Molecular Catalysis 469 (2019) 68–74

Contents lists available at ScienceDirect

Molecular Catalysis

journal homepage: www.elsevier.com/locate/mcat

Selective synthesis of 2-furoic acid and 5-hydroxymethyl-2-furancarboxylic acid from bio-based furans by recombinant *Escherichia coli* cells

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ARTICLE INFO

Keywords:
Aldehyde dehydrogenases
Biocatalysis
Furans
Platform chemicals
Selective oxidation

ABSTRACT

Upgradation of bio-based furans into chemicals and biofuels has received great interest recently. In this work, we reported selective synthesis of furan carboxylic acids from the corresponding aldehydes by recombinant *Escherichia coli* cells expressing 3-succinoylsemialdehyde-pyridine dehydrogenase (SAPDH) from *Comamonas testosteroni* SC1588. The effects of induction and reaction conditions on whole-cell catalytic oxidation of furfural (FF) were studied. High temperature induction resulted in decreased activities of recombinant cells, likely due to improper protein folding. Nonetheless, recombinant cells induced under high temperature enable the byproduct furfuryl alcohol to be faster re-oxidized into 2-furoic acid (FCA) than those induced under low temperature. So the yield and selectivity of FCA were improved significantly by using high temperature induction, at expense of slightly longer reaction periods. The activities of recombinant cells highly depended on pH. The tolerant levels of this recombinant strain toward FF and 5-hydroxymethylfurfural (HMF) were approximately 100 mM. FCA and 5-hydroxymethyl-2-furancarboxylic acid (HMFCA) were obtained with the yields of 95–98%. FCA of up to 147 mM was produced by a fed-batch strategy, in a quantitative yield. In addition, most aromatic aldehydes tested were transformed into the target carboxylic acids by this biocatalytic method, with the yields up to 100%.

1. Introduction

Furfural (FF) and 5-hydroxymethylfurfural (HMF) that can be synthesized via carbohydrate dehydration are top value-added platform chemicals derived from biomass [1]. Recently, upgrading of these furans into commercially interesting chemicals and biofuels has attracted considerable interest [2–6]. 2-Furoic acid (2-furancarboxylic acid, FCA) and 5-hydroxymethyl-2-furancarboxylic acid (HMFCA) are the oxidation products of FF and HMF, respectively. FCA is an important fine chemical, which has wide applications in pharmaceutical, agrochemical, flavor, and fragrance industries [2]. Presently, FCA is industrially produced from FF via Cannizzaro reaction in the presence of NaOH [2]. However, an equal amount of the byproduct furfuryl alcohol forms simultaneously, resulting in the theoretical selectivity of 50%. HMFCA is a promising building block in polymer industry, since it can not only be directly used for manufacturing polyesters [7], but also is a key starting material for the synthesis of renewable terephthalic acid (TPA) [8,9], a monomer in the production of polyethylene terephthalate (PET) that is widely used in the industrial production of synthetic fibers and plastic bottles. In addition, HMFCA is a synthon in the synthesis of an interleukin inhibitor [10]. It was reported that this chemical showed an antitumor activity [11]. Chemical methods are still playing a predominant role in the synthesis of HMFCA and FCA nowadays [2,12].

It is well known that furans are susceptible to polymerization and decomposition under harsh conditions (e.g., high temperature, and the presence of acids), thus resulting in the formation of the byproducts [4,13]. And even rapid aging and decomposition of HMF were observed when storing under room temperature [14]. Hence, developing practical routes remains challenging for selective and efficient conversion of furans into the desired products. Biocatalysis may open up novel possibilities for upgradation of this kind of inherently unstable chemicals [15,16], because biotransformations are generally performed under mild reaction conditions using environmentally friendly biocatalysts, and are exquisitely selective. However, both FF and HMF have a strongly inhibitory and toxic effect on biocatalysts [17,18], which may be one of the biggest barriers for the development of practical biocatalytic processes. Also, it may account for the fact that there are limited studies on biocatalytic upgrading of FF and HMF compared to chemical processes. Krystof et al. reported a chemo-enzymatic route for the synthesis of FCA and HMFCA [19], in which peracids produced *in situ* were capable of selectively oxidizing the formyl group in furans; acids

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https://doi.org/10.1016/j.mcat.2019.03.006
Received 14 December 2018; Received in revised form 28 February 2019; Accepted 4 March 2019

