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Biocatalytic Reduction of HMF to 2,5-Bis(hydroxymethyl)furan by HMF-Tolerant Whole Cells

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Catalytic upgrading of 5-hydroxymethylfurfural (HMF), an important biobased platform chemical for high-value products, is currently of great interest. In this work, a new highly HMF-tolerant yeast strain—*Meyerozyma guilliermondii* SC1103 was isolated, and biocatalytic reduction of HMF to 2,5-bis(hydroxymethyl)furan (BHMF) using its resting cells was reported. Co-substrates exerted a significant effect on the catalytic activity and selectivity of microbial cells as well as their HMF-tolerant levels whereas the nitrogen source and mineral salts had no effects. In addition, *M. guilliermondii* SC1103 cells exhibited good catalytic performances within the range of pH 4.0–10.0. The yeast was highly tolerant to both HMF (up to 110 mM) and BHMF (up to 200 mM). In addition, 100 mM HMF could be selectively reduced to BHMF within 12 h by its resting cells in the presence of 100 mM glucose (as cosubstrate), with a yield of 86% and selectivity of >99%. The production of 191 mM of BHMF was realized within 24.5 h by using a fed-batch strategy, with a productivity of approximately 24 g L^{-1} per day. In addition, this new biocatalytic approach was applied for the reduction of furfural and 5-methylfurfural, affording the corresponding furfuryl alcohols with yields of 83 and 89%, respectively.

Introduction

Recently, utilization of renewable and carbon-neutral biomass has attracted a lot of interest for the production of biobased fuels and platform chemicals.[1] 5-Hydroxymethylfurfural (HMF) obtained through the dehydration of hexoses was recognized by the U.S. Department of Energy as one of "Top 10+4" biobased chemicals.[2] HMF could be converted into various useful chemicals owing to the presence of functional groups such as primary hydroxyl and formyl. For example, HMF could undergo oxidation and esterification as well as etherification.[3] In addition, HMF could be transformed into 2,5-bis(hydroxymethyl)-furan (BHMF), 2,5-dihydroxymethyltetrahydrofuran (DHMTHF), and 2,5-dimethylfuran (DMF) through selective reduction.[3b] BHMF is the hydrogenation product of the formyl group in HMF and is a versatile building block for the synthesis of polymers,[4] drugs,[5] macrocycle polyether compounds,[6] and crown ethers.[7]

To date, BHMF is synthesized mainly by chemical reduction of HMF. Cottier et al. described BHMF synthesis by stoichiometric reduction of HMF using sodium borohydride.[6] Catalytic hydrogenation of HMF to BHMF was reported on noble metal catalysts[8] as well as non-noble metals.[9] The Cannizzaro reaction was used for the synthesis of BHMF from HMF, but the theoretic selectivity toward the desired product was 50% because of the formation of an equimolar byproduct (5-hydroxymethyl-2-furancarboxylic acid, HMFCA).[10] In addition, several groups reported environmentally benign (photo)-electrochemical routes for the reduction of HMF to BHMF.[11]

Biocatalysis represents an attractive and promising option to supplement or replace chemical methods for organic transformations because of a number of advantages such as excellent selectivity, mild reaction conditions, high efficiency, and environmental friendliness.[12] However, biocatalytic valorization of HMF received little attention[13] compared to chemical routes. The reason may be that the costly enzymes have been used generally for the synthesis of enantiopure and high-value building blocks or drugs.[14] Driven by environmental concerns, biocatalytic production of bulk commodities such as biodiesel and biobased polyesters has received growing attention.[15] Recently, our group has reported enzyme-catalyzed oxidation and esterification of HMF.[16]

Biocatalytic reduction of HMF to BHMF can be conducted theoretically using isolated oxidoreductases as well as whole cells. Compared to isolated enzymes, whole cells were preferable for HMF reduction because they are not only inexpensive and more stable but also do not require complex cofactor regeneration systems that are necessary for isolated enzymes.[17] However, efficient synthesis of BHMF from HMF using whole cells is still a great challenge because the substrate HMF is a well-known potent inhibitor to microorganisms.[18] To our knowledge, there are only two reports on whole cell-catalyzed transformation of HMF in the literature,[13f,19] in which 2,5-furandicarboxylic acid (FDCA) was synthesized by oxidation of HMF. Some microorganisms were reported to be capable of transforming HMF in biological detoxification of the inhibitors present in lignocellulosic hydrolysates.[20] Nonetheless, these

