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Integrated biorefinery model based on production of furans using open-ended high yield processes†

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The biodetoxification pathway for the reduction of the fermentation inhibitor furfural was utilized to produce furfuryl alcohol using both a commercial Bakers' yeast and six other native strains, selected for their high tolerance towards the inhibitory effects of furfural. This study explores the potential of the microbial method as an environmentally-benign alternative to the conventional catalytic hydrogenation process for producing furfuryl alcohol used extensively in industry. The microbial method for furfuryl alcohol production provides the benefit of a homogeneous biochemical conversion, devoid of chemical catalysis, in conjunction with other carbohydrate-based processes (*e.g.* production of ethanol). Results showed that the yields of furfuryl alcohol using the laboratory yeast strains exceeded 90% of the theoretical yield at a furfural concentration of 25 g l^{-1}, which are comparable to yields obtained using the catalytic process. Furfuryl alcohol yields progressively declined as the furfural concentration was increased up to 65 g l^{-1}, where the yields averaged over 37%. Piecing together novel high-yield conversion processes for furfural and furfuryl alcohol, an integrated biorefinery model based on the production of furans has been envisioned. Such a facility bypasses the need for high pressure hydrogenation using copper chromite catalysts and hydrogen and azeotropic distillation of furfural to produce dilute streams of both notable platform chemicals.

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Introduction

Biorefineries, furfural and derivatives

It is envisioned that bio-based societies can become a reality when biomass-derived renewable substitutes have been found for the vast array of products currently derived from the processing of crude petroleum and other fossil sources. The design of future biorefineries may seek inspiration from the utility of current petroleum refineries, producing a variety of fuels and value-added chemicals by processing a wide array of biomass feedstock using physical, chemical, and biological unit operations. The integrated biorefinery has been defined as 'a facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass',[1] and there is a consensus among researchers in this area that these are the essential tools for the realization of a bio-based society. While the replacement of fossil-origin fuels with biomass-derived fuel sources might seem overwhelming due to the extremely large volumes of oil utilized (~90 million barrels per day globally)[2] and the commodity nature of the pricing, other products from the refining process may be more easily replaced with renewable sources. The production of synthetic organic materials such as fibers, rubbers, adhesives, coatings, plastics, *etc.* (currently produced primarily from petroleum and natural gas), requires a carbon source, and biomass is the only renewable feedstock that fulfills this requirement.[3]

To identify the most valuable chemicals amidst a cornucopia of available candidates for production in the integrated biorefinery, the concept of the *platform chemical* has been introduced in a study conducted by the United States Department of Energy (US DOE) in 2004,[4] which included compounds such as levulinic acid, glycerol, sorbitol. The study recommended that research into their production and implementation would be a promising first step towards the development of biorefineries. This study was revisited in 2010 by Bozell and Petersen,[5] in which they refined the criteria for the identification of chemicals as 'new top chemical opportunities', and this included chemicals such as ethanol, lactic acid, and furfural.

Furfural, identified as one of the most promising chemicals by Bozell and Peterson,[5] is the natural dehydration product of

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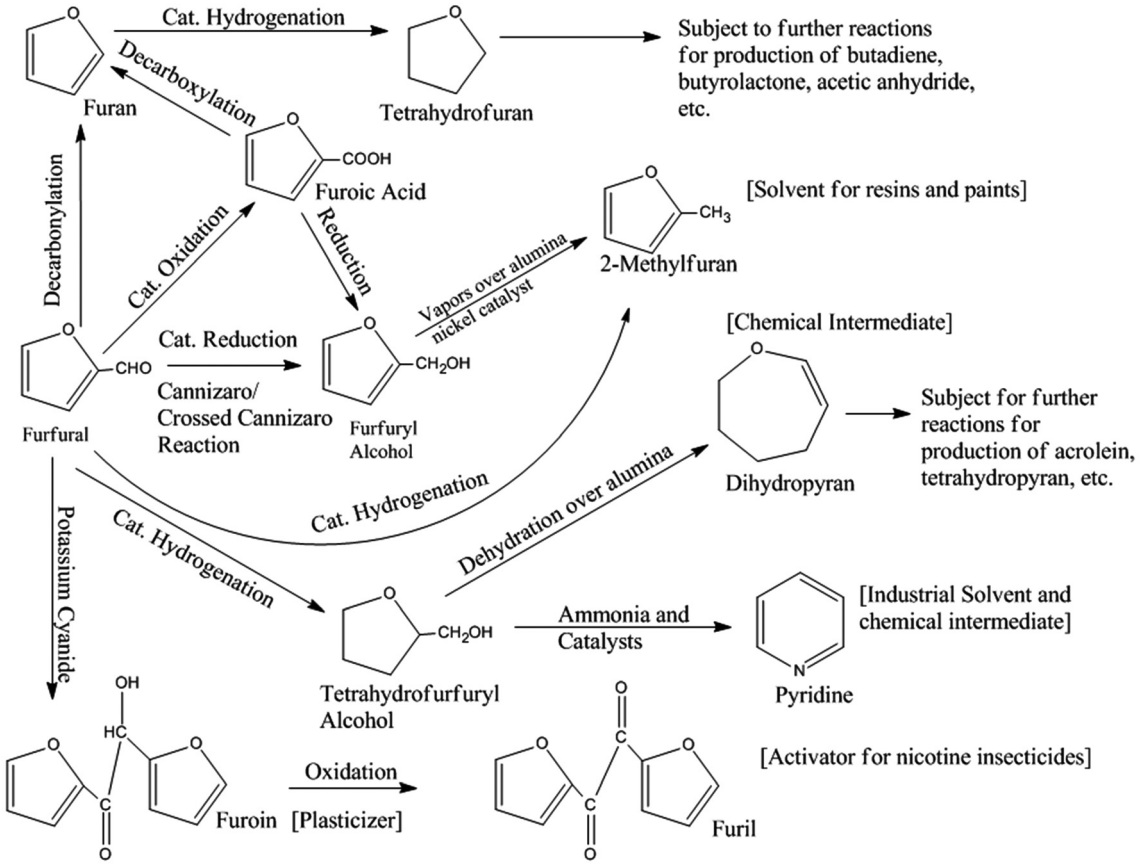