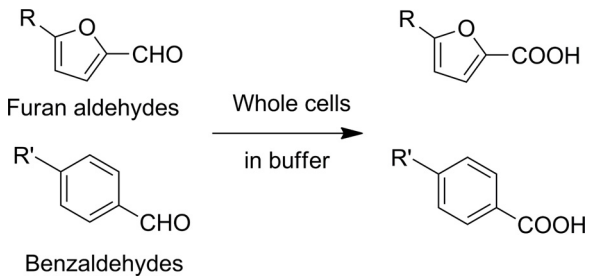
were obtained in the yields of 80–91%. HMF was quickly oxidized into HMFCA with a yield of 94% by using xanthine oxidase [20]. Baeyer-Villiger monooxygenases were also found to enable FF and HMF to be oxidized into the corresponding acids; the conversions were approximately 60–85% [21]. Recently, our group exploited an alcohol dehydrogenase coupled with an efficient $NAD(P)^+$ *in situ* regeneration system for selective synthesis of FCA and HMFCA [22]. In addition to enzymes, whole cells were also used as catalysts for selective oxidation of furan aldehydes into carboxylic acids [23–27]. We recently isolated a HMF-tolerant strain *Comamonas testosteroni* SC1588 for the synthesis of HMFCA; the desired product was afforded in a yield of approximately 98% when the substrate concentration was 160 mM [28].

Aldehyde dehydrogenases (ALDHs) represent the natural catalysts for the oxidation of aldehydes in cells. However, previous examples of synthetic applications of this type of enzymes were scarce [29]. Their biotransformation applications are not starting until very recently [25,30,31]. 3-Succinoylsemialdehyde-pyridine dehydrogenase (SAPDH) is a critical enzyme involved in the catabolism of nicotine, which catalyzes the oxidation of 3-succinoylsemialdehyde-pyridine into 3-succinoyl-pyridine [32]. Wang et al. constructed a whole-cell biocatalyst harboring a SAPDH for the synthesis of 3-succinoyl-pyridine, a valuable feedstock in the production of hypotensive agents [33]. In this work, we broadened the catalytic application of SAPDH to the oxidation of aromatic aldehydes (Scheme 1). A SAPDH responsible for HMF oxidation was identified from *C. testosteroni* SC1588, and heterologously expressed in *Escherichia coli*. Whole-cell catalytic oxidation of FF into FCA was performed using recombinant *E. coli*_CtSAPDH. Effect of induction conditions on the catalytic performances of recombinant cells in FF oxidation was studied. In addition, the biocatalytic process was optimized to obtain a good yield. A fed-batch strategy was applied for accumulating the desired product of a high concentration in the reaction mixture. Besides, this biocatalytic approach was exploited for selective oxidation of other aldehydes into the target carboxylic acids.

2. Experimental

2.1. Materials

FF (99%), 2,5-bis(hydroxymethyl)furan (BHMF, 98%) and benzaldehyde (98.5%) were obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). HMF (98%), 5-formylfuran-2-furancarboxylic acid (FFCA, 98%) and 2,5-furandicarboxylic acid (FDCA, 97%) were purchased from J&K Scientific Ltd. (Guangzhou, China). HMFCA (98%) and 5-methoxymethylfurfural (MMF, 97%) were bought from Adamas Reagent Ltd. (Shanghai, China). Furfuryl alcohol (98%), FCA (98%), 5-methylfurfural (97%), 4-fluorobenzaldehyde (98%), 4-fluorobenzoic acid (98%), 4-fluorobenzyl alcohol (98%), 4-chlorobenzaldehyde (97%), 4-chlorobenzoic acid (99%), 4-chlorobenzyl alcohol (99%), 4-bromobenzaldehyde (97%), 4-bromobenzoic acid (98%), 4-bromobenzyl alcohol (99%) and 2,5-diformylfuran (DFF, > 98%) were bought from TCI (Japan). 5-Methyfurfuryl alcohol (98%) was purchased from Apollo Scientific Ltd. (UK). 5-Methoxymethyl-2-furancarboxylic acid (99%) was bought from ThermoFisher Scientific (Beijing, China). 5-Methyl-2-furancarboxylic acid (97%) was obtained from Sigma-Aldrich (USA). Benzyl alcohol (99%) was purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Benzoic acid (99.5%) was obtained from Damao Chemical Reagent Ltd. (Tianjin, China). The gene sequence of CtSAPDH, and its cloning and expression in *E. coli* are available in supplementary material.