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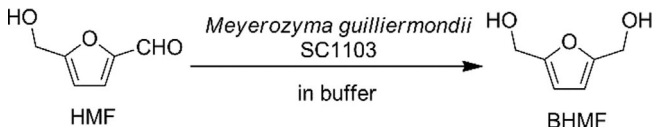
microorganisms were not appropriate biocatalysts for efficient synthesis of BHMF from HMF because of the following reasons: i) Their biodetoxification efficiencies remained low,[21] suggesting that HMF reduction rates were low; for instance, the highest HMF transformation rate was reported to be less than 2 mmol L^{-1} h^{-1}.[20c,21a] ii) Their tolerance to HMF, especially in high concentrations, was poor; for example, HMF of more than 30 mM would exert a significantly deleterious effect on biotransformation, leading to long reaction periods (>48 h).[20c,d] iii) The selectivities were not satisfactory; in addition to BHMF, the oxidation products of HMF were also formed.[21b,22] In this work, we report a new HMF-tolerant yeast strain—*Meyerozyma guilliermondii* SC1103 isolated from soil samples for efficient synthesis of BHMF from HMF (Scheme 1). The effects of some key conditions on whole-cell catalytic synthesis of BHMF were studied to obtain an optimized biocatalytic process. In addition, the tolerance of this strain to the substrate (HMF) and



Scheme 1. Biotransformation of HMF to BHMF in buffer.

product (BHMF) was evaluated, and a substrate feeding strategy was used for the production of a high concentration of BHMF. Besides, the established biocatalytic process was used for the synthesis of other furfuryl alcohols.

Results and Discussion

Effects of cosubstrates, nitrogen source, and mineral salts on BHMF synthesis

Table 1 shows the effects of cosubstrates, nitrogen source, and mineral salts on BHMF synthesis. It was found that cosubstrates exerted a significant effect on the catalytic performance of *M. guilliermondii* SC1103 cells, particularly on the reaction rate and selectivity. The reaction time was much lower using glucose as cosubstrate than using glycerol, although comparable BHMF yields were obtained in both cases (Table 1, entries 2 vs. 1). A yield of 87% was achieved after 7 h of reaction using glucose as cosubstrate (Table 1, entry 2); in addition, the yield changed slightly with the prolongation of the reaction time, suggesting that the product is very stable and cannot be degraded further by the yeast. In addition, the selectivity was higher for glucose compared to the one obtained for glycerol. In the case of glycerol, the lower selectivity (93.1%) was owed to the formation of more HMFCA, an HMF oxidation product. The results indicate that glucose is the preferred cosubstrate for this reduction reaction possibly because, compared to glycerol, glucose is a better carbon source that can sufficiently provide the reduced form of nicotinamide cofactor (NAD(P)H) for HMF reduction.[23] This could be confirmed by the results on glucose concentration effect on BHMF synthesis (Table 1, entries 2–4). The catalytic performances of microbial cells decreased significantly with decreasing glucose concentrations. Lower glucose concentrations led to longer reaction times as well as lower yields and selectivities. The poor results (55% yield and 61% selectivity) were obtained after 24 h in the absence of cosubstrate, suggesting that the cosubstrate plays a key role in whole cell-catalyzed reduction of HMF. In addition to using oxidized cosubstrates (glucose and glycerol) for biocatalytic reduction, we attempted to use acetone as reduced cosubstrate for biocatalytic oxidation of HMF (Figure S1 in the Supporting Information). Unfortunately, BHMF remained to be the major product with a low yield (21%). Both nitrogen source and mineral salts had no significant effects on the reduction reaction (Table 1, entries 5–7). Good yield (89%) and excellent selectivity (99%) were achieved in the absence of both nitrogen source and mineral salts (Table 1, entry 7), which would significantly simplify the reaction mixture and facilitate the downstream product isolation and purification.

Effects of key reaction conditions on BHMF synthesis

The influences of some key conditions on whole cell-catalyzed reduction of HMF were studied (Figure 1). Figure 1a shows the effect of pH value on the reduction of HMF when pH value varies from 4.0 to 10.0. *M. guilliermondii* SC1103 cells exhibited good catalytic performances within the pH range examined. The reaction rates were comparable at pH 4.0–10.0, and the maximal yields of 86–91% were achieved after a reaction time

Table 1. Comparison of the catalytic performances of resting and culturing cells.[a]

