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Significantly improved oxidation of bio-based furans into furan carboxylic acids using substrate-adapted whole cells

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ABSTRACT

Furan carboxylic acids are important building blocks in polymer and fine chemical industries. In this work, a simple substrate adaptation strategy was applied to improve the catalytic performances of *Comamonas testosteroni* SC1588 cells for the synthesis of various furan carboxylic acids. It was found that biocatalytic synthesis of 5-hydroxymethyl-2-furancarboxylic acid (HMFCA) was substantially promoted by adding histidine and increasing cell concentrations. HMFCA was produced in a quantitative yield from 200 mM HMF in 24 h. Besides, the HMFCA yields of 71%–81% were achieved with the substrate concentrations up to 250–300 mM. It was firstly found that 4-tert-butylcatechol (TBC), as the stabilizer present in HMF, exerted a significantly detrimental effect on whole-cell catalytic synthesis of HMFCA at high substrate concentrations (more than 130 mM). In addition, a variety of furan carboxylic acids such as 2-furoic acid, 5-methyl-2-furancarboxylic acid and 5-methoxymethyl-2-furancarboxylic acid were synthesized with the yields up to 98%.

1. Introduction

In the last decade, utilization of renewable and carbon-neutral biomass has attracted considerable interest for the production of biofuels and bio-based chemicals, due to the depletion of fossil resources and increasing concerns about global warming [1,2]. Bio-based furans such as 5-hydroxymethylfurfural (HMF) and furfural are important intermediates bridging the gap between biomass and biofuels as well as between biomass and bio-based chemicals [3,4]. These furans could be upgraded into many useful chemicals through classical chemical transformations, because of the presence of reactive functional groups such as primary hydroxyl and formyl groups. For example, 2,5-furandicarboxylic acid (FDCA), a promising alternative to terephthalic acid (TPA) for the production of bio-based polyethylene 2,5-furandicarboxylate (PEF), could be synthesized via HMF oxidation [5], while selective reduction of HMF afforded 2,5-bis(hydroxymethyl)furan (BHMF) [6,7]. Like FDCA, 5-hydroxymethyl-2-furancarboxylic acid (HMFCA), one of the oxidation derivatives of HMF, is a useful building block for the synthesis of polyesters as well as TPA [8,9]. In addition, HMFCA was used for manufacturing an interleukin inhibitor and served as a potential antitumor therapeutic [10,11]. Also, 2-furoic acid that is the oxidation product of furfural has been widely used in flavor, fragrance, pharmaceutical, and agrochemical industries [4].

Currently, chemical catalysts are still playing a central role in the valorization of bio-based furans [12]. Nonetheless, chemically catalytic processes are generally accompanied by many drawbacks such as unsatisfactory selectivity, harsh reaction conditions, and increased environmental concerns. In recent years, biocatalysis has emerged as a promising alternative to chemical routes for the oxidation of these inherently unstable bio-based furans [13,14], since most of the abovementioned problems can been overcome in biocatalytic oxidations. A variety of enzymes including xanthine oxidase, Baeyer–Villiger monooxygenases, alcohol and aldehyde dehydrogenases, and lipase were reported for selective oxidation of HMF into HMFCA [15–19]. In addition to isolated enzymes, whole cells were exploited for producing HMFCA. *Pseudomonas syringae* SF4-17 was isolated from soil for the synthesis of HMFCA [20]. *Serratia liquefaciens* LF14 enabled selective oxidation of HMF into HMFCA [21]. We reported a HMF-tolerant strain *Comamonas testosteroni* SC1588 for the synthesis of HMFCA [22]. Recently, Sayed et al. [23] reported whole-cell catalytic synthesis of HMFCA using *Gluconobacter oxydans*. With the exception of limited examples [18,22,23], the substrate concentrations used are usually low in biocatalytic oxidation of bio-based furans, because these furans are well-known inhibitors towards enzymes and microorganisms [24]. In addition, their biotransformation efficiencies are unsatisfactory, especially at high substrate concentrations that are highly

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desired in the large-scale production. From a technical viewpoint, therefore, significant challenges remain in biocatalytic upgrading of bio-based furans.

Recently, we demonstrated that *C. testosteroni* SC1588 was a good catalyst for the synthesis of HMFCA, because of its great HMF-tolerance as well as satisfactory catalytic efficiency [22]. To tap the application potential of this wild-type microbe, a simple substrate adaptation strategy that has been well established in environmental microbiology [25–27] was applied to enhance its catalytic performances in this work, where the substrate was supplemented as the inducer during cell cultivation for inducing the expression of enzymes responsible for HMF transformation. It was interestingly found that the catalytic performances of this strain including the HMF-tolerant level and catalytic activity were substantially improved upon substrate adaptation and process optimization. In addition, the sources of HMF exerted a significant effect on the catalytic performances of the cells, especially at high substrate concentrations. Also, the substrate spectrum of this biocatalyst was studied.

