF and NF to create a hybrid process [73].

V. CELL SURFACE TECHNOLOGY FOR BIOBUTANOL PRODUCTION

In order to extract butanol from fermentation broth, various separation methods are now used, including adsorption [62], liquid–liquid extraction [63], gas stripping [64], pervaporation [65], reverse osmosis and perstraction, [66] Because to its effectiveness and energy-saving features as

well as the fact that it has no negative effects on microorganisms, pervaporation (PV) is regarded as having the highest potential of all these separation techniques. Contrary to distillation, pervaporation is a more sophisticated and cost-effective method to separate the water and butanol later [67]. Some of the components in a feed solution can preferentially pass through a membrane surface as it is being passed over the membrane, concentrating as vapors in the permeate. This membrane process, known as pervaporation, can achieve molecular separation for liquid mixtures [68]. The difference in vapor pressure between feed solution and permeate vapor, which is normally maintained by supplying a vacuum on the downstream side, is what drives the pervaporation process.

The separating membrane serves as the process' central component. When selecting a pervaporation membrane for a given mixture, there are two key considerations that must be made: Separation factor and permeate flux, or the mass flow rate per unit membrane area (the membrane's permeate side component to feed side component ratio divided by the permeate side component to feed side component ratio). Based on a membrane's specific characteristics, such as its hydrophilicity and organophilicity, there are many distinct types of pervaporation membranes for varied uses. Two scenarios are often involved when using the method of producing biobutanol through pervaporation. The first involves recovering butanol from low-solvent fermentation broth, where organophilic membranes may be utilized to let solvents flow while keeping water in retentate [69]. The solvent in the retentate must be dehydrated while the hydrophilic membranes must preferentially extract water from the solution in order to dehydrate low-water-content butanol-water combinations. The design and manufacture of intelligent membrane Over the past few years, materials have attracted a lot of interest with the goal of enhancing butanol pervaporation separation's long-term stability and effectiveness. Moreover, a number of scientists have developed various fermentation-pervaporation integrated techniques for making biobutanol [70].

VI. TECHNO-ECONOMIC COST OF BIOETHANOL AND BIOBUTANOL

lignocellulose-based biofuels are essential for preserving agricultural land and reducing the negative effects of global traffic and transportation on the environment [74]. Bioethanol has sparked a lot of interest in recent years as a fuel extender or even as a standalone liquid fuel. Consequently, because of their affordability and high potential for availability, lignocellulosic materials are extremely appealing substrates for the synthesis of bioethanol [75]. It is estimated that it will cost somewhere between 0.13 and 0.81 US dollars to produce one liter of ethanol. The cost of the feedstock, which ranged from 30 to 90 US dollars per metric ton, is one of the key factors affecting the economic outcome. In order to lower the energy needed for the distillation process and other downstream processes, high ethanol yields and concentrations must be attained during fermentation. Making ethanol produced from lignocellulose competitive with ethanol produced from sugar and starch requires improved pretreatment techniques, improved enzymatic hydrolysis using less expensive enhanced fermentation systems, and more effective enzymes, all of which pose considerable scientific challenges. Another method to reduce ethanol production costs overall is process integration, either internally or externally with other plant types, such as heat and power plants [76].

Production of bio-butanol is continuing on a global scale as an alternative to petrochemical fuels. The cost to manufacture butanol from cooked rice is $1.24 per kilogram (Kg), as determined by the techno-chemical analysis of butanol [77]. Costs for liquid-liquid extraction and distillation of butanol from corn biomass, lignocellulose, and sugarcane were 0.74 dollars, 1.19 dollars, and 1.59 dollars per kg, respectively [78]. ABE fermentation utilizing maize as the feedstock is used to produce butanol commercially at the Jilin Cathy industries in China for a cost of $2000 USD per ton, or roughly 70% of the whole cost. Thus, utilizing non-food biomass as a top priority to lower process costs By 2020, it is predicted that there would be a global demand for bio-butanol and other bio-based fuels of over 248 billion US dollars due to its advantages over other biofuels [79]. Butanol and ethanol are currently heavily supplied by petrochemical companies to the worldwide market, which makes its price dependent on the price of crude oil.