Entry	Cosubstrate [mmol L^{-1}]	Nitrogen source [g L^{-1}]	Mineral salts[b]	*t* [h]	BHMF yield [%]	Selectivity [%]
1	glycerol, 30	$(NH_4)_2SO_4$, 2	yes	24	88.0±0.3	93.1±0.1
2	glucose, 30	$(NH_4)_2SO_4$, 2	yes	7	87.1±0.2	98.9±0.0
3	glucose, 15	$(NH_4)_2SO_4$, 2	yes	12	88.7±0.8	97.6±0.2
4	none	$(NH_4)_2SO_4$, 2	yes	24	55.2±1.5	61.4±0.4
5	glucose, 30	none	yes	7	90.5±3.5	99.0±0.1
6	glucose, 30	$(NH_4)_2SO_4$, 2	none	7	85.8±1.5	98.9±0.0
7	glucose, 30	none	none	7	89.3±0.9	99.0±0.0

[a] Reaction conditions: 40 mM HMF, 20 mg mL^{-1} microbial cells, 4 mL phosphate buffer (100 mM, pH 7.2), 200 rpm, 30 °C. [b] $MgCl_2{\cdot}6H_2O$ (0.1 g L^{-1}), EDTA (10 mg L^{-1}), $ZnSO_4{\cdot}7H_2O$ (2 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (1 mg L^{-1}), $FeSO_4{\cdot}7H_2O$ (5 mg L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.2 mg L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.2 mg L^{-1}), $CoCl_2{\cdot}6H_2O$ (0.4 mg L^{-1}), and $MnCl_2{\cdot}2H_2O$ (1 mg L^{-1}).

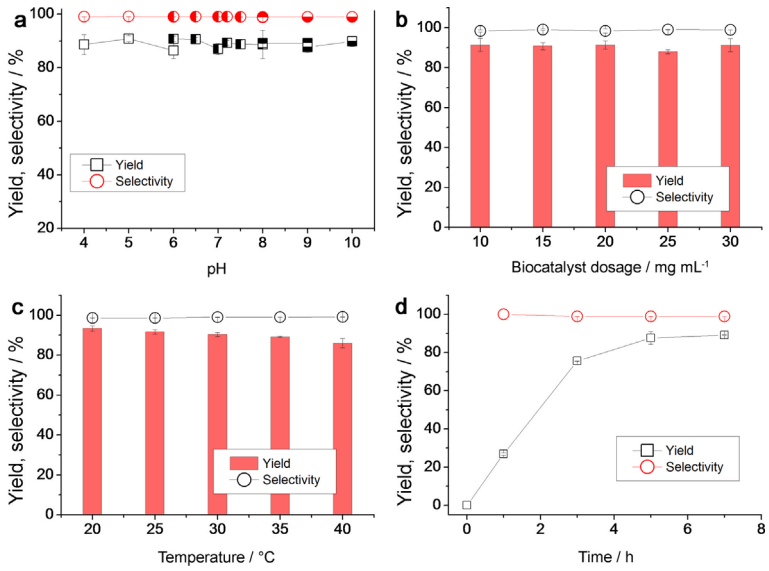


Figure 1. Effects of a) pH, b) biocatalyst dosage, c) temperature, and d) reaction time on BHMF synthesis. The following general conditions applied unless otherwise stated: 40 mM HMF, 30 mM glucose, 20 mg mL^{-1} microbial cells, 4 mL phosphate buffer (100 mM, pH 7.2), 30 °C, 200 rpm; a) buffers used: pH 4.0–6.0 citrate buffer, pH 6.0–8.0 phosphate buffer, pH 8.0–9.0 Tris-HCl buffer, pH 9.0–10.0 glycine-NaOH buffer, 7 h; b) gradual increase of cell dosage from 10 to 30 mg mL^{-1} with corresponding periods of 9, 7, 7, 5, and 5 h; c) gradual increase of temperature from 20 to 40 °C with corresponding periods of 24, 9, 7, 7, and 5 h; d) 35 °C.

of 7 h. In addition, excellent selectivities (ca. 99%) were observed. Figure 1b shows the impact of the cell dosage on BHMF synthesis. Although the cell dosage exerted no significant effect on the maximal yields (88–91%) and selectivities (ca. 99%), its effect on the reaction rates was substantial. For example, a reaction period of 9 h was needed for reaching equilibrium with 10 mg mL^{-1} of cell dosage whereas only 5 h were needed for more than 25 mg mL^{-1} microbial cells. The effect of the reaction temperature on BHMF synthesis is shown in Figure 1c. Similarly, the reaction rates were also affected significantly by the temperature, but its effect on yield and selectivity was slight. The reaction time was reduced significantly by increasing the temperature (e.g., 24 h at 20 °C vs. 5–7 h at >30 °C). These results suggest that *M. guilliermondii* SC1103 cells can exhibit good catalytic performances over a wide range of conditions, which demonstrates its great application potential for HMF reduction. Figure 1d shows the time course of whole-cell catalytic reduction of HMF under optimized conditions. BHMF was synthesized quickly, and a maximal yield of 89% was obtained within 7 h. Moreover, excellent selectivities were retained during the reaction.