2. Experimental

2.1. Materials

C. testosteroni SC1588 maintained in the China Center for Type Culture Collection (CCTCC, Wuhan, China; with CCTCC no. M2016562) was isolated by our laboratory [22]. HMF (99%) and 4-tert-butylcatechol (TBC, 98%) were purchased from Aladdin (Shanghai, China). HMFCA (98%) and 5-methoxymethylfurfural (MMF, 97%) were purchased from Adamas Reagent Ltd (Shanghai, China). HMF (98%), 5-formyl-2-furancarboxylic acid (FFCA, 98%) and FDCA (97%) were from J&K Scientific Ltd (Guangzhou, China). Histidine (99%), BHMF (98%) and furfural (99%) were obtained from Macklin Biochemical Co., Ltd (Shanghai, China). Furfuryl alcohol (98%), 2,5-diformylfuran (DFF, >98%), 2-furoic acid (98%), 4-fluorobenzaldehyde (98%), 4-fluorobenzoic acid (98%), 4-fluorobenzyl alcohol (98%), and 5-methylfurfural (97%) were purchased from TCI (Japan). 5-Methylfurfuryl alcohol (98%) was purchased from Apollo Scientific Ltd (UK). 5-Methyl-2-furancarboxylic acid (97%) was from Sigma-Aldrich (USA). 5-Methoxymethyl-2-furancarboxylic acid (99%) was bought from ThermoFisher Scientific (Beijing, China). Other chemicals are of analytical grade and commercial available.

2.2. Acclimatization and substrate-adapted cultivation of microbial cells

Acclimatization of microbial cells was performed according to a recent method [7], with some modifications. *C. testosteroni* SC1588 cells were pre-cultivated in 1.8% nutrient broth for 12 h at 30 °C and 160 r/min, followed by inoculation of 1% culture into fresh 1.8% nutrient broth containing 5 mM HMF and incubation for 12 h at the same conditions. Then 1% culture was inoculated into fresh 1.8% nutrient broth containing 10 mM HMF and was cultivated for 12 h. Similarly, the sequential cell acclimatization in the nutrient broth containing 15 mM and 20 mM HMF was conducted. Acclimatized cells were screened by streaked plate method, where the culture acclimatized was streaked on nutrient agar containing 20 mM HMF and cultivated for 24 h at 30 °C. The isolated strain was maintained on nutrient agar.

Generally, *C. testosteroni* SC1588 cells acclimatized were pre-cultivated in 1.8% nutrient broth for 5 h at 30 °C and 160 r/min. Then, 1% culture was inoculated into fresh 1.8% nutrient broth containing 5 mM HMF. After being incubated for 8 h under the same conditions, the cells adapted to substrate were harvested by centrifugation (7000 g, 6 min, 4 °C) and washed twice with 0.85% NaCl solution.

2.3. Biocatalytic oxidation of HMF to HMFCA

Typically, 4 mL phosphate buffer (0.2 M, pH 7) containing 100 mM HMF and 30 mg/mL cells (wet weight) was incubated at 30 °C and 160 r/min. Aliquots were withdrawn from the reaction mixtures at specified time intervals and diluted with the corresponding mobile phase prior to HPLC analysis. The conversion was defined as the ratio of the consumed substrate amount to the initial substrate amount (in mol). The yield was defined as the ratio of the measured product amount to the theoretical value based on the initial amount of HMF (in mol). All the experiments were conducted at least in duplicate, and the values were expressed as the means ± standard deviations.

2.4. Whole-cell catalytic oxidation of aromatic aldehydes

Whole-cell catalytic oxidation was performed at 30 °C in a 15 mL vial containing a designated concentration of aldehydes and 70 mg/mL microbial cells (wet weight) adapted to 5 mM HMF in 2 mL phosphate buffer (0.2 M, pH 7). The reaction was stirred at 160 r/min, and aliquots (20 μL) were withdrawn at specified time intervals, and diluted with the corresponding mobile phase prior to HPLC analysis. All the experiments were conducted at least in duplicate, and the values were expressed as the means ± standard deviations.

