Efficient Enantioselective Synthesis of (R)-[3,5-Bis(trifluoromethyl)phenyl] Ethanol by *Leifsonia xyli* CCTCC M 2010241 Using Isopropanol as Co-Substrate

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(R)-[3,5-Bis(trifluoromethyl)phenyl] ethanol is a key chiral intermediate for the synthesis of aprepitant. In this paper, an efficient synthetic process for (R)-[3,5-bis(trifluoromethyl)phenyl] ethanol was developed via the asymmetric reduction of 3,5-bis(trifluoromethyl) acetophenone, catalyzed by *Leifsonia xyli* CCTCC M 2010241 cells using isopropanol as the co-substrate for cofactor recycling. Firstly, the substrate and product solubility and cell membrane permeability of biocatalysts were evaluated with different co-substrate additions into the reaction system, in which isopropanol manifested as the best hydrogen donor of coupled NADH regeneration during the bioreduction of 3,5-bis(trifluoromethyl) acetophenone. Subsequently, the optimization of parameters for the bioreduction were undertaken to improve the effectiveness of the process. The determined efficient reaction system contained 200 mM of 3,5-bis(trifluoromethyl) acetophenone, 20% (v/v) of isopropanol, and 300 g/l of wet cells. The bioreduction was executed at 30°C and 200 rpm for 30 h, and 91.8% of product yield with 99.9% of enantiometric excess (e.e.) was obtained. The established bioreduction reaction system could tolerate higher substrate concentrations of 3,5-bis(trifluoromethyl) acetophenone, and afforded a satisfactory yield and excellent product e.e. for the desired (R)-chiral alcohol, thus providing an alternative to the chemical synthesis of (R)-[3,5-bis(trifluoromethyl)phenyl] ethanol.

**Key words:** *Leifsonia xyli*, enantioselective reduction, 3,5-bis(trifluoromethyl) acetophenone, co-enzyme regeneration, (R)-[3,5-bis(trifluoromethyl)phenyl] ethanol

Enantiomerically pure chemicals are important intermediates for the synthesis of agrochemicals, flavors, and pharmaceutical products [9, 13]. The worldwide sales of enantiopure intermediates or single enantiomer drugs were dramatically increased because of the different biological activity displayed by each enantiomer of chiral compound in many cases [18]. As the typical chiral intermediates, enantiopure alcohols are very useful for pharmaceuticals production [24]. The asymmetric reduction of prochiral ketones, which are catalyzed by biocatalysts or chemical catalysts, is most important and widely applied for optically pure alcohols preparation [13, 14]. Recently, the asymmetrically biocatalytic reduction has gained much attention owing to its outstanding enantioselectivity, mild reaction conditions, and environmental safety [15, 17]. The enantioselective bioreduction was usually performed either by isolated enzymes [8, 17] or whole-cell systems [2, 14]. Bioreduction using isolated enzymes generally required the external addition of a coenzyme (NAD⁺ or NADP⁺) and a corresponding regeneration system [8, 21]. The application of NAD(P)H-dependent oxidoreductases in industrial-scale bioconversion was limited owing to the external addition of cost-expensive cofactors [21]. The whole-cell system is usually more economic, because many whole-cell biocatalysts can afford their internal cofactor regeneration through a “coupled-substrate” approach, such as adding cheap co-substrates or other compounds [4]. In addition, biocatalysis with a whole-cell system could protect target enzymes from inactivation within their natural cellular environment, and avoid the complicated enzyme purification process [12].

(R)-[3,5-Bis(trifluoromethyl)phenyl] ethanol is a crucial intermediate for the synthesis of aprepitant (Emend), which was developed by Merck Corporation and used for the treatment of chemotherapy-induced emesis [5, 19]. The synthesis of (R)-[3,5-bis(trifluoromethyl)phenyl] ethanol via the asymmetrically biocatalytic reduction of 3,5-
bis(trifluoromethyl) acetophenone has been established (Scheme 1), which is highly enantioselective and environmentally friendly. This approach avoids the usage of expensive heavy-metal catalysts such as Ru and Rh, which are usually applied in chemical catalytic processes [3, 7, 11, 20]. As listed in Table 1, a number of microorganisms capable of catalyzing the bioreduction of 3,5-bis(trifluoromethyl) acetophenone to (R)-[3,5-bis(trifluoromethyl)phenyl] ethanol have been investigated. Among them, Candida tropicalis, Lactobacillus kefir, Penicillium expansum, and Leifsonia xyli were identified as the positive reduction microbes with high enantioselectivity. However, like most whole-cell biocatalysts, these microbes could only perform the expected reduction at a relatively low substrate concentration. The presence of excess ketone will inhibit the oxidoreductases [3, 11] and decrease the yield. The usage of a high content of non-natural substrates on the industrial scale [6] with the desired high yield of products via biocatalytic processes could not be handled by such microorganisms. Therefore, a competent whole-cell reaction system has to be developed to conquer this challenge and attain a satisfactory process yield and excellent enantioselectivity at high substrate concentrations.