Scheme 1. Whole-cell catalytic oxidation of aromatic aldehydes into carboxylic acids.

2.2. Cultivation of E. coli_CtSAPDH

The medium for cell cultivation was Luria Bertani (LB) medium containing 10 g/L tryptone, 10 g/L NaCl, and 5 g/L yeast extract (pH 7.2), which was supplemented with 50 mg/mL kanamycin. *E. coli*_CtSAPDH cells were pre-cultivated at 37 °C and 180 r/min for 12 h in 50 mL LB medium. Then, 1% seed culture was inoculated into the fresh LB medium containing 50 mg/L kanamycin and incubated at 37 °C and 180 r/min. When the optical density of the culture at 600 nm reached 0.6-0.8, isopropyl β-D-thiogalactoside (IPTG) as inducer was added to the medium at a final concentration of 0.05 mM, and cultivation was performed at 30 °C and 160 r/min for 20 h. The cells were harvested by centrifugation (8000 r/min, 5 min, 4 °C) and washed twice with 0.85% NaCl solution, followed by re-suspension in phosphate buffer (0.2 M, pH 7) to give a cell concentration of 50 mg (cell wet weight) per mL.

2.3. General procedure for biocatalytic oxidation of FF

Typically, 4 mL of phosphate buffer (0.2 M, pH 7) containing 50 mM FF and 50 mg (cell wet weight) per mL microbial cells 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 formed product amount to the theoretical value based on the initial substrate amount (in mol). The selectivity was defined as the ratio of the formed product amount to the total amount of all products (in mol). All the experiments were conducted at least in duplicate, and the values were expressed as the means ± standard deviations.

2.4. Synthesis of FCA by fed-batch feeding of substrate

The reaction mixture containing 4 mL phosphate buffer (0.2 M, pH 7), 50 mM FF, and 50 mg (cell wet weight)/mL microbial cells was incubated at 30 °C and 160 r/min. When FF was almost used up, FF of approximately 0.2 mmol was repeatedly supplemented into the reaction mixture. Two strategies were applied for controlling pH of reaction mixtures: one was to add $CaCO_3$ (about 2 mol) to reaction mixture at the beginning, and the other was to add $NaHCO_3$ (approximately 0.2 mol) when supplementing FF. The changes in the concentrations of various compounds were monitored by HPLC.

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 using a reversed phase HPLC equipped with a Waters 996 photodiode array detector (Waters, USA). For the analysis of the reaction mixtures of FF biotransformation, the mobile phase was a mixture of acetonitrile/0.4% $(NH_4)_2SO_4$ solution with pH 3.5 (10: 90, v/v) at a flow rate of 0.6 mL/min. The retention times of FCA (maximum absorption wavelength of 245 nm), furfuryl alcohol (216 nm) and FF (278 nm) were 9.2, 14.3 and 16.3 min, respectively. The analytic methods of other compounds and their retention times are available in supplementary material.

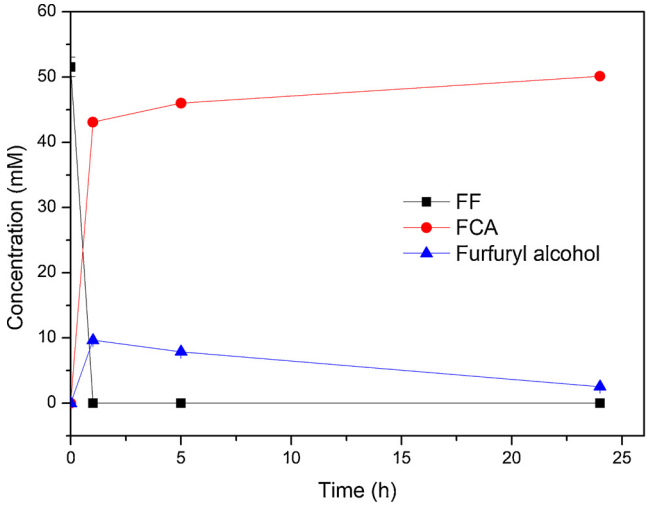


Fig. 1. Progress curve of biocatalytic oxidation of FF. Reaction conditions: approximately 50 mM FF, 50 mg/mL (wet weight) microbial cells, 1 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min.