2.5. HPLC analysis

The reaction mixtures were analyzed on a Zorbax Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm, Agilent, USA) by reversed phase HPLC equipped with a Waters 1525 pump and a 2489 UV detector (Waters, USA). Unless otherwise stated, the mobile phase was usually the mixture of acetonitrile/0.4% $(NH_4)_2SO_4$ solution with pH 3.5 (10/90, v/v) with the flow rate of 0.6 mL/min. For quantifying the oxidized products in the biotransformation of 5-methyfurfural and 5-methoxymethylfurfural, the mixtures of acetonitrile/0.02% phosphoric acid solution (40/60, v/v) with a flow rate of 0.3 mL/min and acetonitrile/0.4% $(NH_4)_2SO_4$ solution with pH 3.5 (20/80, v/v) with the flow rate of 0.8 mL/min were used, respectively. The detection wavelength and retention times of various compounds are available in Table S1.

3. Results and discussion

3.1. Effect of inducer concentrations

Substrate adaptation strategy proved to be effective for enhancing the catalytic performances of microorganisms, due to the induced expression of the relative enzymes. Indeed, we also found that rest cells cultivated in the presence of a low concentration of substrate displayed the markedly improved catalytic performances [7]. Therefore, this strategy was applied for promoting whole-cell catalytic synthesis of HMFCA (Fig. 1), where the cells were cultivated in the presence of 2.5–10 mM HMF. It was found that the substrate conversions of more than 99% were obtained within 24 h in all cases. Nevertheless, the reaction rates with the substrate-adapted cells were much higher than that with the cells free of substrate adaptation, since the substrate conversion was approximately 36% within 6 h in the control, whereas the conversions of 69%–95% were obtained within the same reaction period with the substrate-adapted cells as biocatalysts (Fig. S1a). It might be possible that the cells would produce more kinds and amounts of enzymes to detoxify HMF for their survival when they were cultivated in the presence of HMF. However, no significant differences

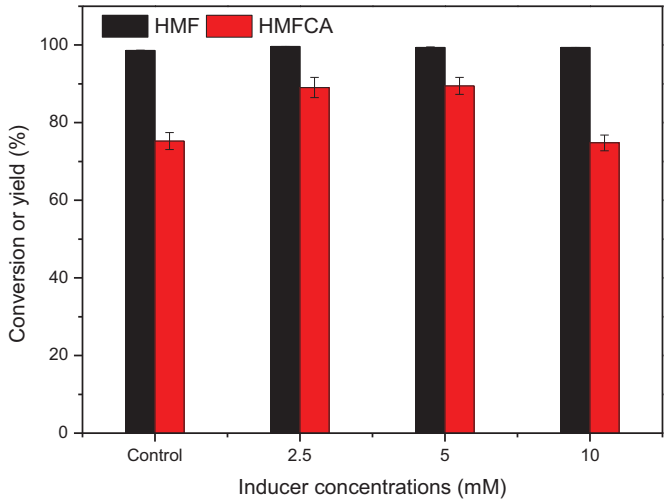

Fig. 1. Effect of inducer concentrations on catalytic performances of the cells in the oxidation of HMF. Reaction conditions: 100 mM HMF, 30 mg/mL cells (wet weight), 4 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min, 24 h.

in the band numbers as well as in the band depth were observed between the supernatant of the cells adapted to 5 mM HMF and that in the control on the basis of SDS-PAGE analysis (Fig. S1b), likely due to the low sensitivity of this technology. So other high-sensitivity technologies such as quantitative reverse transcription PCR assay may be needed to provide direct experimental evidences for this, which is in progress in our laboratory. High concentrations of the inducer HMF would result in significant cell damage (e.g. membrane, chromatin and actin damage, and reduced intracellular ATP level) [28] as well as decreased cell biomass (Fig. S1c), because of the high toxicity of HMF. It might account for the lower catalytic activity of the cells cultivated in the presence of 10 mM HMF compared to those cultivated in the presence of 2.5–5 mM HMF. These results obtained in this work are in good agreement with our recent results [29], where no improvements in the synthesis of furfuryl alcohol were observed with furfural-adapted cells as biocatalysts compared to those with the cells free of substrate adaptation, likely due to the great toxicity of furfural. In the control, the desired product HMFCA was afforded with a 75% yield after 24 h, along with approximately 25% of BHMF as the byproduct (Fig. 1). Interestingly, improved HMFCA yields (approximately 89%) were observed when the cells adapted to 2.5–5 mM HMF acted as biocatalysts. Nonetheless, the yield was around 75% in the case of 10 mM HMF (Fig. 1). Considering the toxicity of the inducer, therefore, the selection of its appropriate concentrations appeared to be critical for enhancing the catalytic performances of the cells.