VII. FUTURE REMARKS

S. cerevisiae metabolic engineering for biofuel production has made great strides in recent years. However, there are still a number of difficulties that need to be addressed before this technology can be fully commercialized. One of the primary obstacles is the high cost of lignocellulosic biomass pretreatment, which is necessary for efficient conversion of this material to biofuels. Future research should focus on developing cost-effective and sustainable pretreatment methods that can increase the economic feasibility of conversion of lignocellulosic biomass.

Another challenge is the low yields of biofuels obtained from *S. cerevisiae*, which limit the scalability of the process. The development of novel biosynthetic pathways, the modification of central carbon metabolism, and the

improvement of fermentation conditions are some of the metabolic engineering techniques that should be investigated in future studies to increase the generation of biofuels. Furthermore, another significant problem that requires attention is the host cell toxicity of biofuels. Future research should aim to develop more robust *S. cerevisiae* strains that can tolerate higher concentrations of biofuels as well as strategies to minimize the toxicity of biofuels during fermentation.

VIII. CONCLUSION

Metabolic engineering of *S. cerevisiae* for 4 and 2 carbon biofuel production holds great promise for the sustainable and cost-effective production of biofuels. By manipulating the yeast's metabolic pathways, researchers have been able to redirect carbon flux towards the production of these valuable biofuels. With the growing demand for renewable energy sources and the need to reduce our reliance on fossil fuels, this approach represents an exciting avenue for future research and development in the field of biofuels. Further optimization of the metabolic pathways and scaling up the production process will be essential to ensure the economic viability and commercial success of this technology.

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REFERENCES

[1] Rocha-Meneses, L., et al., *Second-generation bioethanol production: A review of strategies for waste valorisation.* Agronomy Research, 2017. **15**(3): p. 830-847.

[2] Basics, E., *Clean cities, energy efficiency & renewable energy (EERE)*. 2015.

[3] Smith, A.M., *Prospects for increasing starch and sucrose yields for bioethanol production.* The Plant Journal, 2008. **54**(4): p. 546-558.

[4] Overend, R.P. and E. Chornet, *Fractionation of lignocellulosics by steam-aqueous pretreatments.* Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences, 1987. **321**(1561): p. 523-536.

[5] Mussatto, S., *A closer look at the developments and impact of biofuels in transport and environment; what are the next steps? Biofuel Res. J. 3, 331*. 2016.

[6] Matutes, K.C.B. and R.L. Besagas, *Bioethanol Production from Selected Species of Astera Plants by Simultaneous Saccharification and Fermentation using Dilute Acid and Alkaline Extraction Pretreatment Methods.* 2021.

[7] Salimi, E., et al., *The role of enzyme loading on starch and cellulose hydrolysis of food waste.* Waste and Biomass Valorization, 2019. **10**: p. 3753-3762.

[8] Ibrahim, M.F., S.W. Kim, and S. Abd-Aziz, *Advanced bioprocessing strategies for biobutanol production from biomass.* Renewable and Sustainable Energy Reviews, 2018. **91**: p. 1192-1204.

[9] Taherzadeh, M.J. and K. Karimi, *Enzymatic-based hydrolysis processes for ethanol from lignocellulosic materials: A review.* BioResources, 2007. **2**(4): p. 707-738.

[10] Balat, M. and H. Balat, *Recent trends in global production and utilization of bio-ethanol fuel.* Applied energy, 2009. **86**(11): p. 2273-2282.

[11] Dürre, P., *Biobutanol: an attractive biofuel.* Biotechnology Journal: Healthcare Nutrition Technology, 2007. **2**(12): p. 1525-1534.

[12] Lee, S.Y., et al., *Fermentative butanol production by Clostridia.* Biotechnology and bioengineering, 2008. **101**(2): p. 209-228.

