Biochemical Engineering Journal 101 (2015) 119–125

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Bioreduction of 3,5-bis(trifluoromethyl)acetophenone using ionic liquid as a co-solvent catalyzed by recombinant *Escherichia coli* cells

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ARTICLE INFO

Article history:
Received 23 January 2015
Received in revised form 19 April 2015
Accepted 16 May 2015
Available online 19 May 2015

Keywords:
Asymmetric bioreduction
Recombinant DNA
Bioprocess design
Ionic liquid-containing system
Fed-batch culture

ABSTRACT

We investigated the asymmetric bioreduction of 3,5-bis(trifluoromethyl) acetophenone (BTAP) to (*R*)-[3,5-bis(trifluoromethyl) phenyl] ethanol ((*R*)-BTPE) in a hydrophilic quaternary ammonium-based ionic liquid (IL)-containing system to improve the efficiency of bioreduction catalyzed by recombinant *Escherichia coli* cells overexpressing carbonyl reductase. Based on the low toxicity to microbial cells and moderately increased cell membrane permeability, tetramethylammonium cysteine ([N1,1,1,1][Cys]) was selected and employed as co-solvent. Some key reaction parameters involved in the bioreduction were also investigated in the [N1,1,1,1][Cys]-containing system. The optimum conditions for the process were found to be: 3.5% (w/v) [N1,1,1,1][Cys], 20% (v/v) isopropanol, 1 M BTAP, 12.7 g/L of recombinant *E. coli* cells, pH 6.8, reaction for 12 h at 30 °C. A 98.7% yield (with >99 % of enantiomeric excess (ee)) was obtained under the optimum conditions. The biocatalytic process was scaled up to a 5 L fermentor afforded high reaction yield in IL-containing system. The results demonstrated that the IL [N1,1,1,1][Cys] is a useful co-solvent to improve bioreduction process and may has potential applications in various biocatalytic reactions.

1. Introduction

Chiral alcohols are among the most valuable building blocks for the manufacture of pharmaceuticals. (*R*)-[3,5-bis(trifluoromethyl) phenyl] ethanol ((*R*)-BTPE) is a key chiral intermediate for the synthesis of Aprepitant (Emend®) with an annual sale of approximately 387 million dollars in 2010 and Fosaprepitant (Ivemend®), a tachykinin NK1 receptor antagonist widely administered to cancer patients for the treatment of chemotherapy-induced nausea and vomiting [1–3]. Currently, (*R*)-BTPE is obtained mainly through the transitional metal-catalyzed asymmetric reduction of the 3,5-bis(trifluoromethyl) acetophenone (BTAP), while the drawbacks of this method are the utilization of air sensitive and expensive catalysts, and/or hazardous reagents. In light of the above description, the search for novel approaches for highly efficient synthesis of enantiometrically pure BTPE is becoming increasingly important. Recently, the asymmetric reduction of BTAP using microbial resting cells and various enzymes have attracted more attention due to their environmental friendliness, mild reaction conditions, and excellent regio-, stereo-, and chemoselectivity. Several studies on microbial reduction of BTAP to (*R*)-BTPE have been reported so far by us and other groups [4–9]. However, the product yield is unsatisfactory at high BTAP concentration, possibly owing to the problems arisen from the relatively poor solubility of the substrate in aqueous media, and/or its toxicity to the biocatalyst, which cumbers the practical application of this bioprocess [5,6,10].

Over the past decades, it was found that biocatalysts were also able to work in non-aqueous media such as organic solvents and ionic liquids (ILs) [11–15]. In particular, as clean alternative to conventional organic solvents, ILs can be used as reaction media for performing biocatalytic reactions [16,17]. ILs are a promising new class of functional solvents with unique properties (non-volatile, non-flammable and highly stable), and able to dissolve a variety of polar and nonpolar compounds, thus exhibiting great potential for environment-friendly green solvents. Moreover, the properties of ILs can be easily tuned to match the specific requirements of particular process by altering the cations and/or anions. As a result, the catalytic performance of enzyme or microbial cells may be improved in IL-containing systems [18–19]. Recently, the biocatalytic asymmetric reduction with ILs in reaction systems has been investigated, and some valuable results have been