Fig. 1 Possible furan-based chemicals with furfural as a precursor. Uses of some of the chemicals are provided in the brackets (adapted from Sain *et al.*[7]).

five-carbon sugars, arabinose and xylose. As of 2007, the market price of furfural was reported to be $1450 per ton.[6] Apart from being a valuable platform chemical derived from renewable biomass feedstocks, furfural is the precursor for many furan-based chemicals, and finds itself inextricably involved in largely catalytic transformations leading to higher-value chemicals and solvents.

Furfural offers enormous prospects for the development of a biorefinery geared towards the production of furfuryl alcohol and its derivatives. Importantly, it promises to open up a whole new class of furanic chemicals that can be derived from biomass feedstock, with very well established chemistry that has been comprehensively researched since furfural was first produced in large quantities.

The greatest application of furfural, among many, is its conversion into derivatives such as furfuryl alcohol, furan, furoic acid, *etc.*, and the potential applications for these chemicals. Fig. 1 illustrates a snapshot of some of the potential furan-based chemicals that can be derived from a common precursor, furfural.

Furfuryl alcohol

Around 62% of the furfural produced globally each year is converted into furfuryl alcohol, which finds its largest application in the manufacture of foundry resins. Resins made from cross-linked polymers of furfuryl alcohol with itself and other products (furfural, formaldehyde, phenolic compounds, urea, *etc.*) were shown to possess excellent chemical, thermal and mechanical properties, in addition to withstanding corrosion and solvent action.[8] Furfuryl alcohol has also been used in the manufacture of furan fiber-reinforced plastics (FRP) for use in piping, and is recommended for use for high performance chemical processes when chlorinated aromatics, oxygenated organic solvents, *etc.*, are used, owing to its anti-corrosion properties. Such corrosion-resistant off-the-shelf piping has been available since 1977.[8,9] Recently, applications of furfuryl alcohol have also been explored for impregnating wood (furfurylation) to improve its physical and mechanical properties (hardness, improvement of the moduli of rupture and elasticity, and enhanced dimensional stability.[10,11] Furfurylation has also been shown to impart to the wood-polymer composites exceptional resistance to microbial decay and attack by insects,[11] with the advantage of being non-toxic over other chemical treatment agents. Polymerized furfuryl alcohol was also used as a source of carbon for the preparation of molecular sieves for potential applications as confined nanopore chemical reactors[12] and for the preparation of carbon membranes with gas separation capabilities.[13]

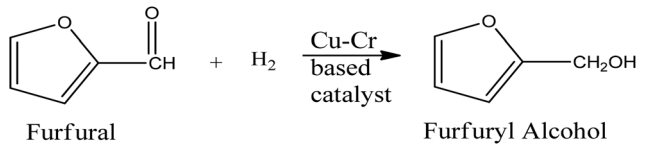

Fig. 2 Copper chromite catalyzed conversion of furfural into furfuryl alcohol (adapted from Wojcik[16]).

Manufacture of furfuryl alcohol

Catalytic hydrogenation of furfural. The catalytic hydrogenation of furfural (Fig. 2) is thought to occur by the selective hydrogenation of the carbonyl group (C═O) on furfural, through an alkoxide intermediate producing furfuryl alcohol.[14] Hydrogenation of the second active group, the unsaturated C═C bond, results in the production of tetrahydrofurfural.[15] Several catalysts have been evaluated for the hydrogenation of furfural into furfuryl alcohol. Copper is a highly selective catalyst for the hydrogenation reaction and is widely used in its chromite form, while decarbonylation becomes the dominant path when using palladium, leading to the production of 2-methylfuran in the latter case.

Commercial production of furfuryl alcohol in the past was carried out using 1–2% copper chromite catalyst at 1000–1500 psi by hydrogenating technical-grade furfural in 110-gallon autoclaves at 175 °C, with reported furfuryl alcohol yields of 96–99%.[16] Today, this process is accomplished almost exclusively in the vapor phase by the hydrogenation of furfural using copper chromite catalysts to produce furfuryl alcohol with yields exceeding 92%.[17] While the use of copper chromite catalysts guarantees exceptional yields, the moderate activity[18,19] and the toxicity[20,21] associated with chromite have generated interest in the evaluation of other possible candidates to catalyze the hydrogenation reaction. In addition, stringent environmental regulations to prevent the disposal of deactivated copper chromite catalysts in landfills have been enacted, and have generated interest in the development of catalysts that do not contain chromium, as replacements.[22] Research has been done on the use of highly selective heterogeneous catalysts such as carbon-supported copper, studied at 150–200 °C and at a hydrogen pressure of ~14 psi.[22,23] RANEY® nickel, modified with the salts of heteropolyacids having Keggin-type structures, has been utilized as a catalyst in a liquid-phase process (~145 psi) to accomplish the production of furfuryl alcohol with high selectivity (98.5%).[21] Lee and Chen[15] worked with ultrafine (0.1–0.01 sq. m g^{-1} surface area) amorphous catalysts prepared using Ni, P and B and found a greater selectivity (over 80% at furfural conversion greater than 80%) in comparison to conventional RANEY® nickel catalyst. The hydrogenation reactions in this study were conducted at 80 °C and at 250 psi., Chromium in the copper chromite catalyst was substituted with MgO to reduce furfural on coprecipitated Cu–MgO catalysts in the vapor phase (250 °C), resulting in the production of furfuryl alcohol at a high selectivity of 98%.[24] Yet catalysts containing copper have the benefit of being milder and are more selective than their nickel and platinum counterparts, which explains their widespread use in industrial hydrogenation reactions.[25]