[13] Qureshi, N., B.C. Saha, and M.A. Cotta, *Butanol production from wheat straw hydrolysate using Clostridium beijerinckii.* Bioprocess and biosystems engineering, 2007. **30**: p. 419-427.

[14] Adegboye, M.F., et al., *Bioprospecting of microbial strains for biofuel production: metabolic engineering, applications, and challenges.* Biotechnology for biofuels, 2021. **14**(1): p. 1-21.

[15] Ko, J.K., et al., *Recent advances and future directions in plant and yeast engineering to improve lignocellulosic biofuel production.* Renewable and Sustainable Energy Reviews, 2020. **134**: p. 110390.

[16] Cunha, J.T., et al., *Engineered Saccharomyces cerevisiae for lignocellulosic valorization: a review and perspectives on bioethanol production.* Bioengineered, 2020. **11**(1): p. 883-903.

[17] Favaro, L., T. Jansen, and W.H. van Zyl, *Exploring industrial and natural Saccharomyces cerevisiae strains for the bio-based economy from biomass: the case of bioethanol.* Critical reviews in biotechnology, 2019. **39**(6): p. 800-816.

[18] Jung, S.-J., S.-H. Kim, and I.-M. Chung, *Comparison of lignin, cellulose, and hemicellulose contents for biofuels utilization among 4 types of lignocellulosic crops.* Biomass and bioenergy, 2015. **83**: p. 322-327.

[19] Hong, J., et al., *Development of a cellulolytic Saccharomyces cerevisiae strain with enhanced cellobiohydrolase activity.* World Journal of Microbiology and Biotechnology, 2014. **30**(11): p. 2985-2993.

[20] Yamada, R., et al., *Cocktail δ-integration: a novel method to construct cellulolytic enzyme expression ratio-optimized yeast strains.* Microbial cell factories, 2010. **9**: p. 1-8.

[21] 21. Yamada, R., et al., *Direct ethanol production from cellulosic materials using a diploid strain of Saccharomyces cerevisiaewith optimized cellulase expression.* Biotechnology for Biofuels, 2011. **4**(1): p. 8.

[22] Yamada, R., et al., *Direct Ethanol Production from Ionic Liquid-Pretreated Lignocellulosic Biomass by Cellulase-Displaying Yeasts.* Applied Biochemistry and Biotechnology, 2017. **182**(1): p. 229-237.

[23] Yang, P., et al., *CRISPR-Cas9 Approach Constructing Cellulase sestc-Engineered Saccharomyces cerevisiae for the Production of Orange Peel Ethanol.* Frontiers in Microbiology, 2018. **9**.

[24] Adebami, G.E., et al., *Genetics and metabolic engineering of yeast strains for efficient ethanol production.* Journal of Food Process Engineering, 2022. **45**(7): p. e13798.

[25] Sakamoto, T., et al., *Direct ethanol production from hemicellulosic materials of rice straw by use of an engineered yeast strain codisplaying three types of hemicellulolytic enzymes on the surface of xylose-utilizing Saccharomyces cerevisiae cells.* Journal of Biotechnology, 2012. **158**(4): p. 203-210.

[26] Mert, M.J., et al., *Engineering of Saccharomyces cerevisiae to utilize xylan as a sole carbohydrate source by co-expression of an endoxylanase, xylosidase and a bacterial xylose isomerase.* Journal of Industrial Microbiology and Biotechnology, 2016. **43**(4): p. 431-440.

[27] Cunha, J.T., et al., *Consolidated bioprocessing of corn cob-derived hemicellulose: engineered industrial Saccharomyces cerevisiae as efficient whole cell biocatalysts.* Biotechnology for Biofuels, 2020. **13**(1): p. 138.

[28] Wang, C., et al., *Coutilization of D-Glucose, D-Xylose, and L-Arabinose in Saccharomyces cerevisiae by Coexpressing the Metabolic Pathways and Evolutionary Engineering.* BioMed Research International, 2017. **2017**: p. 5318232.