In our previous work, hundreds of microbes isolated from soil samples had been evaluated for their asymmetric reduction abilities. Leifsonia xyli HS0904 (CCTCC M 2010241) was selected as an efficient biocatalyst possessing high carbonyl reductase activity and excellent stereoselectivity [20]. However, a comparatively low conversion rate (62%) and low initial substrate concentration (70 mM) had cumbered its practical application. Thus, in this study, an efficient whole-cell biocatalytic system using isopropanol as a co-substrate to strengthen the cofactor recycling has been developed, with better tolerance towards substrate inhibition and affording a satisfactory conversion rate at a higher substrate concentration.

**Materials and Methods**

**Materials**

Leifsonia xyli CCTCC M 2010241 (HS0904) was isolated from soil and preserved in our laboratory [20]. 3,5-Bis(trifluoromethyl) acetophenone (≥99.5%) was provided by Beijing Golden Olive Company, China. (R)-[3,5-Bis(trifluoromethyl)phenyl] ethanol was purchased from Capot Chemical Co., Ltd., China. All other chemicals were obtained commercially and of analytical grade or better level.

**Cultivation of Leifsonia xyli CCTCC M 2010241**

Firstly, Leifsonia xyli CCTCC M 2010241 was inoculated into 100 ml of seed medium (10.0 g/l glucose, 5.0 g/l peptone, 4.0 g/l yeast extract, 2.0 g/l (NH₄)₂SO₄, 1.0 g/l KH₂PO₄, 0.5 g/l NaCl, 0.5 g/l MgSO₄·7H₂O, pH 6.5) and incubated at 30°C and 200 rpm for 24 h.
Subsequently, 8 ml of such overnight culture was transferred into 100 ml of fermentation medium (25.0 g/l glucose, 20.0 g/l yeast extract, 4.0 g/l KH$_2$PO$_4$, 0.4 g/l NaCl, 0.6 g/l MgSO$_4$$\cdot$7H$_2$O, 0.2 g/l Li$_2$SO$_4$, pH 6.5) and cultured at 30°C and 200 rpm for an additional 48 h. The incubated cells were harvested by centrifugation at 10,000 ×g and 4°C for 10 min, and the resulted cell pellets were washed twice with 0.85% saline solution and stored at 4°C for further use.

**Asymmetric Reduction of 3,5-Bis(trifluoromethyl) Acetophenone Catalyzed by Leifsonia xyli CCTCC M 2010241 Cells**

The collected cells were resuspended in 20 ml of potassium phosphate buffer (200 mM, pH 8.0), and then various concentrations of 3,5-bis(trifluoromethyl) acetophenone and co-substrate were added into the reaction buffer. The asymmetric reduction was carried out by shaking at 200 rpm and 30°C for 30 h. Aliquot samples were taken from the reaction system at certain time intervals. The resulted supernatant was extracted twice with ethyl acetate. The obtained ethyl acetate fraction was dried with anhydrous MgSO$_4$ and subjected to GC analysis.

**Analytical Methods**

**NADH regeneration assay.** The incubated cells (10 g wet cell weight) were resuspended in 100 ml of potassium phosphate buffer (200 mM, pH 8.0) and sonicated by an ultrasonic oscillator (Sonic Materials Co., USA) for 30 min. After centrifugation at 13,000 ×g and 4°C to remove cell debris, the supernatant was used as the crude enzyme and quantified by the Bradford method using bovine serum albumin as the standard. The 3 ml assay mixture consisted of 200 mM potassium phosphate buffer (pH 8.0), 0.05 mM 3,5-bis(trifluoromethyl) acetophenone, 0.5 ml of crude enzyme (1.90 mg total protein), 0.3 mM NADH or 0.3 mM NAD$^+$, and 10 g/l or 10% (v/v) co-substrate. The parallel assay using potassium phosphate buffer instead of co-substrate was set as the control. The reaction mixture was pre-incubated at 30°C for 5 min before the addition of crude enzyme. The cofactor regeneration reaction was assayed at 30°C for 30 min by monitoring the absorbance variation of NADH at 340 nm per minute, using a SpectraMax M2e spectrophotometer (Molecular Devices, USA).