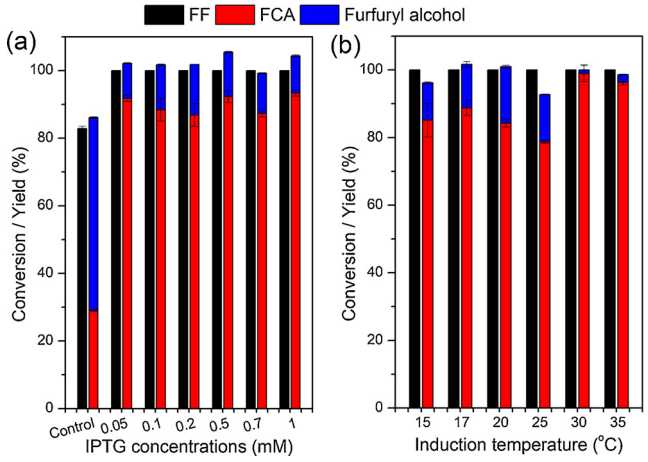


Fig. 2. Effect of IPTG concentrations (a) and induction temperature (b) on selective oxidation of FF. General reaction conditions: 50 mM FF, 50 mg/mL (wet weight) microbial cells, 1 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min, 10 h; (a): The cells induced in the presence of IPTG of 0.05–1 mM at 17 °C for 20 h; (b): The cells induced in the presence of IPTG of 0.05 mM at 15–35 °C for 20 h.

3. Results and discussion

3.1. Time course of whole-cell catalytic oxidation of FF

Fig. 1 shows the progress curve of biocatalytic oxidation of FF with recombinant *E. coli* expressing CtSAPDH. It was found that recombinant *E. coli*_CtSAPDH was capable of efficient transformation of the toxic substrate FF. The substrate was completely converted within 1 h. On the contrary, FF of only 7% was transformed when *E. coli* harboring plain vector was used as biocatalyst (data not shown). It suggests that CtSAPDH heterologously expressed in *E. coli* contributes the transformation of FF. The desired product FCA of approximately 43 mM (the yield of 84%) was produced within 1 h (Fig. 1). In addition, furfuryl alcohol was simultaneously produced as the byproduct, which might stem from the catalytic behaviors of reductases/dehydrogenases inherently present in host cells. Then, furfuryl alcohol was re-oxidized slowly into FCA, which is the limited step in the synthesis of the desired product. It is highly consistent with a previous report [28]. The desired product was furnished in a good yield of 97% after the reaction of 24 h.

3.2. Effect of induction conditions

Induction is crucial for the biosynthesis and folding of heterologous proteins in recombinant cells, so it may affect the catalytic performances of recombinant cells. The influence of induction conditions on whole-cell catalytic oxidation of FF was studied (Fig. 2 and Fig. S1). Fig. 2a shows the effect of the concentrations of IPTG, an inducer of the lactose operon, on the catalytic performances of *E. coli* cells. Compared to that in the control, the biocatalytic synthesis of FCA was significantly improved upon IPTG induction (Fig. 2a), due to the induced expression of active CtSAPDH (Fig. S2b). However, the concentrations of IPTG had no significant impact on the catalytic performances of *E. coli* cells within the concentration range tested. Comparable substrate conversions as well as similar product yields were observed in all cases (Fig. 2a).

Interestingly, induction temperature was found to exert a great effect on the oxidation of FF (Fig. 2b). Microbial cells induced at high temperature (30 and 35 °C) provided excellent FCA yields (96–99%), while the FCA yields (79–89%) were lower in other cases. To uncover the underlying reasons, time courses of FF oxidation catalyzed by whole cells induced under different temperature were monitored (Fig. S3). It was found that recombinant cells induced under 15–20 °C were capable of completely transforming FF in 0.5 h, whereas longer reaction periods (1–5 h) were required to achieve 100% substrate conversions for the cells induced under 25–35 °C. In addition, a clear trend was observed: the transformation rates of FF decreased substantially with increasing induction temperature from 25 to 35 °C (Fig. S3a). Although the biosynthesis of heterologous proteins might accelerate at high induction temperature, improper protein folding occurred easily and thus a large amount of inactive inclusion body formed [34,35], resulting in the decreased apparent activities. On the basis of SDS-PAGE analysis (Fig. S2a), the formation of more inclusion body was indeed found under higher induction temperature (e.g., 30–35 °C) compared to that under lower induction temperature. In contrast, the concentrations of soluble SAPDH appeared to be lower under higher induction temperature than those under lower induction temperature (Fig. S2b). Also, it might be verified by the fact that the FCA yields at 0.5 h reduced with increasing induction temperature (15–20 °C, 59–61%; 25 °C, 44%, 30–35 °C, 36-31%, Fig. S3b).