Substrate inhibition and toxicity toward microbial cells

As described above, HMF is an inhibitory and toxic compound toward microbes because it may result in damage of cell walls and membranes as well as inhibition of the activities of various dehydrogenases and of ribonucleic acid (RNA) synthesis.[20b,24] Therefore, the HMF-tolerant level of microbial cells is critical for efficient synthesis of BHMF from HMF. The tolerance of *M. guilliermondii* SC1103 cells toward HMF was evaluated (Figure 2). As shown in Figure 2a, the highest initial reaction rate (14.4 mmol L^{-1} h^{-1}) was observed at 70 mM HMF in the presence of 30 mM glucose, and further increasing the substrate concentrations resulted in significant decrease of the initial reaction rates. This effect suggests that significant substrate inhibition occurs when HMF concentrations are more than 80 mM. In addition, the yield was recorded at various HMF concentrations in the presence of 30 mM glucose. As shown in Figure 2a, high yields (92–97%) were obtained within 5–7 h, with excellent selectivities (99%) when HMF concentrations were less than 50 mM. Although the selectivity remained high at higher substrate concentrations, the yield decreased significantly to 37–84%.

A yield of only 69% was obtained when the HMF concentration was 70 mM, which could not be explained rationally by

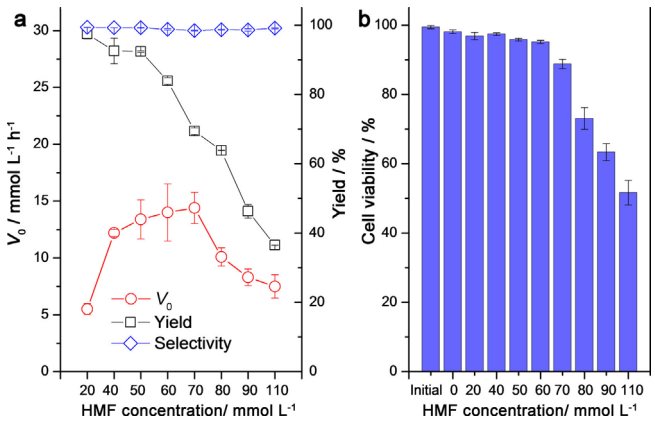


Figure 2. Effects of substrate concentrations on a) BHMF synthesis and b) cell viability. Reaction conditions: 30 mM glucose, 20 mg mL^{-1} microbial cells, 4 mL phosphate buffer (100 mM, pH 7.2), 200 rpm, 35 °C; a) reaction periods: 5, 7, 7, 12, 24, 36, 12 and 7 h, with gradually increasing HMF concentrations from 20 to 110 mM, respectively; b) incubation period of 12 h.

the results on substrate inhibition, because no substrate inhibition occurs at this substrate concentration. Besides inhibiting enzyme activities, HMF might also be toxic to microbial cells. Therefore, cell viability was assayed after the yeast cells were incubated in the presence of various HMF concentrations (Figure 2b). It was found that more than 89% of the cells remained alive after an incubation period of 12 h in the presence of less than 70 mM HMF. However, the cell viability decreased significantly to 52–73% for more than 80 mM HMF, indicating that these concentrations of HMF may be highly toxic to the yeast cells. However, the total number of cells including dead and alive cells did not change considerably before and after incubation in HMF solutions, which suggested that no cell lysis occurred. The above results on HMF inhibition and toxicity suggest that *M. guilliermondii* SC1103 cells may be tolerant to substrate concentrations up to 70 mM in the presence of 30 mM glucose. Nonetheless, the yields (69–84%) were not satisfactory when substrate concentrations were 60–70 mM. It is well known that efficient regeneration of the reduced cofactors (e.g., NAD(P)H) is critical for biocatalytic reduction reactions, which is closely related to the cosubstrates and their concentrations.[17] Accordingly, the changes in glucose concentrations were monitored during the reaction when the HMF concentration was 50 mM (data not shown). It was found that glucose was used up within 3 h. Therefore, we reasoned that the relatively low yields might be owed to insufficient regeneration of the reduced cofactors for the reduction of HMF in the presence of 30 mM glucose, when HMF concentrations were more than 60 mM.