**Solubility test.** The solubility determination of the substrate 3,5-bis(trifluoromethyl) acetophenone and the product (R)-[3,5-bis(trifluoromethyl)phenyl] ethanol in the co-substrate-containing potassium phosphate buffer (200 mM, pH 8.0) mixture was performed with a similar method described in the literature [22]. Calibration curves were first established using the corresponding standard dissolved in n-hexane as the reference. Then, excessive 3,5-bis(trifluoromethyl) acetophenone or (R)-[3,5-bis(trifluoromethyl)phenyl] ethanol was thoroughly mixed with 5 ml of potassium phosphate buffer containing 10 g/l or 10% (v/v) co-substrate in a sealed vial and shaken at 200 rpm, 30°C for 48 h, followed by centrifugation at 10,000 ×g for 10 min. The obtained supernatant was extracted with n-hexane and then subjected to a spectral measurement at 240 nm for 3,5-bis(trifluoromethyl) acetophenone, or at 260 nm for (R)-[3,5-bis(trifluoromethyl)phenyl] ethanol. The control containing the same amount of co-substrate in the buffer without substrate or product was used as the reference. The solubility of substrate or product in pure water was measured with the same protocol.

**Cell membrane permeability assay.** The cell membrane permeability assay was carried out using a similar procedure described previously [12]. Briefly, Leifsonia xyli CCTCC M 2010241 cells were resuspended in 5 ml of potassium phosphate buffer (200 mM, pH 8.0) with 10 g/l or 10% (v/v) co-substrate to a final cell concentration of 100 g/l. The assay mixture was incubated at 30°C and 200 rpm, and aliquot samples were taken from the mixture at 0 h and 24 h, respectively. Samples were diluted and measured at 260 nm and 280 nm, respectively. The net increase of OD$_{260}$ and OD$_{280}$ from 0 h to 24 h could reflect the release of intracellular components (primarily nucleic acids and proteins) into the medium during the incubation with the supplement of co-substrate [12].

**GC analysis.** The enantiometric excess (e.e.) and yield of the product were determined by gas chromatography (Shimadzu GC-2014; Japan) equipped with a flame ionization detector and a Chirasil-Dex column from Varian (USA). The column temperature was increased from 80°C to 180°C at a rate of 4°C/min, and then kept constant for 10 min. The temperature of the injector and the detector were 250°C. Nitrogen was used as carrier gas and its flow rate was 2 ml/min. Fig. 1 shows the GC chromatographic profile of the 3,5-bis(trifluoromethyl) acetophenone and 3,5-bis(trifluoromethyl)phenyl ethanol enantiomers.

**RESULTS AND DISCUSSION**

**Effects of Sugars and Alcohols as Co-Substrate on Whole-Cell Bioreduction**

Although different sugars have varied metabolic pathways, most of them could provide cofactors for reduction reactions. Many carbohydrates, such as glucose, xylose, arabinose, sucrose, and maltose, have been used as co-substrates to regenerate the cofactors [21]. On the other hand, an alternative hydrogen-transfer bioreduction process
was also characterized, involving the NADH-dependent *Leifsonia* sp. alcohol dehydrogenase (LSADH) with no need for an additional coenzyme regeneration system. LSADH can effectively regenerate NADH by transferring hydrogen from isopropanol [1]. Therefore, whole-cell biocatalytic reduction with two series of co-substrates, involving sugars and alcohols, was conducted to investigate their effects on the asymmetric reduction.