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http://dx.doi.org/10.1016/j.bej.2015.05.013
1369-703X/

obtained in terms of the activity [15,20], enantioselectivity [21] and stability [22] of biocatalysts. However, the reported types of ILs used in reaction systems for whole cell-mediated biocatalysis remain limited, and mainly focus on conventional imidazolium and pyridinium-based ILs. Moreover, most of the ILs used, with recombinant *Escherichia coli* cells as biocatalyst, were hydrophobic ILs and mainly selected for the IL/water two-phase system [23,24]. Hydrophilic ILs, especially used as co-solvent in aqueous system for recombinant *E. coli*–mediated biocatalytic reaction, has largely been unexplored.

Hydrophilic surfactant ILs (e.g., alkylimidazolium-based ILs), as a new type of functional ILs and co-solvent, have been investigated in the bioreduction sometimes with remarkable results. The surfactant ILs employed in reaction system can make cell membrane more permeable, not only change the intracellular concentration of substrate and product, but also reduce their toxicity to the biocatalysts, thus enhancing the biocatalytic efficiency. He et al. [25] described the use of hydrophilic IL 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) as co-solvent for asymmetric bioreduction of ethyl acetoacetate to ethyl (*R*)-3-hydroxybutyrate catalyzed by *Pichia membranaefaciens* Hansen ZJPH07 cells, and it was found that the addition of [BMIM][BF_4] in aqueous system can markedly reduce the substrate inhibition and moderately improve the enantioselectivity. Similarly, the presence of 1-(2′-hydroxyl) ethyl-3-methylimidazolium nitrate (C_2OHMIM·NO_3) as co-solvent in reaction system can facilitate the biocatalytic reduction of 4′-methoxyacetophenone to (*S*)-1-(4-methoxyphenyl) ethanol with immobilized *Rhodotorula* sp. AS2.2241, and provide significant increase in product yield [26]. In some cases, hydrophilic ILs can not only improve the enzyme stability but also act as enzyme activators, and lead to the improvement of productivity [13,16]. Therefore, it seems that hydrophilic IL is a promising and attractive co-solvent for whole cell-mediated biocatalytic processes.

Similar to alkylimidazolium-based ILs, quaternary ammonium-based ILs also exhibited surface-active properties, and were regarded as surfactant ILs [27,28]. To date, only a few studies have been reported about the performances of quaternary ammonium-based surfactant ILs in biocatalytic reactions [29].

In the present study, we focus on the performance evaluation of eight quaternary ammonium-based hydrophilic ILs for efficient synthesis of (*R*)-BTPE by recombinant *E. coli* cells overexpressing an engineered carbonyl reductase (LXCAR-S154Y) from *Leifsonia xyli* HS0904 for the first time. It was found that the bioreduction of BTAP to (*R*)-BTPE could be markedly improved by adding tetramethylammonium cysteine ([N1,1,1,1][Cys]) as co-solvent in aqueous system. To optimize the bioreduction in this developed [N1,1,1,1][Cys]-containing buffer system, the effects of some crucial reaction parameters for the synthesis of (*R*)-BTPE were subsequently investigated, such as [N1,1,1,1][Cys] content, buffer pH, reaction temperature, substrate concentration and cell concentration. Moreover, a comparative study was performed either in the presence or in the absence of [N1,1,1,1][Cys], higher reaction yield was achieved in [N1,1,1,1][Cys]-containing buffer system.

2. Material and methods

2.1. Chemicals

Substrate BTAP was supplied by Beijing Golden Olive Company, China. Product (*R*)-BTPE and (*S*)-BTPE were purchased from Capot Chemical Co., Ltd., China. The ILs synthesized in this research were entrusted to Shanghai Chengjie Chemical Co., Ltd. China. All other chemicals were from commercial sources and were of analytical grade.

2.2. Strain and fed-batch cultivation

Recombinant *E. coli* BL21 (DE3), overexpressing an engineered carbonyl reductase (LXCAR-S154Y) from *L. xyli* HS0904, was obtained by directed evolution in our previous study [30].