Despite the promise of these catalytic reactions, they lack conformity towards green chemistry principles and are not suitable for integration into lignocellulosic biorefineries. If the value of furfural as a platform chemical must be effectively realized, greener and much milder alternative routes of furfuryl alcohol production need to be explored and established, which this study attempts to undertake.

Microbial conversion of furfural. A relatively understudied area of furfuryl alcohol production involves the microbial reduction of furfural. Much of the literature published in this area pertains to conversion of furfural as a fermentation inhibitor into the less toxic furfuryl alcohol. Lignocellulose, by virtue of its structure comprising of the crystalline cellulose structure enclosed in a lignin shell bonded by the hemicellulose (acting as glue), is notoriously resistant to chemical and biological attacks that seek to break it down, a phenomenon referred to as biocalcitrance.[26,27] Following an acid-catalyzed pretreatment of lignocellulosic biomass during the production of cellulosic ethanol, inhibitors such as furfural, 5-hydroxymethylfurfural (HMF), acetic acid, formic acid, vanillin, levulinic acid, 4-hydroxybenzaldehyde, *etc.* are produced.[27] Of these, furfural and HMF have been identified to be potent inhibitors to microbial growth and activity during fermentation processes. It should be noted that alkaline pretreatments do not form these moieties due to the absence of the acid catalyst, but this is not the topic of this paper.

The *Saccharomyces cerevisiae* yeast strain 354 has been reported to convert pure furfural to furfuryl alcohol[28] and tolerate furfural concentrations up to 3%. Using molasses as the sugar source, the authors report furfuryl alcohol yields of 96% at conditions much milder than the catalytic process. De Villegas *et al.*[29] investigated the effects of aeration and stirring on the production of furfuryl alcohol using *S. cerevisiae* 354, and obtained a conversion of 70% with a final furfural concentration of 35% by slow addition. Palmqvist *et al.*[30] conducted small-scale fermentation (150 ml) studies by growing Baker's yeast in the presence of up to 53 mM (~5.1 g l^{-1}) furfural with glucose as the carbon source. Larger-scale fermentations (3 l) were also carried out in this study at a furfural concentration of 29 mM (~2.8 g l^{-1}) and furfuryl alcohol yields of ~97% were reported. A corresponding decrease in cell mass yields and a slight increase in the ethanol yields on glucose were also found in this study. Higher concentrations of furfural in the reaction mixture were shown to have adverse effects on the specific growth rate of the organisms. Studies have also been conducted using diverse organisms such as *Methanococcus deltae* ΔLH,[31] *Coniochaeta lignaria*,[32] engineered strains of *Escherichia coli* and *Klebsiella oxytoca*,[33] *etc.*, with the specific intent of abating the toxicity of furfural by converting it into furfuryl alcohol. It is, therefore, evident that furfural can be converted into furfuryl alcohol in very high yields by employing microbial processes, which offer the advantages of milder, non-catalytic conditions. While studies have focused exclusively on biodetoxification of lignocellulosic

hydrolysates for fermentation, use of this pathway for the production of furfuryl alcohol is resonant of a truly green process that is in line with the goals of the integrated biorefinery.

Mechanism

Researchers have sought to elucidate the mechanism by which *S. cerevisiae* accomplishes the reduction of furfural into furfuryl alcohol, and to identify the enzymes involved. Banerjee *et al.*[34] assayed several glycolytic enzymes in the presence of furfural and reported that dehydrogenases were the most susceptible to inhibition by furfural. In particular, the enzymes glyceraldehyde 3-phosphate dehydrogenase and alcohol dehydrogenase (ADH) were observed to have been the most inhibited due to the presence of furfural. In another study, these researchers concluded that the activity of ADH II, responsible for the oxidation of ethanol, seemed to have been stimulated, leading to an increase in total ADH activity.[35] This was augmented by the work of Weigert *et al.*,[36] who concluded that ADH was the enzyme most responsible for the conversion of furfural into furfuryl alcohol in the organism *Scheffersomyces stipitis*. In batch culture, the specific growth rate of *S. cerevisiae* was found to decrease with increasing furfural concentration (0 to 2 g l^{-1}) and inoculum size.[37] It was observed that the inhibitory effect of furfural on growth was much smaller than its effect on ethanol production, because growth may be influenced by several metabolic cycles, while furfural was thought to stimulate some processes which provided energy for growth. This is likely because during anaerobic fermentation, organisms utilize glucose mostly for the production of energy in the form of the energy carrier molecule, ATP (adenosine triphosphate) and very little (~5%) of this energy goes towards growth. Glycolysis and oxidative phosphorylation are seen as the two catabolic pathways that lead to the production of ATP in *S. cerevisiae*, where glucose is consumed in order to produce ATP and ethanol.[38]