Besides, the induction temperature appeared to have an effect on the expression of reductases/dehydrogenases present in host cells. Its effect on the apparent reduction activities of recombinant cells had the same trend as that on the apparent oxidation activities (Fig. S3b and c). For example, the yields of furfuryl alcohol were 35–41% at 0.5 h with the cells induced under 15–20 °C, whereas being 27%, 20% and 9% with those induced under 25, 30 and 35 °C, respectively (Fig. S3c). More interestingly, it was found that furfuryl alcohol formed was re-oxidized faster by the cells induced under higher temperature (30–35 °C) than by those induced under 15–25 °C (Fig. S3c). It indicates that induction temperature may also affect the expression of enzymes responsible for the re-oxidation of furfuryl alcohol. To verify our assumption, direct oxidation of furfuryl alcohol was conducted with recombinant cells induced under various temperature (Fig. 3). As shown in Fig. 3, the cells induced under 25–35 °C indeed exhibited much higher catalytic activities than those induced under 17–20 °C. In addition to inducer concentrations and induction temperature, induction time is also one of parameters affecting the enzyme expression. So the effect of induction periods on the oxidation of FF was investigated when induction periods varied from 12 to 28 h (Fig. S1). It was observed that the cells induced for different periods showed similar catalytic performances in the oxidation of FF. FCA was produced in the yields of 93–95%, along with trace furfuryl alcohol (< 2%). It suggests that induction periods have no significant influence on the catalytic performances of recombinant cells.

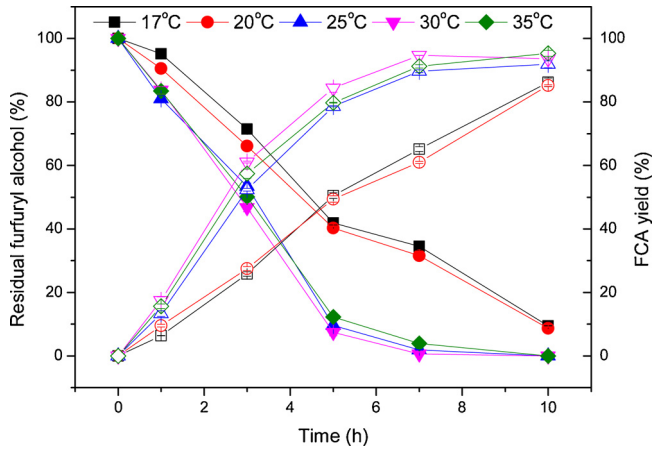


Fig. 3. Effect of induction temperature on the oxidation of furfuryl alcohol (solid symbols) into FCA (open symbols). Reaction conditions: 20 mM furfuryl alcohol, 50 mg/mL (wet weight) microbial cells, 2 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min.

3.3. Effect of pH and temperature

To obtain a good yield, the reaction conditions including reaction temperature and pH were optimized (Fig. 4). Fig. 4a shows the effect of reaction temperature on biocatalytic oxidation of FF. The *E. coli*_CtSAPDH cells displayed comparable activities at 20–35 °C, since the substrate was completely converted within 5 h (data not shown). However, the substrate conversion was approximately 73% within the same period when the reaction was performed at 40 °C, despite 95% conversion in 10 h. It indicates that the reaction temperature as high as 40 °C is unfavorable for transformation of FF by this biocatalyst, likely due to partial thermal inactivation of enzymes. To identify whether cell lysis occurred at high temperature, the cell viability was determined after incubation of 6 h at different temperature in the absence and presence of FF (Fig. S4). No significant decreases in the cell viability were observed after 6 h, regardless of whether the substrate is present or not as well as of temperature. So cell lysis was not a major cause for the low activity of whole-cell biocatalyst at 40 °C. As shown in Fig. 4a, the cells displayed the best catalytic performance at 30 °C. The desired product FCA was achieved in the yield of 95%. More importantly, its selectivity was up to 100%, since the byproduct furfuryl alcohol was completely re-oxidized into FCA in 10 h.