To verify our assumption, the reduction of HMF was conducted in the presence of 100 mM glucose (Figure 3). The biocatalytic reduction of HMF was improved significantly when a high concentration of glucose was supplemented. For example, the yield of 87% was obtained in the case of 70 mM HMF in the presence of 100 mM glucose, which is much higher than that in the presence of 30 mM glucose (87% vs. 69%). More importantly, the HMF-tolerant level of microbial cells became higher with 100 mM glucose as cosubstrate (Figure 3): good yields (ca. 85%) were achieved in 12 h when HMF concentrations were 80–100 mM. Even microbial cells were capable of smoothly reducing HMF in the concentration up to 110 mM to the desired product, resulting in a yield of 87% after 36 h. To our knowledge, this is the highest HMF-tolerant concentration of microbial cells ever reported. In general, the effects of furan on microbial cells could be explained by a redirection of microbial energy for repairing the damage, relieving enzyme inhibition, and regenerating the cofactors.[25] Therefore, supplementation of excess cosubstrate might favor the regeneration of NAD(P)H as well as fixing the damage, thus significantly enhancing HMF-tolerance of microbial cells.[23] However, low yields (42–51%) were obtained when HMF concentrations were higher than 150 mM (Figure 3). In addition, no significant improvements in the yields were observed when higher concentrations of glucose (150–200 mM) were supplemented (Figure S2, available in the Supporting Information), suggesting significant substrate inhibition and toxicity at substrate concentrations of above 150 mM. It is worth noting that the excellent selectivities (>99%) remained in the range of the substrate concentrations tested.

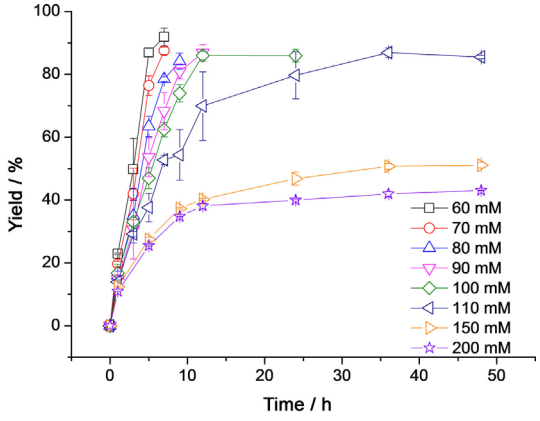


Figure 3. Whole cell-catalyzed reduction of HMF in the presence of 100 mM glucose. Conditions: HMF of the designated concentration, 20 mg mL^{-1} microbial cells, 4 mL phosphate buffer (100 mM, pH 7.2), 200 rpm, 35 °C.

Product inhibition and toxicity toward microbial cells

In addition to substrate inhibition and toxicity, the products also may exert such negative effects on biocatalytic reduction reactions.[17] Product inhibition and toxicity toward microbial cells were studied. As shown in Figure 4, no significant product inhibition was observed when BHMF concentrations were less than 150 mM because the initial reaction rates (13.4 and 14.4 mmol L^{-1} h^{-1}) were comparable. The initial reaction rate slightly decreased to 10.6 mmol L^{-1} h^{-1} when the BHMF concentration was increased to 300 mM. In addition, BHMF had almost no toxicity toward microbial cells when its concentrations were less than 100 mM and slight toxicity was found at 150 mM BHMF (82% of cell viability). Cell viability (63%) remained moderate at BHMF concentration up to 300 mM. Simi-

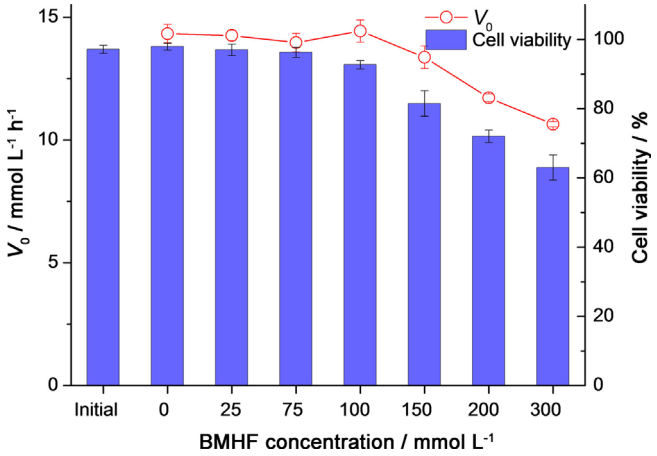


Figure 4. Effect of the product concentrations of BHMF synthesis and cell viability. Conditions: 50 mM HMF, 30 mM glucose, BHMF of the designated concentration, 20 mg mL^{-1} microbial cells, 4 mL phosphate buffer (100 mM, pH 7.2), 200 rpm, 35 °C; after microbial cells were incubated for 12 h under the above conditions without HMF, cell viability was measured.