3.2. Effect of substrate sources

To further enhance HMFCA synthesis, optimization of reaction temperature, pH and cell cultivation periods was conducted (Figs. S2 and S3). Unfortunately, no significant improvements in the HMFCA yields were observed. Then, the substrate tolerance of the substrate-adapted cells was evaluated (Fig. 2(a)), since high substrate concentrations are desirable for achieving satisfactory productivities in the large-scale production. Surprisingly, poor results were obtained when the concentrations of HMF obtained from J&K Scientific were more than 130 mM, evidenced by low HMFCA yields (31%–54%). It is opposite to our previous results [22]. On the contrary, the HMFCA yield remained high when the concentration of HMF from Aladdin Co. was up to 160 mM. To uncover the reason for these results, HMF of different sources was characterized. It was reported that HMF with the purity of 97%–99% was susceptible to rapid aging and decomposition upon storage at room temperature in an oil form [30], thus leading to the formation of the dimer and larger oligomers. These substances might exert negative effects on the catalytic performances of the cells. Because HMF from J&K Scientific has been stored at 4 °C for a long period in our laboratory, we reason that the abovementioned impurities may be produced. Hence, it was analyzed by ^{1}H NMR (Fig. S4), with HMF newly obtained from Aladdin as the control. It was found that the HMF purity remained high, regardless of its sources, and that no significant amounts of these impurities were observed on the basis of ^{1}H NMR spectra. It suggests that HMF appears to be stable when it is stored at low temperature. On the other hand, metals might be present in commercially available HMF, which depended on its synthetic methods. It is well known that metal ions have a significant effect on the catalytic performances of enzymes. Therefore, the contents of metals present in HMF were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (Table S2). It was found that the metal contents were extremely low in two samples. After carefully reviewing Certificate of Analysis of HMF, we found that 0.5% TBC was supplemented as the stabilizer in HMF obtained from J&K Scientific, while this stabilizer was absent in HMF from Aladdin. Indeed, the peak of CH_3 of tert-butyl in TBC appeared at 1.3 ppm (Fig. S4), thereby demonstrating the presence of this stabilizer in HMF from J&K Scientific. So the presence of TBC was assumed to exert a negative effect on biocatalytic oxidation of HMF. To verify our assumption, biocatalytic oxidation of HMF (obtained from Aladdin) was conducted in the presence of 0.5% TBC (Fig. 2(b)). As expected, a low HMFCA yield (44%) as well as an unsatisfactory HMF conversion was furnished, which is almost consistent with the results obtained in biocatalytic oxidation of 160 mM of HMF from J&K Scientific. To our knowledge, it is the first time to unveil the negative effect of TBC on biocatalysis. HMF from Aladdin was used in the subsequent studies, due to the absence of TBC.

3.3. Effect of substrate concentrations

Fig. 3 shows the effect of the substrate concentrations on biocatalytic oxidation of HMF. It was found that the desired product was obtained in a 94% yield when the substrate concentration was 150 mM (Fig. 3(a)). However, further increases in the substrate concentrations resulted in significantly reduced catalytic performances of the cells, likely due to substantially inhibitory and toxic effects of high concentrations of HMF on whole-cell biocatalysts. For example, the maximal HMFCA yield substantially decreased to approximately 56% in the case of 200 mM HMF, although the substrate was almost used up after 48 h. In addition, almost no HMF was converted when its concentration was up to 250 mM. Previously, we found that the catalytic performances of *C. testosteroni* SC1588 cells were considerably improved in the presence of histidine [22]. Therefore, the addition of histidine was carried out (Fig. 3(b)). The HMFCA yield was found to be 90% within 36 h upon the addition of histidine when the substrate concentration was 150 mM. This yield is slightly lower than the corresponding value (98%) using furfuryl alcohol-adapted whole cells [22], likely due to the lower toxicity of furfuryl alcohol than that of HMF. More importantly, the tolerant level of whole cells toward substrate was greatly improved in the presence of histidine. The HMFCA yield was remarkably improved to approximately 87% in the presence of histidine when the HMF concentration was 200 mM. Similarly, the addition of histidine led to an improved maximal yield (around 46%) as well as an increased substrate conversion (about 91%) in the case of 250 mM HMF. Nonetheless, the HMFCA yield remained extremely poor with the substrate concentration up to 300 mM (Fig. 3(b)).