Fig. 3 shows a simplified scheme of pathways for glycolysis, leading to growth and the production of ethanol, in the presence of furfural, constructed by Palmqvist *et al.*[30] as part of a carbon mass-balance. The coefficients α, β and γ are part of this carbon balance with regard to the quantity of NADH oxidized to NAD^+ during various pathways. Furfural, by the action of ADH, can be reduced to furfuryl alcohol, with the H^+ derived from the oxidation of NADH into NAD^+. An interesting observation from this study was that the presence of furfural reduced glycerol production, leading to the oxidation of an equivalent amount of NADH (corresponding to the amount of glycerol that would have been produced in the absence of furfural). The reduction of furfural into furfuryl alcohol acts as a redox sink for the regeneration of NAD^+, reducing the need for glycerol production to serve this purpose.[39] The excess NADH formed as a result of biosynthesis was oxidized by both the reduction of furfural, and glycerol production, but the latter occurred only after all the furfural was depleted.[30]

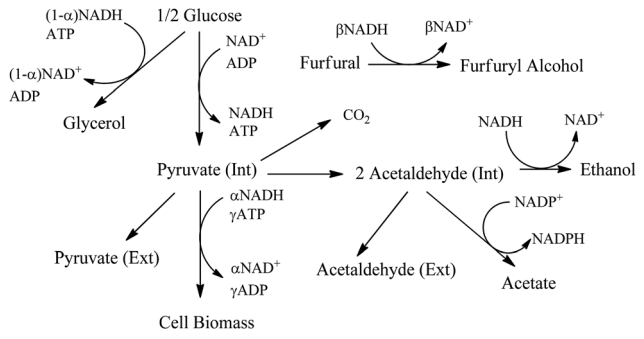

Fig. 3 Proposed pathway for the transformation of furfural into furfuryl alcohol in conjunction with the fermentation of glucose (adapted from Palmqvist *et al.*[30]); 'Int' and 'Ext' refer to intracellular and extracellular, respectively.

In addition, the presence of furfural led to the production of significant amounts of extracellular acetaldehyde, which can limit growth, but not metabolic activity. The depletion of intracellular acetaldehyde (due to excretion) is reflected in the reduced amounts of acetate observed (which was reported to be low in the beginning of the reaction due to the inhibition of aldehyde dehydrogenase (ALDH) in the presence of high concentrations of acetaldehyde). The excretion of acetaldehyde is also reflected in the reduced amounts of ethanol produced (by the reduction of acetaldehyde by the action of ADH) in the presence of furfural. The formation of furoic acid as a byproduct has been shown to be the effect of oxidation of furfural by ALDH, even though its activity towards furfural is low.[30]

Experimental

Materials

Initial experiments performed to establish growth and fermentation conditions utilized generic Bakers' yeast (Red Star® Active dry yeast, Lesaffre Yeast Corp., Milwaukee, WI). These served as a follow-up for further fermentation experiments using yeast strains that exhibited tolerance to furfural.

For medium preparation and sterilization, 20 g l^{-1} of glucose (Acros Organics, New Jersey) was sterilized separately in distilled water (with furfural added depending on whether the reactor was not the control), and 5X YP (50 g L^{-1} yeast extract, 100 g L^{-1} peptone) was sterilized separately by mixing 100 g l^{-1} bacto-peptone and 50 g l^{-1} yeast extract in distilled water, so that when added back to the glucose–furfural mixture, the YP concentration would be reduced to 10 g l^{-1} yeast extract and 20 g l^{-1} bacto-peptone. This step of performing the sterilization separately was done to prevent the occurrence of caramelization[40] when the three chemicals were sterilized together. Yeast extract and bacto-peptone were procured from Fisher Scientific (Fair Lawn, New Jersey).

Selection of yeast species

96-well plate growth phenotyping of *S. cerevisiae* strains. To identify *S. cerevisiae* strains with growth tolerance to furfural, 57 *S. cerevisiae* strains were prepared as previously described.[41] In brief, strains were individually inoculated into a 96-deep well block (NUNC) containing 500 μl of YPD media (10 g L^{-1}

yeast extract, 20 g L^{-1} peptone, 20 g L^{-1} dextrose) with a multichannel pipettor. After inoculation, the deep well block was sealed with breathable tape (Axygen), covered with a lid and incubated in 30 °C platform shaker. After 48 h of growth, 10 μl of saturated cultures from the deep well block were used to inoculate a standard 96-well plate (NUNC) containing 190 μl of YPD + 50 mM Furfural (Sigma-Aldrich).

The inoculated 96-well plate was placed in Tecan F500 plate reader maintaining an interior chamber temperature of 30 °C. Plates were shaken for 10 s and optical density at 595 nm (OD_{595}) measured from each well every 10 minutes for approximately 24 h with no shaking. The total change in cell density for each strain was calculated by subtracting the initial cell density (OD_{595} at t = 0 h) from the final cell density (OD_{595} at t = 24 h). Average changes in cell density and standard deviations were determined from three independent biological replicates.

Aerobic growth and fermentation

Cultures were first grown as starter solutions for a period of 24 hours aerobically with stirring. Inoculation was performed by suspending yeast grown on YP agar (10 g l^{-1} yeast extract, 20 g l^{-1} bacto-peptone and 20 g l^{-1} agar) media plates into YPD liquid medium (10 g l^{-1} yeast extract, 20 g l^{-1} bacto-peptone and 20 g l^{-1} dextrose)[42] in sterile conditions.