For the cultivation of recombinant *E. coli* as whole-cell biocatalyst, a fed-batch process was developed. Single colony of recombinant *E. coli* was selected from agar plate, and inoculated into a 500 ml flask containing 100 ml Luria–Bertani (LB) medium (tryptone 10 g/L, yeast extract 5 g/L and NaCl 10 g/L) supplemented with 50 μg/m kanamycin, and incubated for 12 h on a rotary shaker at 37 °C and 200 rpm as the seed culture. The obtained seed culture (4%, v/v) was then transferred into a 5 L Biostat® B bioreactor (Braun Biotech Int., Germany) containing 2.5 L fermentation medium, and incubated at 37 °C. The fermentation medium contained: citric acid 3 g/L, glucose 20 g/L, tryptone 30 g/L, yeast extract 20 g/L, NH_4Cl 0.1 g/L, $Na_2HPO_4{\cdot}12H_2O$ 3 g/L, KH_2PO_4 6.75 g/L, $(NH_4)_2SO_4$ 5 g/L, $MgSO_4{\cdot}7H_2O$ 1.5 g/L and 6 ml/L of trace metal solution ($FeSO_4{\cdot}7H_2O$ 10 g/L, $ZnSO_4{\cdot}7H_2O$ 2.25 g/L, $MnSO_4{\cdot}5H_2O$ 0.5 g/L, $CaCl_2{\cdot}2H_2O$ 1.35 g/L, $CuCl_2{\cdot}2H_2O$ 1 g/L, $NaMoO_4{\cdot}2H_2O$ 0.1 g/L and H_3BO_3 0.2 g/L). The agitation speed and aeration was set at 800 rpm and 5 L/min. The expression of carbonyl reductase was induced at 33 °C by the addition of lactose to a final concentration of 20 g/L when the OD_{600} reached around 25 (about at 5 h). The pH value of the culture was kept constant at 7.1 throughout the whole process by adding ammonia water or nutrient feeding solution. When an increase in pH signaled the complete consumption of glucose, the nutrient feeding solution was fed automatically by peristaltic pump. The nutrient feeding solution contained 700 g/L of glucose. After induction for 28 h, the incubated recombinant *E. coli* cells were harvested by centrifugation at 4 °C, 9000 rpm for 10 min, and washed twice with saline, and then subjected to biocatalytic reduction.

2.3. Selection of ILs

Eight quaternary ammonium-based hydrophilic ILs (shown in Table 1) were evaluated for their performances in the asymmetric reduction of BTAP to (*R*)-BTPE catalyzed by recombinant *E. coli* cells. The bioreduction was performed in 50 ml Erlenmeyer flasks. The reaction mixtures (total volume of 5 ml) contained 3.82 ml phosphate buffer (200 mM, pH 7.2), 5 g/L recombinant *E. coli* cells (DCW), 1.0 ml isopropanol (20%, v/v), 200 mM BTAP, and 1.5% (w/v) ILs. The reaction mixtures were incubated at 30 °C and 200 rpm. After reaction for 10 min and 2 h, samples were taken to determine the initial reaction rate and yield. The yield and ee value of the product were assayed by chiral-GC analysis described in our previous report [9].

2.4. Cell viability assay

The viability of recombinant *E. coli* cells was defined as the ratio of the consumed glucose amount by cells pretreated in various IL-containing buffer systems [16,25]. The recombinant *E. coli* cells were pre-incubated at 30 °C, 200 rpm for 6 in various 1.5% (w/v) IL-phosphate buffer (200 mM, pH 7.2) systems in the presence of 200 mM BTAP or not, then adding 10 g/L glucose and cultured for an additional 4 h. The glucose concentrations in the medium were then assayed using biological sensing analyzer.

2.5. Cell membrane permeability assay

The recombinant *E. coli* cells were incubated at 30 °C, 200 rpm in various IL-phosphate buffer (200 mM, pH 7.2) systems, or phosphate buffer (200 mM, pH 7.2) solution only. The cell-free supernatants containing ILs and released intracellular components (primarily nucleic acids and proteins) were withdrawn from the