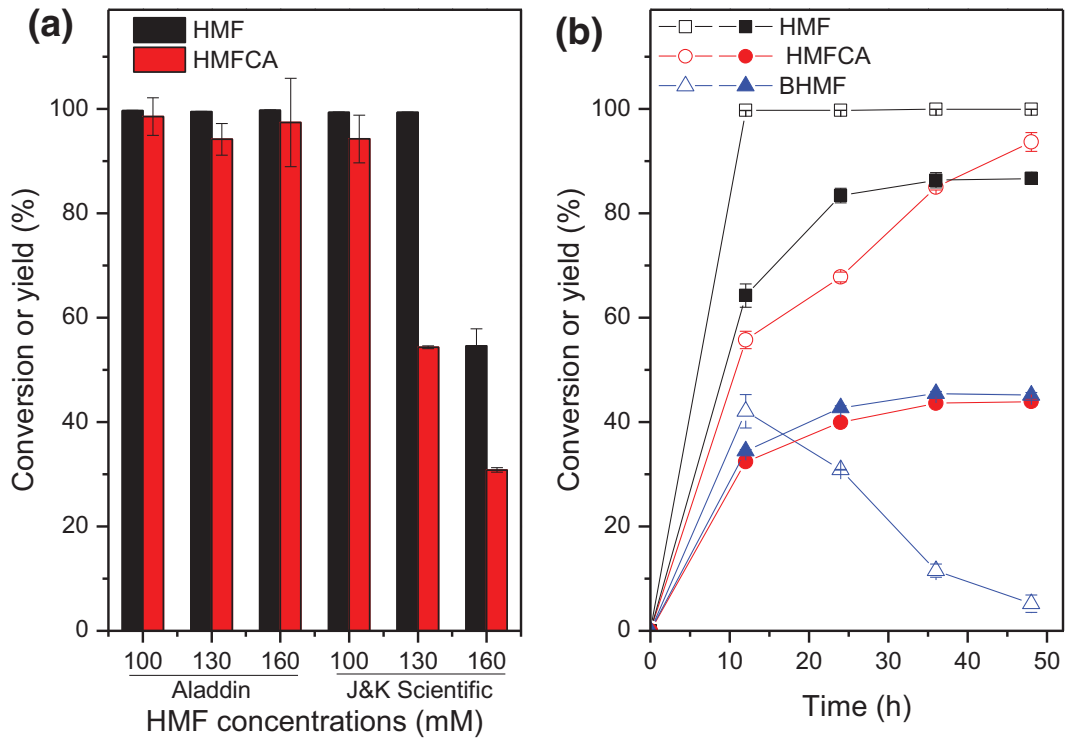

Fig. 2. Effect of substrate sources (a) and TBC (b) on biocatalytic oxidation of HMF. General conditions unless otherwise stated: 160 mM HMF, 30 mg/mL cells (wet weight), 4 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min, tuning pH of the reaction mixture to approximately 7 every 24 h. (a) 100–160 mM HMF; reaction periods are 24, 48 and 48 h with gradually increasing HMF concentrations from 100 to 160 mM, respectively; (b) the absence (open symbols) and presence (solid symbols) of 0.5% TBC.