Data analysis

The furfural and furfuryl alcohol concentrations in the hydrolysate were analyzed through a Dionex ICS-3000 Chromatography System. The samples were centrifuged, decanted and then filtered through a PTFE 0.20 μm filter (Millipore) to remove solids. The samples were further diluted and injected into a Supelcogel C-610H HPLC column (particle size 9 μm, Sigma-Aldrich) maintained at 30 °C. Detection was performed by an Ultraviolet (UV) detector with maximum absorbance at 220. Methanol solution of 15 vol%, which was adjusted to pH 4.0 by adding 4 wt% H_2SO_4, was used as the eluent at a rate of 1.0 ml min^{-1}. HPLC grade furfural, furfuryl alcohol and furoic acid were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and used to generate standards for analysis.

Results

Fermentation: Bakers' yeast

Active dry Bakers' yeast was used to establish experimentation criteria initially and to assess the conversion potential and tolerance of generic yeast towards furfural. Fermentation flasks were dosed with furfural at concentrations of 5, 10, 15, 30 and 40 g l^{-1}, while the concentration of yeast was kept constant at 10 g l^{-1} (~2×10^8 cells ml^{-1}). Samples taken immediately after inoculating the media with yeast indicated a much lower concentration of furfural presented in solution, leading to infer that the yeast readily took up furfural and/or there was a loss of furfural due to condensation reactions during sterilization. Later experiments confirmed the latter as the reason for the decreased concentration of furfural, so the results from these experiments factor in that loss.

Identification of furfural tolerant *S. cerevisiae* strains

Recent studies have determined that wild *S. cerevisiae* strains isolated from diverse ecological niches display a range of phenotypic responses to environmental stress conditions[43,44] due, in part, to their genetic differences. Given this, it was speculated that wild *S. cerevisiae* strains with wide-ranging genetic variation might also display a range of abilities to detoxify furfural, including differences in enzymatic activities for conversion into furfuryl alcohol and abilities to regenerate cellular NADH. Given that yeast cell growth is limited by furfural, it was speculated that diverse wild strains would grow at different rates and to different cell densities in the presence of furfural. To test for this, a panel of 57 wild and domesticated *S. cerevisiae* strains were screened for those displaying the greatest change in cell density on lab media (YPD) containing 50 mM furfural within 24 h (Fig. 4 and ESI Table 1†). Indeed, a range of growth phenotypes were observed; very little change in growth was seen with commonly-used lab strains BY4741, S288c, CEN.PK2-1D, and W303 while large increases in growth were observed by clinical isolates 273614N and YJM421, natural isolates UWOPS87-2421 and DBVPG6765, fermentation strains DBVPG6040, DBVPG6044, NC-02 and UC5, and NCYC361. Interestingly, one lab strain, FL100, grew robustly in the presence of furfural, while a number of other fermentation strains and clinical isolates did not show similar growth. This suggests that specific environmental niches that the strains were isolated from do not necessarily correlate with furfural tolerance.

From this work six strains with higher growth in the presence of furfural compared to common lab strains were selected for further analysis. The six strains were cultured in YPD media with 50 mM furfural with their growth rate plotted with time. Growth rate was measured as the OD at 595 nm with the background absorbance subtracted. The results indicate that the 6 strains showed similar growth rates, with an initial lag before exponential growth after 12 hours (Fig. 5). Based on these results all six strains displayed similar capacity to reproduce in the presence of furfural and were suitable for fermentation experiments.

Fermentation: furfural-tolerant strains

The screening experiments for furfural tolerance were conducted at a furfural concentration of 50 mM (~4.8 g l^{-1}) to identify the yeast strains capable of tolerating high concentrations of furfural, possibly through greater abilities to reduce furfural. The screening was followed up with fermentation experiments (in duplicates) using the strains that exhibited the greatest furfural tolerance, and the furfuryl alcohol yields for the various strains are shown in Fig. 6.

The greatest furfuryl alcohol yield (~83%) was obtained using the strain UWOPS87-2421, while the lowest yield (~73%) was obtained using the strain UC5. Results did not indicate