[29] Tran Nguyen Hoang, P., et al., *Genomic and phenotypic characterization of a refactored xylose-utilizing Saccharomyces cerevisiae strain for lignocellulosic biofuel production.* Biotechnology for biofuels, 2018. **11**(1): p. 1-13.

[30] Jagtap, R.S., et al., *Improving ethanol yields in sugarcane molasses fermentation by engineering the high osmolarity glycerol pathway while maintaining osmotolerance in Saccharomyces cerevisiae.* Applied Microbiology and Biotechnology, 2019. **103**(2): p. 1031-1042.

[31] Zhu, L., et al., *Overexpression of SFA1 in engineered Saccharomyces cerevisiae to increase xylose utilization and ethanol production from different lignocellulose hydrolysates.* Bioresource Technology, 2020. **313**: p. 123724.

[32] Xu, H., et al., *PHO13 deletion-induced transcriptional activation prevents sedoheptulose accumulation during xylose metabolism in engineered Saccharomyces cerevisiae.* Metabolic Engineering, 2016. **34**: p. 88-96.

[33] Papapetridis, I., et al., *Improving ethanol yield in acetate-reducing Saccharomyces cerevisiae by cofactor engineering of 6-phosphogluconate dehydrogenase and deletion of ALD6.* Microbial Cell Factories, 2016. **15**: p. 1-16.

[34] Wu, R., et al., *Enhanced ethanol production from sugarcane molasses by industrially engineered Saccharomyces cerevisiae via replacement of the PHO4 gene.* RSC Advances, 2020. **10**(4): p. 2267-2276.

[35] Hasunuma, T., T. Sakamoto, and A. Kondo, *Inverse metabolic engineering based on transient acclimation of yeast improves acid-containing xylose fermentation and tolerance to formic and acetic acids.* Applied Microbiology and Biotechnology, 2016. **100**(2): p. 1027-1038.

[36] Li, Y.-J., et al., *Engineered yeast with a CO2-fixation pathway to improve the bio-ethanol production from xylose-mixed sugars.* Scientific reports, 2017. **7**(1): p. 43875.

[37] Wei, N., et al., *Enhanced biofuel production through coupled acetic acid and xylose consumption by engineered yeast.* Nature communications, 2013. **4**(1): p. 2580.

[38] Zhang, G.-C., et al., *Optimization of an acetate reduction pathway for producing cellulosic ethanol by engineered yeast.* Biotechnology and Bioengineering, 2016. **113**(12): p. 2587-2596.

[39] Kuroda, K. and M. Ueda, *Cellular and molecular engineering of yeast Saccharomyces cerevisiae for advanced biobutanol production.* FEMS Microbiology Letters, 2016. **363**(3).

[40] Hussain, A., et al., *Advances in microbial metabolic engineering for the production of butanol isomers (isobutanol and 1-butanol) from a various biomass.* BioEnergy Research, 2022. **15**(4): p. 1854-1871.

[41] Schadeweg, V. and E. Boles, *n-Butanol production in Saccharomyces cerevisiae is limited by the availability of coenzyme A and cytosolic acetyl-CoA.* Biotechnology for Biofuels, 2016. **9**(1): p. 1-12.

[42] Liu, R., et al., *Metabolic engineering of fatty acyl-ACP reductase-dependent pathway to improve fatty alcohol production in Escherichia coli.* Metabolic engineering, 2014. **22**: p. 10-21.

[43] Krivoruchko, A., et al., *Improving biobutanol production in engineered Saccharomyces cerevisiae by manipulation of acetyl-CoA metabolism.* Journal of Industrial Microbiology and Biotechnology, 2013. **40**(9): p. 1051-1056.

[44] Hong, K.-K. and J. Nielsen, *Metabolic engineering of Saccharomyces cerevisiae: a key cell factory platform for future biorefineries.* Cellular and Molecular Life Sciences, 2012. **69**: p. 2671-2690.