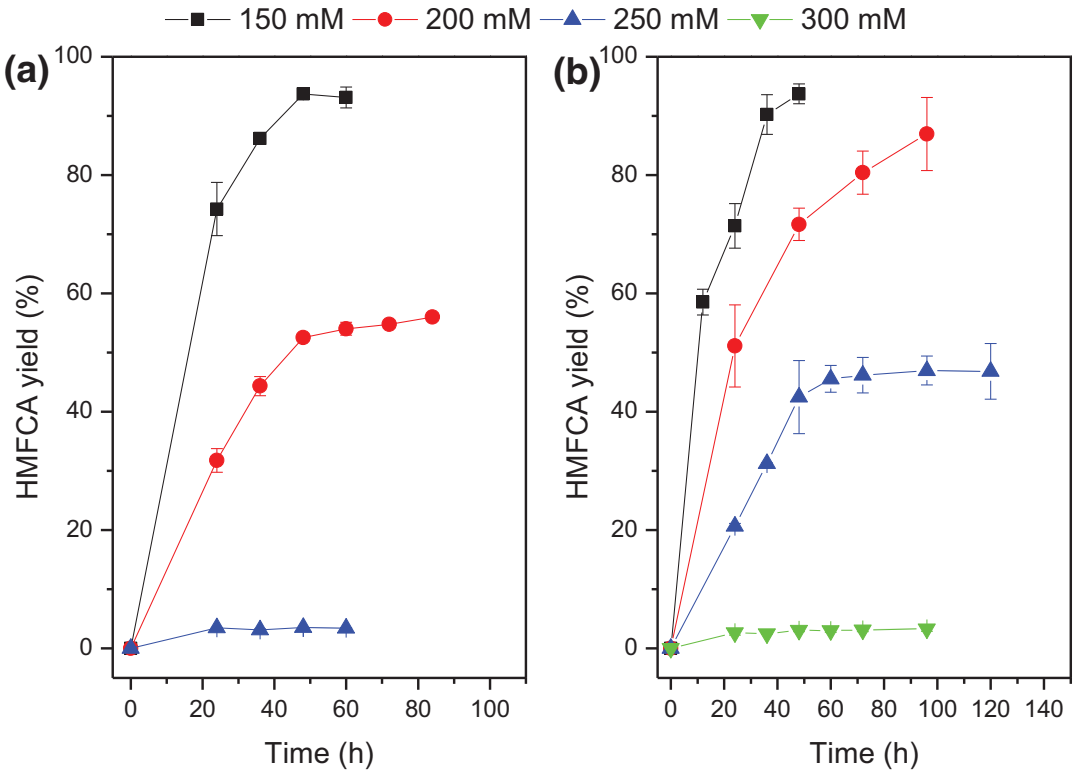

Fig. 3. Effect of substrate concentrations on biocatalytic oxidation of HMF in the absence (a) and presence (b) of histidine. Reaction conditions: 150–300 mM HMF, 30 mg/mL cells (wet weight), 20 mM histidine, 4 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min, tuning pH of the reaction mixture to approximately 7 every 24 h.

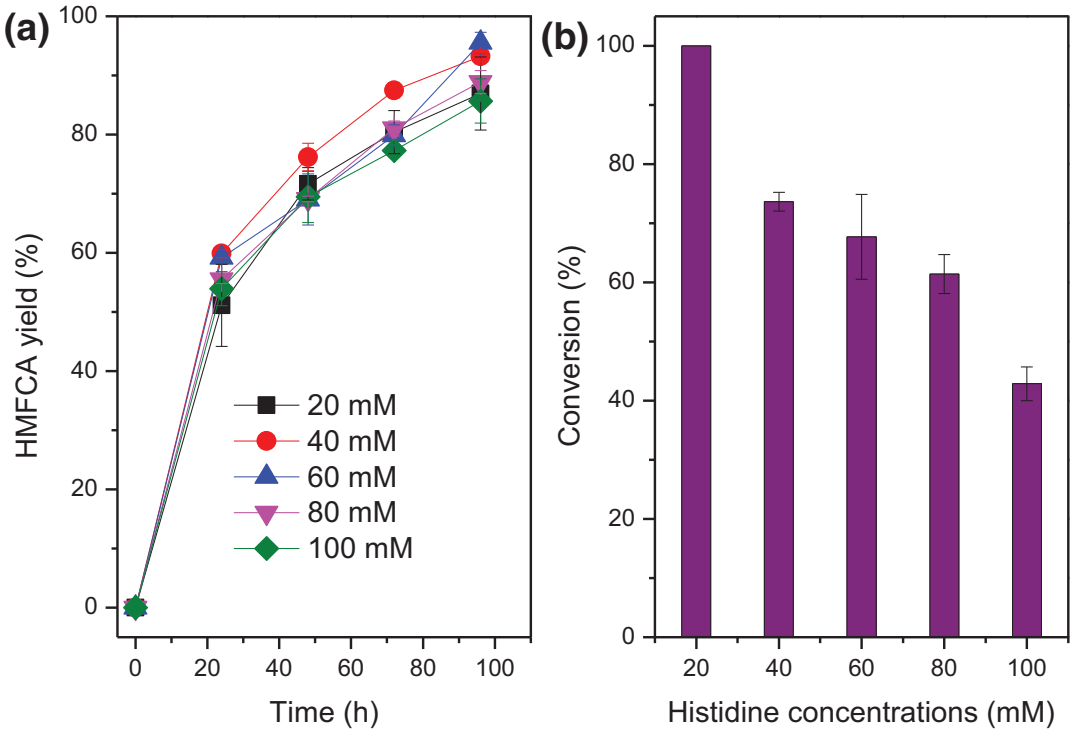

Fig. 4. Effect of histidine concentrations on biocatalytic oxidation of HMF (a) and histidine consumption during biotransformation (b). Reaction conditions: 200 mM HMF, 30 mg/mL cells (wet weight), 20–100 mM histidine, 4 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min, tuning pH of the reaction mixture to approximately 7 every 24 h.

3.4. Effect of histidine concentrations

The effect of the histidine concentrations on biocatalytic oxidation of HMF was studied (Fig. 4(a)). No significant improvements in the reaction rates as well as in the HMFCA yields were observed with the increment of the histidine concentrations. In addition, the changes in the histidine concentrations were determined upon the reaction (Fig. 4(b)). It was found that only part of histidine was consumed when its concentration was more than 40 mM. It suggests that histidine appears not to be a good carbon source for *C. testosteroni* SC1588 cells, although this microorganism was found to be able to grow on histidine [22]. Besides, the addition of excess histidine seems to be unnecessary for promoting biocatalytic synthesis of HMFCA. Furans are well known to have diverse detrimental effects on microorganisms including DNA damage, inhibition of glycolytic enzymes and dehydrogenases, disruption of cell membranes, and perturbation of redox balance [28]. Previously, we found that the cell viability was higher in the presence of histidine than that in the absence of this chemical [22]. Based on the genome sequence of *C. testosteroni* SC1588, a variety of enzymes responsible for amino acid metabolism were present in this bacterium. So, histidine might be involved in cell damage repair through its cellular metabolism, resulting in the enhanced cell stability and thus improving the substrate tolerance. Also, Ujor et al. [31] reported that glycerol supplementation enhanced in situ detoxification of furfural by *Clostridium beijerinckii* during butanol fermentation.