Three sugars including glucose, sucrose, and maltose, as well as four alcohols including glycerol, butanol, ethanol, and isopropanol, were tested in the whole-cell biocatalytic system (Table 2). In the absence of co-substrate, the yield was only about 28.4 ± 1.1% with 80.8 ± 0.01% of product e.e. value. The supplement of co-substrate improved the e.e. value of the product at different levels, but the variation of yield was distinctly diversified. Among the three sugars, only maltose presented a negative influence on product yield, and glucose showed the best performance to support the reduction. The promoted product yield (50.8%) and e.e. value (99.3%) with glucose as co-substrate may be the results of NADH regeneration by glucose via the Embden-Meyerhof degradation pathway [16]. However, isopropanol was the only one among the four tested alcohols to be beneficial to the bioreduction. The yield and enantioselectivity were significantly increased in the presence of isopropanol in the reaction system. The achieved product yield reached 85.6%, which was 3-folds compared with the control (without the addition of co-substrate), and the best e.e. value of 99.9% was obtained among all tested co-substrates. The comparison of different co-substrates suggested that isopropanol is an optimal and cost-effective hydrogen donor for biocatalytically asymmetric reduction with satisfactory efficiency.

**Effects of Co-Substrate Addition on NADH Regeneration, Substrate Solubility, and Cell Membrane Permeability in *Leifsonia xyli* CCTCC M 2010241 Cells**

To investigate the NADH regenerating process in the whole-cell system, an activity assay system containing crude enzyme derived from *Leifsonia xyli* CCTCC M 2010241 cells, substrate, NAD⁺, and co-substrate was monitored by its absorbance variation at 340 nm (Fig. 2). When NADH was added into the assay system without the addition of co-substrate, the absorbance decreased gradually, which further proved that the reduction catalyzed by oxidoreductase derived from *Leifsonia xyli* CCTCC M 2010241 cells was an NADH-dependent process [1, 24]. The addition of glucose, or glycerol, or phosphate buffer in the assay mixture would not obviously alter the absorbance. However, in the presence of isopropanol as a co-substrate, the absorbance increased rapidly, which suggested the regeneration of NADH from NAD⁺ offered by the crude enzyme itself.

The effect of co-substrate on the solubility of substrate and product in potassium phosphate buffer was also examined. Among three candidates of co-substrates, isopropanol presented the best support to improve the solubility of both the substrate and product (Table 3). Because of the impacts of co-substrate, substrate, and product on the permeability of the cell membrane, the transportation of substrate and release of products would be restricted and the yields of products might be depressed [10, 12]. Thus, in our study, an assay method to measure the net increase of $OD_{260}$ and $OD_{280}$ values from the mixture during a period of time was applied to analyze the change of cell membrane permeability [12]. When different co-substrates were added into the whole-cell reduction system, the cell membrane permeability was

![Fig. 2. NADH regeneration assay.](image)

The assay system (3 ml) contained 200 mM potassium phosphate buffer (pH 8.0), 0.5 ml of crude enzyme (total protein 1.90 mg), 0.05 mM 3,5-bis(trifluoromethyl) acetophenone, 10% (v/v) or 10 g/l co-substrate, and 0.3 mM NAD⁺ or NADH. The reaction was conducted for 30 min.

**Table 2. Effects of co-substrate on the yield and e.e. value.**

<table>
<thead>
<tr>
<th>Co-substrate</th>
<th>Co-substrate conc.</th>
<th>Yield (%)</th>
<th>e.e. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>100 g/l</td>
<td>50.8 ± 1.2</td>
<td>99.3 ± 0.01</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100 g/l</td>
<td>32.4 ± 1.4</td>
<td>99.0 ± 0.01</td>
</tr>
<tr>
<td>Maltose</td>
<td>100 g/l</td>
<td>14.4 ± 0.5</td>
<td>92.5 ± 0.02</td>
</tr>
<tr>
<td>Glycerol</td>
<td>10% (v/v)</td>
<td>8.9 ± 0.5</td>
<td>82.1 ± 0.01</td>
</tr>
<tr>
<td>Butanol</td>
<td>10% (v/v)</td>
<td>11.1 ± 0.3</td>
<td>86.5 ± 0.02</td>
</tr>
<tr>
<td>Ethanol</td>
<td>10% (v/v)</td>
<td>13.5 ± 0.4</td>
<td>88.4 ± 0.02</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>10% (v/v)</td>
<td>85.6 ± 1.3</td>
<td>99.9 ± 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>28.4 ± 1.1</td>
<td>80.8 ± 0.01</td>
</tr>
</tbody>
</table>

*a The co-substrate concentration.
*b The maximum yield.
*c The product e.e.