[45] González-Ramos, D., et al., *Genome-scale analyses of butanol tolerance in Saccharomyces cerevisiae reveal an essential role of protein degradation.* Biotechnology for biofuels, 2013. **6**(1): p. 1-18.

[46] Hahn, H., et al., *Butanols//Ullmann's Encyclopedia of Industrial Chemistry.* Wiley.—2013.—DOI, 2010. **10**(14356007): p. a04_463.

[47] Chen, X., et al., *Increased isobutanol production in Saccharomyces cerevisiae by overexpression of genes in valine metabolism.* Biotechnology for biofuels, 2011. **4**(1): p. 1-12.

[48] Kondo, T., et al., *Genetic engineering to enhance the Ehrlich pathway and alter carbon flux for increased isobutanol production from glucose by Saccharomyces cerevisiae.* Journal of biotechnology, 2012. **159**(1-2): p. 32-37.

[49] Lee, W.-H., et al., *Isobutanol production in engineered Saccharomyces cerevisiae by overexpression of 2-ketoisovalerate decarboxylase and valine biosynthetic enzymes.* Bioprocess and biosystems engineering, 2012. **35**: p. 1467-1475.

[50] Brat, D., et al., *Cytosolic re-localization and optimization of valine synthesis and catabolism enables increased isobutanol production with the yeast Saccharomyces cerevisiae.* Biotechnology for biofuels, 2012. **5**: p. 1-16.