3.5. Effect of cell concentrations

Fig. 5(a) shows the effect of the cell concentrations on biocatalytic oxidation of HMF. It was found that the reaction rates were significantly improved with increasing the cell concentrations in the synthesis of HMFCA, resulting in the reduced reaction periods. For example, the reaction period for achieving the maximal HMFCA yield was approximately 96 h with 30 mg/mL of the cells, while being 24 h with 70 mg/mL of the cells. An almost quantitative HMFCA yield was obtained with 70 mg/mL of the cells (Fig. 5(a)). With the optimal conditions in hand, the improved synthesis of HMFCA was performed when the substrate concentrations were 250–300 mM (Fig. 5(b)). It was interestingly found that a good yield (81%) was achieved in the case of 250 mM HMF, in spite of a long reaction period. This value is much higher than that obtained in Fig. 3(b) (81% vs. 46%), suggesting that the oxidation of HMF into the desired product would be significantly enhanced with a high catalyst concentration. Also, the HMFCA yield (approximately 71%) remained moderate when the substrate concentration was up to 300 mM (Fig. 5(b)).

3.6. Biocatalytic oxidation of aromatic aldehydes

The substrate spectrum of this whole-cell biocatalyst was studied, where a variety of aromatic aldehydes including bio-based furans and benzaldehydes were examined (Table 1). 5-Methylfurfural that can be directly synthesized from carbohydrates [32] is a useful intermediate in pharmaceutical and perfume industries. This furan was found to be a good substrate for this strain, since it was completely transformed after 48 h, affording the desired product with a 92% yield (Table 1, Entry 1). 2-Furoic acid was obtained in a moderate yield (64%) when the furfural concentration was 100 mM (Entry 2), likely due to the potent toxicity and inhibition of this substrate against microbial cells. When its concentration decreased to 50 mM, indeed, it was quickly transformed into the desired product with a good conversion (100%, Entry 3). 5-Methoxymethylfurfural (MMF) is a structural analog of HMF, but the former is more stable upon storage than the latter. As shown in Table 1, Entry 4, 5-methoxymethyl-2-furancarboxylic acid was furnished with an excellent yield (98%). The reaction rate in the oxidation of MMF seemed to be much lower than that in the oxidation of HMF, which is evidenced by comparing the reaction