larly, no significant changes in the total cell populations were observed before and after incubation in BHMF solutions. The results indicate that the product is less inhibitory and toxic to the yeast cells than the substrate, which is in good agreement with a previous study.[20a]

Synthesis of BHMF by a fed-batch strategy

Continuous accumulation of a high concentration of the product in the reactor is highly desirable because it can not only significantly improve the reactor productivity but also facilitate the product purification. Inspired by the above results on inhibition and toxicity, biocatalytic synthesis of BHMF was conducted by fed-batch feeding of the substrate (Figure 5). BHMF

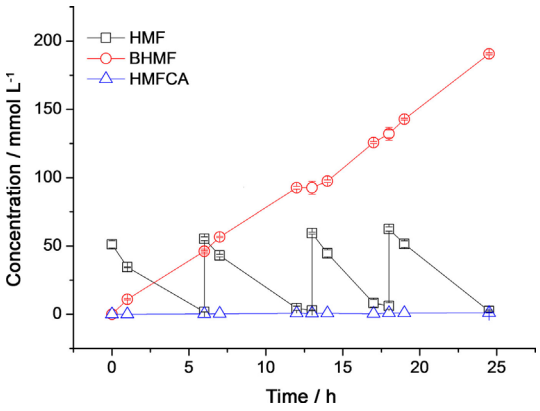


Figure 5. Synthesis of BHMF by a fed-batch strategy. Reaction conditions: 50 mM HMF, 30 mM glucose, 20 mg mL^{-1} microbial cells, 4 mL phosphate buffer (100 mM, pH 7.2), 200 rpm, 35 °C. After HMF was almost used up, 0.2 mmol HMF and 0.12 mmol glucose were supplemented.

up to 191 mM was produced within 24.5 h after three-batch feeding of HMF, and the total yield was approximately 88%. The productivity of approximately 24 g L^{-1} per day was obtained. In addition, only traces of HMFCA (about 1 mM) were formed as the sole byproduct. The selectivity toward the desired product reached >99%.

Reduction of furfurals to furfuryl alcohols

In addition to HMF reduction, the biocatalytic process established in this work was applied for the reduction of other platform chemicals—furfural and 5-methylfurfural (Figure 6). Furfuryl alcohol is an important intermediate for the production of thermostatic resins, synthetic fibers, farm chemicals, and plasticizers.[26] It is well known that furfural is a much stronger inhibitory compound to microorganisms than HMF.[27] Interestingly, it was found that this compound was also a good substrate of *M. guilliermondii* SC1103 and furfuryl alcohol was obtained with a yield of 83% in 5 h, along with 2-furoic acid as the byproduct (Figure 6). The selectivity was approximately 96%. In addition, *M. guilliermondii* SC1103 could efficiently catalyze the reduction of 5-methylfurfural, affording 5-methylfurfuryl alcohol with a yield of 89% and selectivity of >99%.

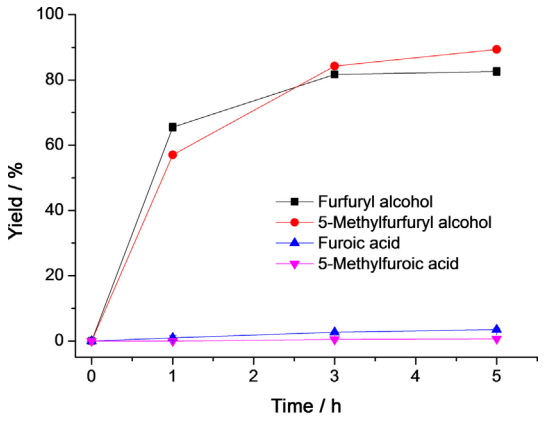


Figure 6. Biocatalytic reduction of furfurals to furfuryl alcohols. Reaction conditions: 50 mM furfurals, 30 mM glucose, 20 mg mL^{-1} microbial cells, 4 mL phosphate buffer (100 mM, pH 7.2), 200 rpm, 35 °C.