[51] Matsuda, F., et al., *Construction of an artificial pathway for isobutanol biosynthesis in the cytosol of Saccharomyces cerevisiae.* Bioscience, biotechnology, and biochemistry, 2012. **76**(11): p. 2139-2141.
[52] Matsuda, F., et al., *Increased isobutanol production in Saccharomyces cerevisiae by eliminating competing pathways and resolving cofactor imbalance.* Microbial cell factories, 2013. **12**(1): p. 1-11.
[53] Park, S.-H., S. Kim, and J.-S. Hahn, *Metabolic engineering of Saccharomyces cerevisiae for the production of isobutanol and 3-methyl-1-butanol.* Applied microbiology and biotechnology, 2014. **98**: p. 9139-9147.
[54] Park, S.-H., S. Kim, and J.-S. Hahn, *Improvement of isobutanol production in Saccharomyces cerevisiae by increasing mitochondrial import of pyruvate through mitochondrial pyruvate carrier.* Applied microbiology and biotechnology, 2016. **100**(17): p. 7591-7598.
[55] Lee, K.-M., et al., *Elimination of biosynthetic pathways for l-valine and l-isoleucine in mitochondria enhances isobutanol production in engineered Saccharomyces cerevisiae.* Bioresource technology, 2018. **268**: p. 271-277.
[56] Wess, J., M. Brinek, and E. Boles, *Improving isobutanol production with the yeast Saccharomyces cerevisiae by successively blocking competing metabolic pathways as well as ethanol and glycerol formation.* Biotechnology for biofuels, 2019. **12**(1): p. 1-15.
[57] Brat, D. and E. Boles, *Isobutanol production from D-xylose by recombinant Saccharomyces cerevisiae.* FEMS yeast research, 2013. **13**(2): p. 241-244.
[58] Zhang, Y., et al., *Xylose utilization stimulates mitochondrial production of isobutanol and 2-methyl-1-butanol in Saccharomyces cerevisiae.* Biotechnology for Biofuels, 2019. **12**: p. 1-15.
[59] Steen, E.J., et al., *Metabolic engineering of Saccharomyces cerevisiae for the production of n-butanol.* Microbial cell factories, 2008. **7**: p. 1-8.
[60] Sakuragi, H., et al., *Enhanced butanol production by eukaryotic Saccharomyces cerevisiae engineered to contain an improved pathway.* Bioscience, Biotechnology, and Biochemistry, 2015. **79**(2): p. 314-320.
[61] Shi, S., et al., *Metabolic engineering of a synergistic pathway for n-butanol production in Saccharomyces cerevisiae.* Scientific Reports, 2016. **6**(1): p. 25675.
[62] Ennis, B., N. Qureshi, and I. Maddox, *In-line toxic product removal during solvent production by continuous fermentation using immobilized Clostridium acetobutylicum.* Enzyme and microbial Technology, 1987. **9**(11): p. 672-675.
[63] Grobben, N.G., et al., *Production of acetone, butanol and ethanol (ABE) from potato wastes: fermentation with integrated membrane extraction.* Applied Microbiology and Biotechnology, 1993. **39**: p. 494-498.
[64] Ezeji, T., N. Qureshi, and H. Blaschek, *Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping.* Applied microbiology and biotechnology, 2004. **63**: p. 653-658.
[65] Van Hecke, W., et al., *Integrated bioprocess for long-term continuous cultivation of Clostridium acetobutylicum coupled to pervaporation with PDMS composite membranes.* Bioresource technology, 2012. **111**: p. 368-377.
[66] Garcia III, A., E.L. Iannotti, and J.L. Fischer, *Butanol fermentation liquor production and separation by reverse osmosis.* Biotechnology and bioengineering, 1986. **28**(6): p. 785-791.
[67] Qureshi, N. and T.C. Ezeji, *Butanol,'a superior biofuel'production from agricultural residues (renewable biomass): recent progress in technology.* Biofuels, Bioproducts and Biorefining: Innovation for a sustainable economy, 2008. **2**(4): p. 319-330.
[68] Feng, X. and R.Y. Huang, *Liquid separation by membrane pervaporation: a review.* Industrial & Engineering Chemistry Research, 1997. **36**(4): p. 1048-1066.
[69] Lipnizki, F., et al., *Organophilic pervaporation: prospects and performance.* Chemical Engineering Journal, 1999. **73**(2): p. 113-129.
[70] Chapman, P.D., et al., *Membranes for the dehydration of solvents by pervaporation.* Journal of Membrane Science, 2008. **318**(1-2): p. 5-37.
[71] Vane, L.M., *A review of pervaporation for product recovery from biomass fermentation processes.* Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology, 2005. **80**(6): p. 603-629.
[72] Rothman, H., R. Greenshields, and F. Rosillo Callé, *The alcohol economy: Fuel ethanol and the Brazilian experience*. 1983: Frances Pinter (Publishers).
[73] Wei, P., et al., *A review of membrane technology for bioethanol production.* Renewable and Sustainable Energy Reviews, 2014. **30**: p. 388-400.
[74] Dempfle, D., O. Kröcher, and M.H.-P. Studer, *Techno-economic assessment of bioethanol production from lignocellulose by consortium-based consolidated bioprocessing at industrial scale.* New biotechnology, 2021. **65**: p. 53-60.
[75] Piccolo, C. and F. Bezzo, *A techno-economic comparison between two technologies for bioethanol production from lignocellulose.* Biomass and bioenergy, 2009. **33**(3): p. 478-491.
[76] Rai, M., et al., *Emerging role of nanobiocatalysts in hydrolysis of lignocellulosic biomass leading to sustainable bioethanol production.* Catalysis Reviews, 2019. **61**(1): p. 1-26.
[77] Ozturk, A.B., et al., *Techno-economic analysis of a two-step fermentation process for bio-butanol production from cooked rice.* Sustainable Energy & Fuels, 2021. **5**(14): p. 3705-3718.
[78] Mailaram, S. and S.K. Maity, *Dual liquid–liquid extraction versus distillation for the production of bio-butanol from corn, sugarcane, and lignocellulose biomass: A techno-economic analysis using pinch technology.* Fuel, 2022. **312**: p. 122932.
[79] Xin, F., et al., *Biobutanol production from crystalline cellulose through consolidated bioprocessing.* Trends in biotechnology, 2019. **37**(2): p. 167-180.