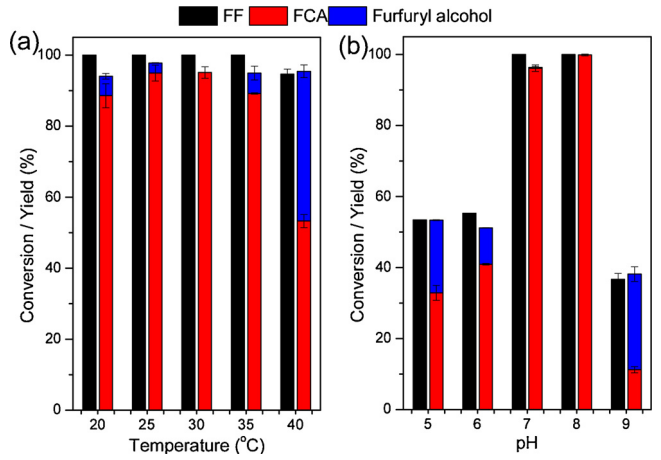


Fig. 4. Effect of reaction temperature (a) and pH (b) on selective oxidation of FF. General reaction conditions: 50 mM FF, 50 mg/mL (wet weight) microbial cells, 2 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min, 10 h; (a): 20–40 °C; (b): phosphate buffer (0.2 M, pH 5–8), and Tris – HCl buffer (0.2 M, pH 9).

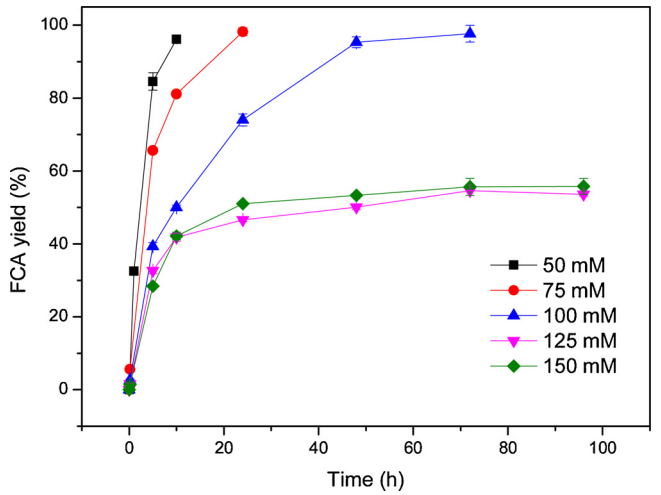


Fig. 5. Effect of substrate concentrations on the synthesis of FCA. Reaction conditions: FF of a designated concentration, 50 mg/mL (wet weight) microbial cells, 2 mmol $CaCO_3$, 4 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min.

As shown in Fig. 4b, pH exerted a significant effect on biocatalytic oxidation of FF. Recombinant cells displayed good catalytic activities only within a narrow pH range (pH 7–8). Similarly, wild-type *C. testosteroni* SC1588 that contains SAPDH also showed narrow pH tolerance [28]. The substrate conversions were 53–55% at pH 5 and 6, while being 100% at pH 7 and 8. In addition, significant effect of pH on the reaction selectivities was observed. FCA selectivities (62–80%) were unsatisfactory at pH 5–6, while absolute selectivities (100%) were achieved at pH 7-8. And even, furfuryl alcohol rather than FCA was produced as the major product at pH 9.

3.4. Effect of substrate concentrations

Good substrate tolerance of biocatalysts is of great importance for their synthetic applications, since high substrate concentrations are highly desirable for achieving satisfactory productivities in large-scale production. With the optimal reaction conditions in hand, therefore, the substrate tolerance of this recombinant strain was evaluated (Fig. 5). It was found that FF could be smoothly transformed into the desired product by this biocatalyst when the substrate concentrations were less than 100 mM. The substrate conversions were up to 100%; FCA was obtained in excellent yields (96–98%) and absolute selectivities (100%). However, the substrate conversions decreased to approximately 74% when the FF concentrations were more than 125 mM (data not shown). FCA yields were around 55%. These results suggested that recombinant *E. coli*_CtSAPDH was able to tolerate FF of up to 100 mM under the tested reaction conditions. This substrate concentration is higher than those used in most of the existing biocatalytic processes for the synthesis of FCA from FF [19,21,24,26,36].