Reaction conditions: 2.0 g of wet cells (0.4 g dry weight) was added into 10 ml of 200 mM potassium phosphate buffer (pH 8.0) containing different kinds of co-substrate, and 100 mM 3,5-bis(trifluoromethyl) acetophenone. Bioreduction was carried out at 30°C and 200 rpm for 24 h.
ENANTIOSELECTIVE SYNTHESIS OF CHIRAL ALCOHOL

Isopropanol still gave the best performance for the improvement of cell membrane permeability. This characteristic could allow faster transportation of the substrate and product, which could facilitate the bioreduction and accelerate the reaction [10].

These assay results had clearly indicated that the addition of isopropanol as a co-substrate can obviously improve the whole-cell bioreduction. It seems that the high efficiency of isopropanol addition in the reduction system may be the results of its contribution to the regeneration of essential cofactor NADH, and the improvement of solubility of substrate and product, as well as the cell membrane permeability.

Effect of Isopropanol Concentration on the Whole-Cell Bioreduction

The effect of isopropanol concentration on the reduction of 3,5-bis(trifluoromethyl) acetophenone was investigated in the range from 5% to 30% (v/v). The results indicated that isopropanol had a remarkable influence on the yield but hardly affected the e.e. value of the product (Fig. 3). The yield of (R)-3,5-bis(trifluoromethyl)phenyl ethanol was steadily increased with the increasing isopropanol concentration when below 20% (v/v), and reached its peak of 98.3% yield with 99.9% e.e. at 20% (v/v) isopropanol. Further increase of the isopropanol concentration resulted in the decline of yield. This may be caused by the detrimental effects of excessive isopropanol and its oxidative derivative acetone, which is acting as a competitive substrate of the reaction.

Table 3. Effects of co-substrates on the solubility of the substrate and product.

<table>
<thead>
<tr>
<th>Co-substrate</th>
<th>Co-substrate conc.</th>
<th>Substrate (g/l)</th>
<th>Product (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer</td>
<td>-</td>
<td>0.0173 ± 0.0008</td>
<td>0.1196 ± 0.0054</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>0.0241 ± 0.0010</td>
<td>0.2090 ± 0.0027</td>
</tr>
<tr>
<td>Glucose</td>
<td>10 g/l</td>
<td>0.0180 ± 0.0006</td>
<td>0.1397 ± 0.0063</td>
</tr>
<tr>
<td>Glycerol</td>
<td>10% (v/v)</td>
<td>0.0211 ± 0.0005</td>
<td>0.1800 ± 0.0086</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>10% (v/v)</td>
<td>0.0255 ± 0.0009</td>
<td>0.2184 ± 0.0092</td>
</tr>
</tbody>
</table>

*The co-substrate concentration.

3,5-Bis(trifluoromethyl) acetophenone.

(R)-3,5-Bis(trifluoromethyl)phenyl ethanol.

Table 4. Effects of co-substrates on the cell membrane permeability of Leifsonia xyli CCTCC M 2010241 cells.

<table>
<thead>
<tr>
<th>Co-substrate</th>
<th>Co-substrate conc.</th>
<th>Net OD&lt;sub&gt;260&lt;/sub&gt;</th>
<th>Net OD&lt;sub&gt;280&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer</td>
<td>-</td>
<td>1.213 ± 0.065</td>
<td>0.584 ± 0.045</td>
</tr>
<tr>
<td>Glucose</td>
<td>10 g/l</td>
<td>8.062 ± 0.122</td>
<td>3.374 ± 0.014</td>
</tr>
<tr>
<td>Glycerol</td>
<td>10% (v/v)</td>
<td>7.018 ± 0.157</td>
<td>2.983 ± 0.022</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>10% (v/v)</td>
<td>20.852 ± 0.589</td>
<td>13.083 ± 0.062</td>
</tr>
</tbody>
</table>

*The co-substrate concentration.

Reaction conditions: 0.5 g/l of wet cells was resuspended in 5 ml of 200 mM potassium phosphate buffer (pH 8.0) containing 10 g/l or 10% (v/v) co-substrate. The mixture was incubated in a 100 ml Erlenmeyer flask at 30°C and 200 rpm. The samples were withdrawn from the reaction system at 0 h and 24 h, respectively.

variously improved (Table 4). Isopropanol still gave the best performance for the improvement of cell membrane permeability. This characteristic could allow faster transportation of the substrate and product, which could facilitate the bioreduction and accelerate the reaction [10].

