Bioresource Technology 262 (2018) 177–183

Contents lists available at ScienceDirect

Bioresource Technology

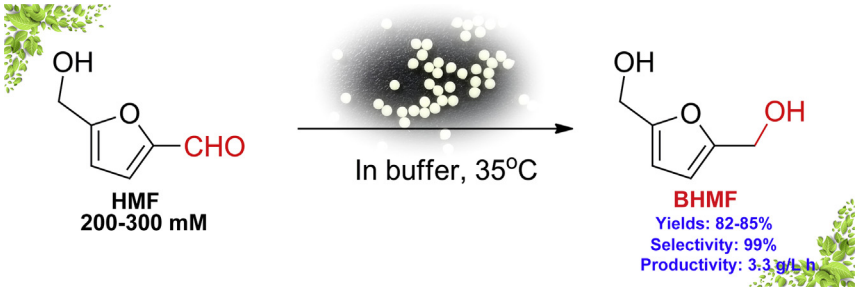
journal homepage: www.elsevier.com/locate/biortech

Improved synthesis of 2,5-bis(hydroxymethyl)furan from 5-hydroxymethylfurfural using acclimatized whole cells entrapped in calcium alginate

Zhong-Hua Xu[a], Ai-Di Cheng[a], Xu-Pu Xing[a], Min-Hua Zong[a], Yun-Peng Bai[b], Ning Li[a,*]

[a] School of Food Science and Engineering, South China University of Technology, 381 Wushan Road, Guangzhou 510640, China
[b] State Key Laboratory of Bioreactor Engineering, Shanghai Collaborative Innovation Center for Biomanufacturing, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Biocatalysis
Biobased platform chemicals
Immobilization
Reduction
Whole cells

ABSTRACT

Upgrading of biomass-derived 5-hydroxymethylfurfural (HMF) has attracted considerable interest recently. In this work, efficient synthesis of 2,5-bis(hydroxymethyl)furan (BHMF) from HMF was reported with the acclimatized *Meyerozyma guilliermondii* SC1103 cells entrapped in calcium alginate beads. Catalytic activities of the cells as well as their HMF-tolerant level increased significantly upon acclimatization and immobilization. BHMF was obtained within 7–24 h with good yields (82–85%) and excellent selectivities (99%) when the substrate concentrations were 200–300 mM. In scale-up synthesis, BHMF of up to 181 mM was produced within 7 h, and its productivity was approximately 3.3 g/L h. In addition, the immobilized biocatalyst showed satisfactory operational stability; the cell viability of 70% was retained after reuse 4 times. With rice straw hydrolysate as co-substrate, both the reaction rate and selectivity decreased, likely due to the deleterious influence of xylose in the hydrolysate.

1. Introduction

In recent years, the production of biofuels and chemicals from renewable biomass, especially lignocelluloses, is of great interest (Sheldon, 2011; Tuck et al., 2012). 5-Hydroxymethylfurfural (HMF) that can be synthesized via carbohydrate dehydration is one of top value biobased platform chemicals (Bozell and Petersen, 2010). It offers a set of functionalities, and may readily undergo a variety of transformations, thus affording many value-added chemicals. For example, selective oxidation of HMF would afford 2,5-diformylfuran (DFF), 5-hydroxymethyl-2-furancarboxylic acid (HMFCA), and 2,5-furandicarboxylic acid (FDCA), which are useful building blocks for the

* Corresponding author.
E-mail address: lining@scut.edu.cn (N. Li).

https://doi.org/10.1016/j.biortech.2018.04.077
Received 3 April 2018; Received in revised form 19 April 2018; Accepted 20 April 2018
Available online 22 April 2018
0960-8524/

production of polymers and pharmaceuticals (Rosatella et al., 2011; van Putten et al., 2013). Likewise, HMF could be selectively reduced into 2,5-bis(hydroxymethyl)furan (BHMF), 2,5-dimethylfuran and 2,5-dimethyltetrahydrofuran (Climent et al., 2014). BHMF is an interesting diol for the synthesis of polymers such as polyesters and polyurethanes (Jiang et al., 2014; Zeng et al., 2013, 2014; Zhang & Dumont, 2017), biologically active compounds (Yan et al., 2016), macrocycle polyether compounds (Cottier et al., 2003), and crown ethers (Timko & Cram, 1974).

To date, chemically catalytic synthesis of BHMF remains dominant, and significant progress has been achieved (Hu et al., 2018). Particularly, considerable effort has been dedicated to the development of a variety of metal catalysts for selective hydrogenation of HMF to BHMF (Hu et al., 2018). Recently, biocatalysis has emerged as a promising alternative to chemical approaches for green and clean upgradation of furans (Domínguez de María and Guajardo, 2017), due to a number of advantages such as mild reaction conditions, environmental friendliness, excellent selectivity and high efficiency. Biocatalytic selective oxidation of HMF received growing attention, and the synthesis of value-added DFF, HMFCA and FDCA was reported by using enzymes and microbial cells (Carro et al., 2015; Dijkman et al., 2014; Jia et al., 2017; Koopman et al., 2010; Krystof et al., 2013; McKenna et al., 2017; Qin et al., 2015; Zhang et al., 2017). On contrary, the reports on biocatalytic synthesis of BHMF from HMF remain limited in the literature. Previously, some microorganisms were found to be capable of reducing HMF in biodetoxification of lignocellulosic hydrolysates (Liu et al., 2004, 2005; Ra et al., 2013). Unfortunately, these reported microbes were not good catalyst candidates for the synthesis of BHMF, due to low transformation rates, poor HMF tolerance, and unsatisfactory selectivities (Feldman et al., 2015; Liu et al., 2005; Zhang et al., 2013). Interestingly, we isolated a HMF-tolerant yeast—*Meyerozyma guilliermondii* SC1103 from soil for the synthesis of BHMF from HMF; a good yield (86%) and an excellent selectivity (99%) were obtained in 12 h, when the substrate concentration was 100 mM (Li et al., 2017). Recently, He et al. found that the recombinant *Escherichia coli* CCZU-K14 displayed a higher HMF-tolerant level as well as good catalytic activities in the HMF reduction; BHMF was afforded with a high yield of approximately 91% in 72 h when the substrate concentration was up to 200 mM (He et al., 2018). Despite exciting results, a number of co-substrates and additives were required for effective transformation of HMF in He's process, which may have significantly negative effects on the economy of the biocatalytic process as well as on the downstream product purification.