The results obtained in this work were compared with selected literature results. As shown in Table 2, HMF was efficiently hydrogenated to BHMF with an excellent yield and selectivity using Pt/MCM-41 as catalyst under mild conditions.[8d] Compared to noble metals, non-noble metal catalysts usually required relatively harsh reaction conditions (e.g., higher temperature) for BHMF synthesis owing to lower reactivity.[9a,c] The efficiency of electrocatalytic hydrogenation of HMF to BHMF remained low[11c] although the method appeared to be environmentally friendly and selective. Biocatalytic reduction of HMF is of considerable interest owing to its many advantages such as not needing to use metal catalysts, H_2, and organic solvents; being highly selective; and requiring mild reaction conditions. Although culturing cells of some microorganisms were reported to be HMF-tolerant, their transformation efficacies were very poor (>48 h required for complete conversion of HMF).[20c,d,21b] And the quantitative results on the selectivity remained unknown in most of the previous publications although qualitative results were described. For example, Feldman et al. reported that both BHMF and FDCA were formed during detoxification by culturing cells of *Pleurotus ostreatus*.[21b] *M. guilliermondii* SC1103 was proven to be a good biocatalyst for the reduction of HMF to BHMF because it is highly tolerant to both HMF and BHMF. Moreover, a good yield as well as an excellent selectivity was obtained in 12 h when the HMF concentration was up to 100 mM. The biocatalytic approach established in this work appeared to be more eco-friendly than metal-mediated chemical methods, in spite of a lower productivity, because the biocatalyst was environmentally benign, and no volatile organic solvent and H_2 were used. In addition, this biocatalytic process was energy efficient and highly selective. Its productivity and substrate concentrations may be improved significantly by increasing cell dosage and using a biphasic system, and the work is currently in progress.

Conclusions

We developed an efficient and selective biocatalytic approach for the synthesis of 2,5-bis(hydroxymethyl)furan (BHMF) from

Table 2. HMF reduction catalyzed by various catalysts.

Catalysts	Reaction conditions	*t* [h]	C/Y [%][a]	BHMF selectivity [%]	Ref.
Pt/MCM-41	~2 M HMF, 50 mg mL^{-1} catalyst, 0.8 MPa H_2, 35 °C, in H_2O	2	98.9 (Y)	98.9	[8d]
$ZrO(OH)_2$	~0.2 M HMF, 25 mg mL^{-1} catalyst, 150 °C, in ethanol (as both hydrogen donor and solvent)	2.5	94.1 (C)	88.9	[9c]
Ag catalytic electrode	0.02 M HMF, $E=-1.3$ V vs Ag/AgCl, 60 C charge passed, pH 9.2, in borate buffer	n.a.[b]	100 (C)	>99	[11c]
Pleurotus ostreatus	0.03 M HMF, 30 °C, 180 rpm, in a GP medium[c]	48	100 (C)	n.a.	[21b]
Saccharomyces cerevisiae 307-12H60	0.06 M HMF, 30 °C, 250 rpm, 1% of the inoculate culture, in a complete synthetic medium	48	100 (C)	n.a.	[20c]
Scheffersomyces stipitis KCTC 7228	0.06 M HMF, 30 °C, pH 5.5, 220 rpm, in seaweed hydrolysates of 10% (*w/v*) slurry	60	100 (C)	n.a.	[20d]
M. guilliermondii SC1103	0.1 M HMF, 20 mg mL^{-1} cells, 100 mM glucose as cosubstrate, pH 7.2, 35 °C, 200 rpm, in phosphate buffer	12	86.0 (Y)	99.2	This work

[a] Y: Yield, C: conversion. [b] n.a.: Not available. [c] GP: glucose peptone.

5-hydroxymethylfurfural (HMF) using resting cells of *Meyerozyma guilliermondii* SC1103. The new isolated *M. guilliermondii* SC1103 was highly tolerant to HMF as well as BHMF and proved to be an excellent biocatalyst for the reduction of HMF. The resting cells of *M. guilliermondii* SC1103 still retained good catalytic performances over a wide range of conditions. BHMF up to 191 mM was synthesized in 24.5 h by a fed-batch strategy, which is promising for the development of an industrially sound biocatalytic process for HMF reduction. Moreover, the yeast cells were able to efficiently transform furfural and 5-methyfurfural into target furfuryl alcohols with good selectivities. In addition to the reduction of furfurals, *M. guilliermondii* SC1103 may have promising application potential in biological detoxification because of its high detoxification efficiency.

Experimental Section

Biological and chemical materials

M. guilliermondii SC1103 was isolated from soil samples obtained from the grounds of an industrial plant. Based on the analysis of the D1/D2 domain of nuclear large subunit (26S) ribosomal DNA (available in the Supporting Information) as well as physiological and biochemical characteristics, the strain was identified as *M. guilliermondii*. The phylogenetic tree of this train is shown in Figure S3 in the Supporting Information. *M. guilliermondii* SC1103 (CCTCC No. M2016144) was maintained in the China Center for Type Culture Collection (CCTCC, Wuhan, P.R. China).