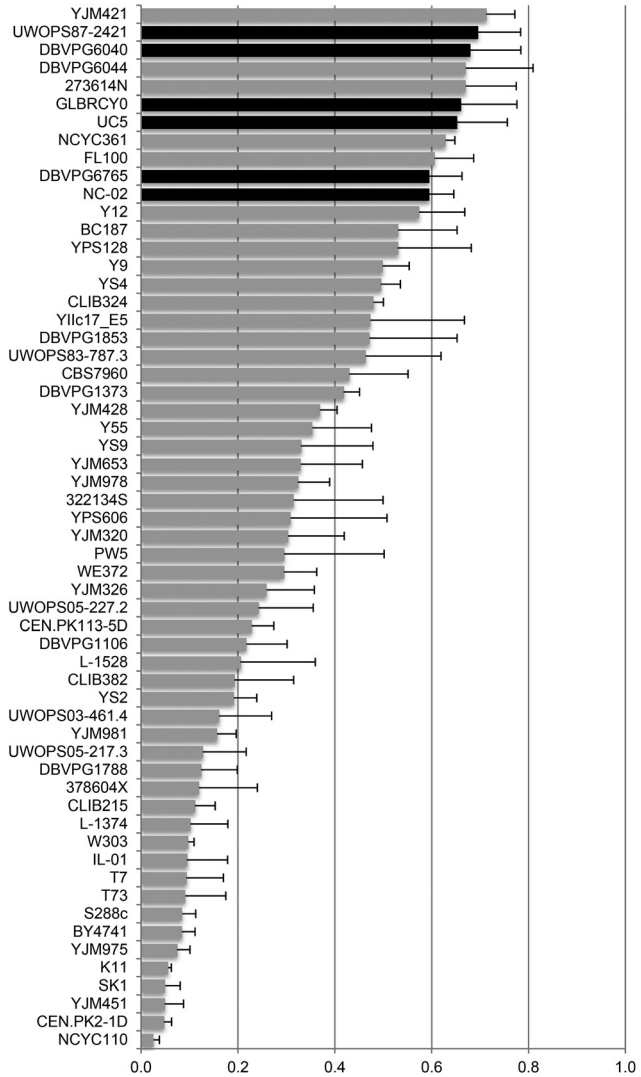

Fig. 4 The average change in total cell density (final OD_{595} – initial OD_{595}) for each strain is plotted with standard deviations from 3 independent biological replicates. Strains identified for growth tolerance to furfural and selected for additional study are shown in bold.

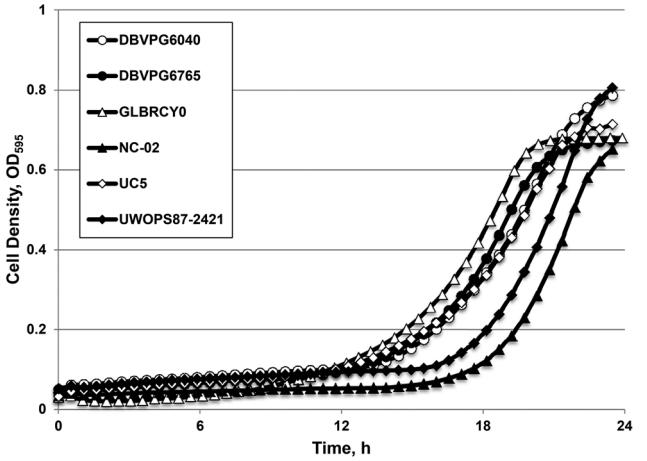

Fig. 5 Representative growth curves of indicated strains cultured in YPD media with 50 mM furfural are plotted as cell density (background subtracted OD_{595}) over time in hours. Cell density readings every 30 minutes are shown for clarity.

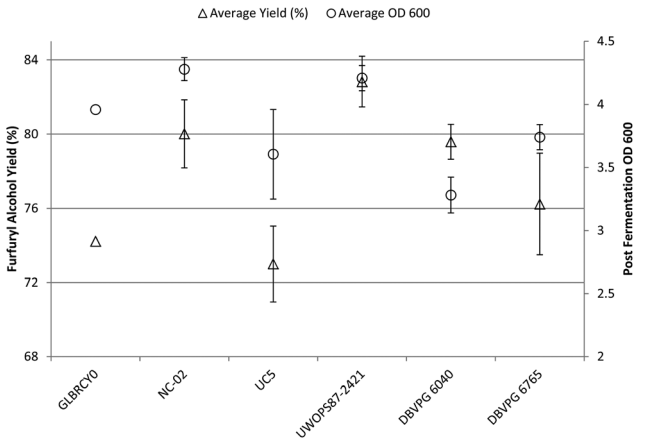

Fig. 6 Yield of furfuryl alcohol and the optical density of the yeast strains following fermentation in the presence of 2.4 g l^{-1} furfural (with standard errors for duplicate experiments).

any obvious and meaningful correlations between furfuryl alcohol yields and the optical density of the organisms following fermentation. However, prior research has established that the inhibition of cell growth by furfural is a function of furfural concentration, as well as the cell density.[30] Navarro *et al.*[45] have observed that the inhibition effects of furfural were nullified at high initial cell concentrations as the furfural was taken up and reduced quickly (up to 5 g l^{-1} furfural). Similar results with regard to faster uptake of furfural with higher inoculum levels are reported by Boyer *et al.*[37] for furfural concentrations up to 2 g l^{-1}.

Following favorable results, the six strains were dosed with furfural at much higher concentrations (25 to 65 g l^{-1}), and the yields of furfuryl alcohol ranged between ~38 and ~94% (Fig. 7). From the results, it appears that the greatest furfuryl alcohol yield is obtained at the lowest furfural concentration of 25 g l^{-1} across individual strains, with a steady decline as the concentration is increased. A logarithmic relationship provided with the best fit for the decline in yields with increasing furfural concentration (for average yields for all six strains varied across the furfural concentration levels). Although UWOPS87-2421 showed the highest yield across the entire range of furfural concentrations tested, the overall behavior of the six strains was quite similar at the furfural concentrations tested. The similar behavior between the six strains suggests that being better at growth in the presence of furfural does not mean greater reduction of furfural. It is likely that growth tolerance is furfural is not just by reduction to the alcohol, but other molecular mechanisms.