3.5. Synthesis of FCA by a fed-batch strategy

Accumulation of high concentrations of product in the reaction mixture facilitates its purification. Nevertheless, direct production of the desired products is usually hard to be achieved from high concentrations of such substrates as FF and HMF, due to their significant toxicity and inhibition against biocatalysts [17,18]. Although the recombinant strain was demonstrated to tolerate FF of up to 100 mM (Fig. 5), the reaction rate was unsatisfactory and a long reaction period was required for achieving a good yield. Considering the substrate toxicity and inhibitory effect, a fed-batch strategy in which approximately 50 mM FF was fed was applied for accumulating a high concentration of FCA in the mixture (Fig. 6). The product FCA is a carboxylic acid. So pH of the reaction mixtures will decrease with its

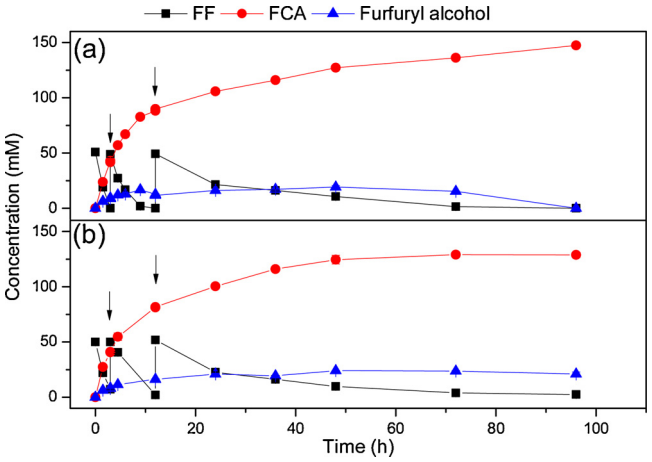


Fig. 6. Continuous synthesis of FCA in the presence of $CaCO_3$ (a) and by adding $NaHCO_3$ (b). General reaction conditions: 50 mM FF, 50 mg/mL (wet weight) microbial cells, 4 mL phosphate buffer (0.2 M, pH 7), 30 °C, 160 r/min. (a) in the presence of 2 mmol $CaCO_3$; (b) supplementing $NaHCO_3$ of 0.2 mmol when feeding FF. Arrows indicate the feed of approximately 0.2 mmol FF.

gradual accumulation, which may exert a negative effect on the activity of biocatalyst. Indeed, it was found that the final FCA concentration of only 65 mM was achieved without the addition of bases (Fig. S5). So two weak bases including $CaCO_3$ and $NaHCO_3$ were supplemented to control pH of the reaction mixtures. Insoluble $CaCO_3$ was added at the beginning, while $NaHCO_3$ was simultaneously added with FF feed. Their effects on biocatalytic oxidation of FF were compared. As shown in Fig. 6, the substrate transformation became much slower with the increment of FF feed times, indicating that FF may cause cell damage. However, the toxic and inhibitory effect of substrate on biocatalyst was significantly relieved by using a fed-batch strategy, which may be evidenced by the reduced reaction period in biotransformation of 100 mM FF (12 h vs 48 h without FF feed in Fig. 5). As shown in Fig. 6a, FCA was obtained within 96 h in a quantitative yield (100%) in the presence of $CaCO_3$. The final concentration of FCA (129 mM) was slightly lower with the addition of $NaHCO_3$ than that with adding $CaCO_3$ (147 mM). In addition, furfuryl alcohol produced (around 24 mM) could not be re-oxidized into FCA (Fig. 6b). It indicates that insoluble $CaCO_3$ seems to be advantageous over $NaHCO_3$ for the synthesis of FCA, because the former is able to adjust pH instantaneously, thus reducing the negative effect of pH change on biocatalyst.