Although good results were achieved recently by us, the biocatalytic approach to BHMF suffered from handling and recovering the biocatalyst difficultly (due to use of the free cells), an unsatisfied substrate concentration and thus a low productivity. Recently, we found that the catalytic performances of microbial cells increased significantly when they were cultivated in the presence of the substrate of a low concentration, likely because of the improved induction expression of the enzyme(s) responsible for the HMF oxidation (Zhang et al., 2017). Also, acclimatization proved to be a well-established strategy for enhancing the resistance of the wild-type strains against environmental stress as well as for improving their catalytic activities (Kalme et al., 2007; Wijekoon et al., 2011). In this work, therefore, acclimatization was applied for improving the catalytic performances of the wild-type *M. guilliermondii* SC1103. To improve the productivity, the acclimatized cells were immobilized with a high density in biocompatible calcium alginate (Ca-alginate) beads. In addition, the immobilized cells are preferred to the free ones for industrial biotransformations, because of higher stability, easier recovery, and using in continuous processes (Park & Chang, 2000). Moreover, successful scale-up production and simple isolation of BHMF verified the practicality of this biocatalytic approach. The co-substrate glucose was replaced with cost-effective rice straw hydrolysate, thus allowing the development of an economic bioprocess.

2. Materials and methods

2.1. Materials

M. guilliermondii SC1103 maintained in the China Center for Type Culture Collection (CCTCC, Wuhan, China; with CCTCC No. M2016144) was isolated by our laboratory (Li et al., 2017). HMF (98%) was purchased from J&K Scientific Ltd. (Guangzhou, China). BHMF (98%) was obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). HMFCA (98%) was purchased from Adamas Reagent Ltd. (Shanghai, China). Rice straw hydrolysate, a gift provided by Dr. Xue-Dan Hou of Guangdong University of Technology, was prepared via enzymatic hydrolysis of deep eutectic solvent-pretreated rice straw (Hou et al., 2018). Other chemicals were of the highest purity commercially available.

2.2. Acclimatization and cultivation of microbial cells

The culture was gradually exposed to HMF of the increasing concentrations to acclimatize *M. guilliermondii* SC1103. Briefly, *M. guilliermondii* SC1103 maintained on potato dextrose agar (PDA) was inoculated to the yeast extract peptone dextrose medium (YPD, 1% yeast extract, 2% peptone and 2% glucose) containing 5 mM HMF, and incubated at 30 °C and 200 r/min for 12 h. Then, the 2% culture was inoculated to the fresh YPD medium containing 5 mM HMF followed by incubation at 30 °C and 200 r/min for 12 h. Similarly, the sequential acclimatization towards 10 mM and 15 mM HMF was conducted according to the steps described above. After acclimatization, the yeast was maintained on PDA.

The acclimatized cells were cultivated according to a recent method (Zhang et al., 2017), with slight modifications. Briefly, the cells were pre-cultivated at 30 °C and 200 r/min for 12 h in the YPD medium containing 15 mM HMF. Then, the 2% seed culture was inoculated to the fresh YPD medium containing 15 mM HMF. After incubation at 30 °C and 200 r/min for 12 h, the cells were harvested by centrifugation (6000 r/min, 10 min, 4 °C) and washed twice with distilled water. Unless otherwise stated, the cells used in this work were subjected to acclimatization.

2.3. Immobilization of microbial cells

Cell immobilization was carried out according to a previous method (Chen et al., 2012), with slight modifications. Typically, 2.0–5.0 g cells (cell wet weight) were mixed with 10 mL 2.5% (w/v) sodium alginate. Then, the mixture was added drop-wise from a syringe to 0.2 M $CaCl_2$ solution. The resulting gel beads (with the diameters of approximately 1.4 mm) were hardened at room temperature for 4 h. Then, the beads were washed three times with Tris-HCl buffer (100 mM, pH 7.2) and stored at 4 °C in this buffer until use. For additional coating on Ca-alginate, the obtained beads were added into 0.9% (w/v) chitosan solution (pH 5.0) or into 2 mg/mL dopamine solution (dissolved in 10 mM, pH 8.5 Tris-HCl buffer), followed by stirring gently for 20 min and 3 h, respectively, for the membrane formation. The beads were washed three times with Tris-HCl buffer (100 mM, pH 7.2) and stored at 4 °C in this buffer.

2.4. General procedure for biocatalytic synthesis of BHMF

Typically, 4 mL Tris-HCl buffer (100 mM, pH 8.0) containing 200 mM HMF, 120 mM glucose, and immobilized cells (containing 50 mg cells (cell wet weight) per mL buffer) was incubated at 35 °C and 200 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 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