De Villegas *et al.*[29] have observed furfuryl alcohol yields up to 70% using *S. cerevisiae* at furfural concentrations of 35 g l^{-1}

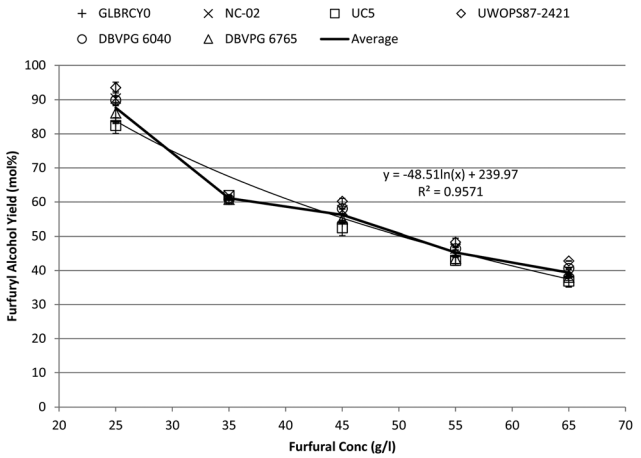

Fig. 7 Chart showing the furfuryl alcohol yield obtained using individual yeast strains and average yield for all six strains at various concentrations of furfural (with standard errors for duplicate experiments).

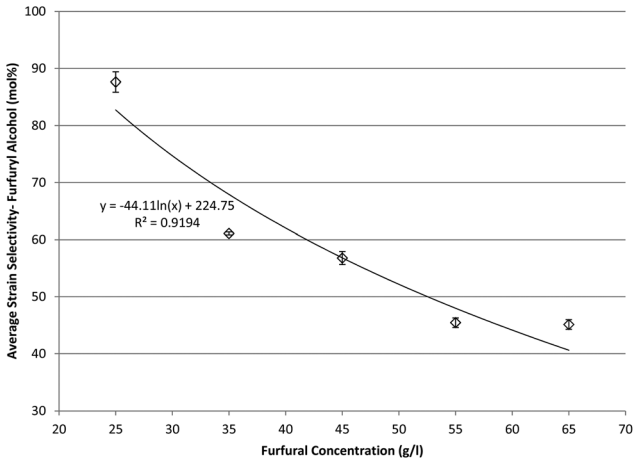

Fig. 8 Average selectivity of the six strains towards the production of primary product furfuryl alcohol, and the byproduct, furoic acid, with standard error.

in fed-batch mode, inferring that uptake and assimilation of small amounts of furfural occurred rapidly, allowing for the addition of furfural at various intervals. Similar results have been reported by Villa *et al.*[28] for furfural concentrations up to 30 g l^{-1} in fed-batch mode, and furfuryl alcohol yields of 96% using *S. cerevisiae*, without any apparent effects on the growth or morphology of yeast cells. The lower yields in the current study were possibly due to the experiments performed in batch mode, causing the organisms to be overwhelmed by the high initial concentration of furfural in solution, unlike in the fed-batch mode where limited amounts of furfural were fed to the yeast at specific intervals.

Sensitivity towards furfural production was also similar between strains, and fell sharply as the concentration of furfural was increased (Fig. 8). While furfural conversion was quite high even at the highest concentration (85.2 to 88.2 mol% and higher), the selectivity towards furfuryl alcohol suffered, indicating the inability of the yeast to effectively convert at higher concentrations. Good selectivity (~88%) at a furfural concentration of 25 g l^{-1}, however, indicates efficient conversion at lower concentrations of furfural in batch mode. Selectivity towards the production of furfuryl alcohol at low furfural concentrations (25%) is comparable to that achieved using novel chemical catalysts (Ni–P–B[15]), having been accomplished at ambient conditions as opposed to high temperature and pressure hydrogenation reactions.

Furoic acid was also produced as a byproduct in small amounts from all of the strains but in very small yields (1.7–5.1 mol%). The yields and selectivity towards the generation of furoic acid, as a byproduct are not substantial, and little furfural is scavenged towards this end. Researchers have observed the production of small amounts of furoic acid,[28] furoin and furil[46,47] alongside furfuryl alcohol during the biotransformation process at low furfural concentrations. Use of HPLC with an ultraviolet detector to study the compounds produced did not reveal peaks that suggested the existence of any other compounds aside from furfural, furfuryl alcohol and furoic acid. It was also not possible to account for the fate of all of the furfural in solution at higher doses. Apart from the production of furfuryl alcohol and furoic acid, it is thought that some furfural is taken up and retained within unviable yeast cells. Chung and Lee[48] have documented the effects of furfural concentration and initial inoculum level on cell viability, and have identified a yeast cell concentration of 10^8 initial cells mL^{-1} as the threshold above which the organisms were able to overcome cell death, use up the furfural in solution and proceed towards fermentation. Extrapolating from these observations, it is speculated that at high furfural concentrations, the organisms were able to convert small amounts of furfural into furfuryl alcohol and some furoic acid, but were not able to release the remainder of the furfural back into solution before furfural toxicity led to cell death.

Conclusions

Furfural was converted into furfuryl alcohol, along with furoic acid as a byproduct, using both generic Bakers' yeast and six furfural-resistant wild *S. cerevisiae* strains, with yields from the latter ranging from ~93 to ~37% with increasing furfural concentrations from 25 to 65 g l^{-1}. All six strains exhibited similar yields at the various furfural concentrations tested for, with strain UWOPS87-2721 producing furfuryl alcohol at the highest yield.

It was found that selectivity towards the production of furfuryl alcohol decreased with increasing furfural concentrations, while that of furoic acid remained relatively constant. All of the furfural introduced into the solution could not be accounted for at higher concentrations, and it is presumed that some of it taken up by the cells and not released back into solution as either product due to toxicity-associated death.