HMF (98%) was purchased from J&K Scientific Ltd. (Guangzhou, P.R. China). BHMF (98%) and furfural (99%) were obtained from Macklin Biochemical Co., Ltd. (Shanghai, P.R. China). HMFCA (98%) was purchased from Adamas Reagent Ltd. (Shanghai, P.R. China). Furfuryl alcohol (98%) was obtained from Alfa Aesar (Tianjin, P.R. China). 2-Furoic acid (98%) and 5-methylfurfural (97%) were purchased from TCI (Japan). 5-Methyl-2-furoic acid (97%) was obtained from Sigma–Aldrich (USA). 5-Methylfurfuryl alcohol (98%) was purchased from Apollo Scientific Ltd. (UK).

Cultivation of *M. guilliermondii* SC1103 cells

M. guilliermondii SC1103 cells were precultivated at 30 °C and 200 rpm for 12 h in the yeast extract peptone dextrose (YPD) medium containing 1% yeast extract, 2% peptone, and 2% glucose. Then, the 2% seed culture was inoculated to the fresh YPD medium. After incubation at 30 °C and 200 rpm for 12 h, the cells were harvested by centrifugation (3500 rpm, 15 min, 4 °C) and washed twice with distilled water, followed by dispersing in phosphate buffer to yield cell concentrations of 10–30 mg (cell wet weight) per mL.

General procedure for biocatalytic reduction of HMF

Typically, phosphate buffer (4 mL, 100 mM, pH 7.2) containing 50 mM HMF, 30 mM glucose and 20 mg (cell wet weight) per mL microbial cells was incubated at 35 °C and 200 rpm. Aliquots were withdrawn from the reaction mixtures at specified time intervals and diluted with the corresponding mobile phase prior to HPLC analysis. The initial reaction rate (V_0) was calculated based on the decrease in HMF concentrations at the initial reaction stage. The yield was defined as the ratio of the measured product amount to the theoretical product amount based on the initial amount of HMF. The selectivity was defined as the ratio of BHMF amount (in mmol) to the sum of all the products. All experiments were conducted at least in duplicate, and the values were expressed as the means ± standard deviations.

HPLC analysis

The reaction mixtures were analyzed using an Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm, Agilent, USA) by reversed-phase HPLC equipped with a Waters 996 photodiode array detector (Waters, USA). The mobile phase was the mixture of acetonitrile and 4.0% $(NH_4)_2SO_4$ solution (10:90, *v/v*) with a flow rate of 0.6 mL min^{-1}. The retention times of HMFCA, BHMF, and HMF were 6.1, 8.3, and 9.9 min, respectively.

Cell viability assay

Yeast cell viability was measured using the methylene-blue staining method.[28] Briefly, the cell suspension (100 μL) was withdrawn and diluted 40 times with phosphate buffer (100 mM, pH 7.2) after incubation for 12 h under the designated conditions. Then, the diluted cell suspension (50 μL) was added into 0.1% methylene blue dissolved in physiological saline (2 mL). After staining for 5 min, blue dead and colorless viable cells were counted using a blood

counting chamber. The cell viability was expressed as the percentage of viable cells in the total cells, and the values were expressed as the means ± standard deviations ($n = 3$).

Optimized synthesis of BHMF by fed-batch feeding of substrate

The reaction mixture containing 4 mL phosphate buffer (100 mM, pH 7.2), 50 mM HMF, 30 mM glucose and 20 mg mL^{-1} microbial cells was incubated at 35 °C and 200 rpm. After HMF was almost used up, 0.2 mmol HMF and 0.12 mmol glucose were repeatedly supplemented into the reaction mixture. The changes in the concentrations of various compounds were monitored by HPLC.

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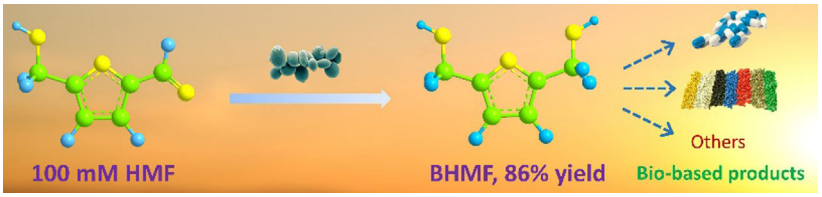
Biocatalytic Reduction of HMF to 2,5-Bis(hydroxymethyl)furan by HMF-Tolerant Whole Cells



High HMF tolerance and selectivity! An efficient and selective biocatalytic approach for the synthesis of 2,5-bis(hydroxymethyl)furan (BHMF) from 5-hydroxymethylfurfural (HMF) is successfully developed by using highly HMF-tolerant *Meyerozyma guilliermondii* SC1103 cells for the first time. A fed-bath strategy was used for the synthesis of BHMF, obtaining a good yield and excellent selectivity.