3.6. Biocatalytic oxidation of aromatic aldehydes

To uncover the substrate spectrum of CtSAPDH, whole-cell catalytic oxidation of various aromatic aldehydes was performed (Table 1). HMF was a good substrate for *E. coli*_CtSAPDH cells. A high yield of HMFCA (95%) was obtained within 5 h when the substrate concentration was 50 mM (Table 1, entry 1). However, the reaction became very slow when its concentration increased to 100 mM (Entry 2), although the yield remained high. With DFF as substrate, FDCA, a promising bio-based alternative to TPA [37], was produced as the major product (Entry 3), together with FFCA. However, the substrate conversion (less than 20%) was pretty poor when the concentration of DFF increased to 50 mM (data not shown), due to great toxicity and inhibition of this furan dialdehyde against whole-cell biocatalyst. FFCA was quickly oxidized into FDCA with an excellent conversion (Entry 4). Also, the desired product 5-methyl-2-furancarboxylic acid was obtained in a quantitative yield when 5-methylfurfural acted as substrate (Entry 5). MMF obtained via carbohydrate dehydration in the presence of methanol is an analog of HMF [38,39], but the former has much better storage stability than the latter. Recently, Carro et al. reported a self-sustained enzyme cascade for the production of FDCA from this chemical [40]. As shown in Table 1, MMF was found to be an appropriate substrate (Entry 6); 5-methoxymethyl-2-furancarboxylic acid, a feedstock for the synthesis of bio-based TPA [9], was afforded in a 95% yield. Compared to the wild type strain [28], recombinant cells exhibited much higher catalytic activities in the oxidation of furans including FF, DFF and 5-methylfurfural, which may be clearly verified by the considerably reduced reaction periods. These results may highlight the superiority of recombinant cells as catalysts to wild-type cells for biotransformation of these furans. As shown in Table 1, both benzaldehyde and its fluorinated derivative were transformed into the target acids with good yields (90–97%, entries 7 and 8). 4-Chlorobenzaldehyde and 4-bromobenzaldehyde were tested with low concentrations (Entries 9–11), because of their poor aqueous solubility. 4-Chlorobenzoic acid was obtained with a 100% yield when the substrate concentration was 10 mM (Entry 9). When the substrate concentration was increased to 20 mM under the assistance of dimethyl sulphoxide (DMSO), unsatisfactory results were obtained (Entries 10 and 11), which may be partially attributed to the deleterious effect of DMSO on the cells. To evaluate whether SAPDH has a chiral recognition ability, kinetic resolution of racemic 2-phenylpropionaldehyde was performed by using this recombinant strain. Unfortunately, (*R*)-acid was furnished with an enantiomeric excess (e.e.) value of approximately 13% (data not shown). Similarly, ALDHs of other sources displayed poor enantioselectivities [25].

4. Conclusions

A biocatalytic approach was successfully established for selective oxidation of a series of aromatic aldehydes into the corresponding carboxylic acids by using recombinant *E. coli* cells harboring a SAPDH from *C. testosteroni* SC1588. This recombinant strain exhibited satisfactory tolerance toward FF and HMF (up to 100 mM), well-known potent inhibitors against microorganisms. Of induction conditions examined, induction temperature was the most significant factor affecting the catalytic performances of recombinant cells. This recombinant strain showed good catalytic activities in a narrow pH range (pH 7–8). So pH control appeared to be of great importance in biocatalytic synthesis of carboxylic acids using this strain. FCA of up to 147 mM was synthesized in a fed-batch process, in a quantitative yield. In addition, this biocatalyst had a broad substrate spectrum, and was capable of efficiently transforming a variety of furan and benzyl aldehydes into the target carboxylic acids with good yields. However, this ALDH has no chiral recognition ability in kinetic resolution of a racemic aldehyde. In spite of good catalytic performances, some problems associated with this recombinant biocatalyst have to be addressed in the future. For example, the long-term stability should be enhanced significantly, which may be realized by reaction engineering strategies (e.g., cell immobilization and medium engineering) and protein engineering [41]. The improved substrate tolerance is highly desired for moving this green technology forward to the industrial applications.

Declarations of interest

none.

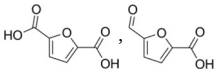
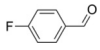
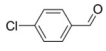
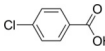
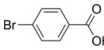
Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (21676103), the Natural Science Foundation of Guangdong Province (2017A030313056), and the Science and Technology Project of Guangzhou City (201804010179).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mcat.2019.03.006.

Table 1
Oxidation of aldehydes into acids using *E. coli*_CtSAPDH cells.

Entry	Substrate	Product	Concentration (mM)	Time (h)	Conversion (%)	Yield (%)
1			50	5	100	95 ± 1
2			100[a]	48	100	95 ± 2
3			20	12	100	77 ± 1/ 32 ± 1
4			50	12	100	100
5			50[a]	5	100	100
6			50	5	100	95
7			50[b]	24	100	97 ± 1
8			50[b]	24	100	90 ± 0
9			10	24	100	100
10			20[c]	24	83 ± 0	59 ± 1
11			20[d]	24	61 ± 0	47 ± 0

Reaction conditions: aldehyde of a designated concentration, 50 mg/mL (wet weight) microbial cells, 4 mL phosphate buffer (0.2 M, pH7.0), 30 °C, 160 r/min.
[a] addition of 500 mM $CaCO_3$.
[b] addition of 5% DMSO.
[c] addition of 15% DMSO.
[d] addition of 20% DMSO.

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