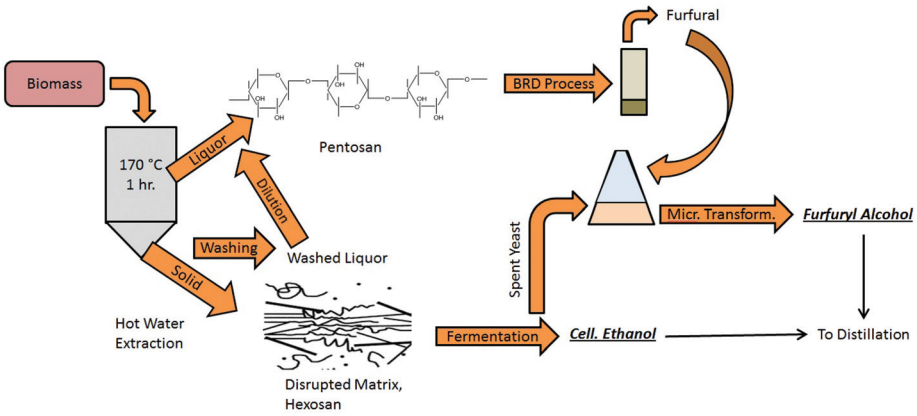

Fig. 9 Proposed model biorefinery for the production of furans in an aqueous, green high-yield process.

The biodetoxification pathway for the reduction of furfural was shown to be of potential use for the production of furfuryl alcohol in relatively high yields at low concentrations of furfural of 25 g L^{-1} or less.

The work shows that the pathway resonates with the stated goals of biocatalysis of safety in lieu of the mild conditions needed (in comparison to catalytic hydrogenation), high selectivity exceeding 85%, and the natural origin of the organisms meaning the biocatalysts are reproducible and biodegradable.[49]

Next steps

Based on the favorable results obtained for the production of furfuryl alcohol in high yields using a non-catalytic aqueous process that can be implemented at ambient conditions, the concept of a biorefinery is proposed for the production of furfural and furfuryl alcohol in relatively high yields (Fig. 9).

Earlier work[50–52] demonstrated that pentose sugars may be recovered in substantial amounts (19.5 to 33% of initial xylose present in the biomass) in the liquid fraction from a range of biomass feedstock (hybrid poplar, miscanthus, switchgrass and corn stover) using liquid hot water extraction without degrading the cellulose fraction by reacting the feedstock at 170 °C for one hour. These hot water extracts contained pentose sugars on the order of 14 to 21 g l^{-1}, characterized as total pentose available for conversion into furfural, while only 3 to 5 g l^{-1} of the hexose sugars (as total hexose) was extracted, indicating that cellulose degradation was minimal. Batch reactive distillation (BRD) was employed to convert these diluted (to 5 g l^{-1}) pentose-rich streams into furfural in high yield (85 to 94%), albeit at very low concentrations.[51,52] Furfural solutions produced by the BRD process (3.86 g l^{-1}) were converted into furfuryl alcohol at lower yields approaching 60% as reported earlier (due to losses encountered during sterilization).

The biorefinery loop is closed by fermenting the solid fraction biomass following hot-water extraction (research on this aspect has not been performed in this study, but has been previously reported[53–55]). Negro *et al.*[56] have shown that hydrothermal pretreatment techniques (both liquid hot water and steam explosion) are effective towards enhancing the enzymatic hydrolysis of the extracted solids (using poplar). In this regard, the water-extracted solids present a very suitable substrate for fermentation and production of cellulosic ethanol using the SSF (simultaneous saccharification and fermentation) configuration.

'Spent yeast' refers to yeast that has been obtained from recycling of the organisms from fermentation of the extracted solids. Further research may be directed at exploring the feasibility of using this yeast as a low cost 'catalyst' at high loading rates (as was done in the experiments in this study) to convert the BRD furfural product into furfuryl alcohol instead of culturing an expensive, inhibitor-resistant yeast strain. Recycling of yeast has been shown to be beneficial as it allows for adaptation of the organisms towards inhibitors,[57] so it is plausible that the yeast recycled from fermentation of the extracted solids may display more resistance to inhibitors. Researchers[58,59] have used recycled yeast (*S. stipitis*) to produce ethanol by the fermentation of red oak hydrolysates and it has been suggested[60] that the use of recycled yeast offers the benefits of adaptation and can aid fermentation (for galactose fermentation using several organisms in this particular study).

The furfuryl alcohol produced from the microbial conversion can then be distilled (more economical than the azeotropic distillation of furfural) to obtain a purified product, and the model biorefinery is thus capable of producing cellulosic ethanol and furfuryl alcohol in separate streams, following liquid hot-water extraction. Production of the furan compounds will thus add value to an existing cellulosic ethanol plant and diversify the range of products that can be produced at such a facility. Advantages conferred over the conventional catalytic process include extremely benign reaction conditions (temperature and pressure) at the expense of time, bypassing the need for heterogeneous catalysts or for azeotropic distillation (for furfural) as furfuryl alcohol can be directly distilled and purified following conversion, along with ethanol. This biorefinery model also accords other advantages compared to some of the newer technologies described in literature[6,61–63]

due to its completely aqueous nature as opposed to the use of expensive and potentially hazardous solvents (toluene, γ-valerolactone, *etc.*), circumvent the need for expensive solid-acid and zeolite catalysts, giving rise to the possibility of retrofitting conventional liquid acid reactors to the BRD configuration to improve furfural yields, and finally, a system that is truly green in concept and open-ended to be readily integrated into existing bioprocessing facilities (ethanol and furfural plants).

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