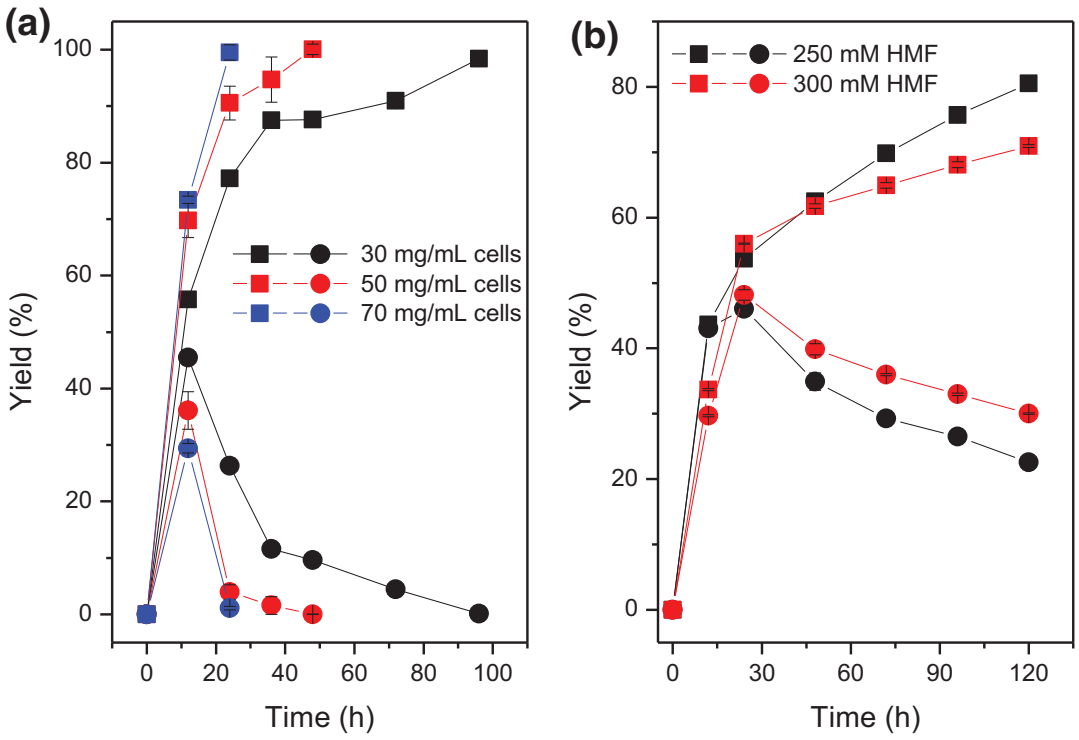

Fig. 5. Effect of cell concentrations on biocatalytic oxidation of HMF (a) and improved synthesis of HMFCA with 70 mg/mL of cells (b). Symbols: HMFCA (square), BHMF (circle). General reaction conditions unless otherwise stated: 200 mM HMF, 70 mg/mL cells (wet weight), 40 mM histidine, 4 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min, tuning pH of the reaction mixture to approximately 7 every 24 h; (a): 30–70 mg/mL cells; (b): 250–300 mM HMF.

Table 1. Whole-cell catalytic oxidation of aromatic aldehydes.

Entry	Substrates	Products	Time (h)	Conversion (%)	Yield (%)
1	5-methylfurfural	5-methyl-2-furancarboxylic acid	48	100	92 ± 0
2	Furfural	2-furoic acid	120	99 ± 1	64 ± 1
3	Furfural[a]	2-furoic acid	24	100	96 ± 2
4	MMF	5-methoxymethyl-2-furancarboxylic acid	48	100	98 ± 2
5	DFF[a]	FDCA/HMFCA	72	100	63 ± 0/29 ± 1
6	DFF[b]	FDCA/HMFCA	24	100	60 ± 1/41 ± 1
7	FFCA[c]	FDCA	24	100	97 ± 2
8	Benzaldehyde[b]	Benzoic acid	24	100	97 ± 2
9	4-fluorobenzaldehyde[b]	4-fluorobenzoic acid	24	100	97 ± 0

Reaction conditions: 70 mg/mL cells (wet weight), 100 mM substrate, 2 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min.
[a] 50 mM.
[b] 20 mM.
[c] Tuning pH to approximately 7 prior to biotransformation.

periods (48 h for transforming 100 mM MMF vs 24 h for transforming 200 mM HMF). It might be ascribed to the presence of the extra methyl group in MMF, which resulted in the steric hindrance as well as the improved hydrophobicity and toxicity. The relative studies for unveiling the underlying reasons are undergone in our laboratory. The mixtures of FDCA and HMFCA were produced with DFF as the substrate (Entries 5 and 6), in which FDCA was the major product (60%–63%). FFCA proved to be a suitable substrate (Entry 7), which was rapidly oxidized into FDCA with a 97% yield within 24 h. Besides, benzaldehydes were tested with a low concentration (20 mM), due to their limited aqueous solubility (Entries 8 and 9). It was found that the desired products were obtained in high yields (97%) within 24 h. We also attempted to evaluate the catalytic performances of whole-cell biocatalyst in the oxidation of benzaldehydes of a high concentration (50 mM) under the assistance of 5% dimethyl sulphoxide (DMSO). Unfortunately, no biotransformations occurred in the presence of 5% DMSO, indicating that this strain was completed inactivated by DMSO.

4. Conclusions

In summary, an efficient biocatalytic approach to furan carboxylic acids has been established using substrate-adapted whole cells of *C. testosteroni* SC1588 in this work. The substrate adaptation strategy proved to be simple and effective for improving the catalytic performances (e.g. substrate tolerance and catalytic efficiency) of the cells in the oxidation of HMF. In addition, biocatalytic synthesis of HMFCA was significantly promoted in the presence of histidine. The stabilizer 4-tert-butylcatechol (TBC) present in HMF had a significantly detrimental effect on whole-cell catalytic synthesis of HMFCA, especially at high substrate concentrations. The desired product HMFCA was furnished with a quantitative yield when the HMF concentration was 200 mM. Increasing HMF concentrations (250–300 mM) resulted in moderate product yields (71%–81%). Most of bio-based furans tested were transformed into the target carboxylic acids with good yields by this whole-cell biocatalyst. Identification and overexpression of the enzymes responsible for HMF oxidation in *Escherichia coli* [33] as

well as reaction engineering strategies (e.g. cell immobilization and in situ product removal) [34] may be promising routes for improving the HMFCA synthesis at higher substrate concentrations.

Acknowledgments

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Declarations of interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jechem.2019.04.025.

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