Effect of Isopropanol Concentration on the Whole-Cell Bioreduction

The effect of isopropanol concentration on the reduction of 3,5-bis(trifluoromethyl) acetophenone was investigated in the range from 5% to 30% (v/v). The results indicated that isopropanol had a remarkable influence on the yield but hardly affected the e.e. value of the product (Fig. 3). The yield of (R)-3,5-bis(trifluoromethyl)phenyl ethanol was steadily increased with the increasing isopropanol concentration when below 20% (v/v), and reached its peak of 98.3% yield with 99.9% e.e. at 20% (v/v) isopropanol. Further increase of the isopropanol concentration resulted in the decline of yield. This may be caused by the detrimental effects of excessive isopropanol and its oxidative derivative acetone, which is acting as a competitive substrate of the reaction.

![Graph showing the effect of isopropanol concentration on asymmetric reduction yield and e.e.](image-url)
dehydrogenase [3]. With the optimal 20% (v/v) isopropanol, the yield of (R)-[3,5-bis(trifluoromethyl)phenyl] ethanol was increased by 59.4%, compared to our previous report of only 62% yield with glucose as a co-substrate [20].

**Effect of 3,5-Bis(trifluoromethyl) Acetophenone Concentration on the Bioreduction**

The concentration of substrate is an indispensable parameter in biocatalytic reactions. Moreover, aromatic ketones usually are toxic to microbial cells, and therefore the whole-cell bioreduction could only perform at relatively low substrate concentrations (ordinarily 10 to 30 mM) [23]. Thus, the effect of 3,5-bis(trifluoromethyl) acetophenone concentration on the bioreduction was investigated in the range of 50 to 300 mM (Fig. 4). The results showed that the yield of product decreased with the increasing substrate concentration, but the e.e. value remained intact. At low concentration in the range of 50 to 100 mM, the yield declined slowly, but with the increase of substrate concentration, the product yield decreased much faster. This observation was similar to the results reported previously [3, 11]. Using *Leifsonia xyli* CCTCC M 2010241 cell as the biocatalyst, the product yield still remained 85.8% when the substrate concentration reached 200 mM. This output was competent enough compared with other reported systems (Table 1). Considering the absolute yield of reaction, 200 mM of substrate concentration was preferred.

**Effect of Cell Concentration on the Bioreduction**

To obtain the optimal reduction system, different cell concentrations ranging from 100 to 400 g/l were also tested (Fig. 5). The yield was slightly improved with increased cell amount until it reached 300 g/l, which gave the highest yield of 90.5%, and further increase of cell concentration presented a negative effect on the product yield. However, the effect of cell concentration on the enantioselectivity was not obvious. Since the best yield of 90.5% with 99.9% e.e. value of the product was obtained, 300 g/l of cell concentration was selected for further optimization.
Time Course of the Bioreduction of 3,5-Bis(trifluoromethyl)acetophenone to (R)-3,5-Bis(trifluoromethyl)phenyl ethanol

The time course of asymmetric bioreduction of 3,5-bis (trifluoromethyl) acetophenone to (R)-3,5-bis(trifluoromethyl)phenyl ethanol using Leifsonia xyi CCTCC M2010241 cells was presented in Fig. 6. The yield of product increased nearly proportionally from 0 h to 24 h, after which the bioreduction was almost stagnant. High enantioselectivity (over 99.9% e.e.) was obtained throughout the whole process. After 30 h of bioreduction, 91.8% of the maximum yield of product with a nearly 100% e.e. was achieved, which was obviously more efficient than the reported Penicillium expansum EBK-9 system [15] and our previous study [20].

In this study, the efficient preparation of (R)-3,5-bis(trifluoromethyl)phenyl ethanol at 200 mM of 3,5-bis(trifluoromethyl) acetophenone was successfully performed via asymmetric reduction using isopropanol as the cosubstrate and catalyzed by Leifsonia xyi CCTCC M2010241 cells, a promising biocatalyst for the bioreduction of aromatic ketones. The established process can tolerate higher concentrations of substrate, and obtain a satisfactory product yield (91.8%) with excellent e.e. value (99.9%). Adding isopropanol as the hydrogen donor efficiently regenerates NADH during the bioreduction. Furthermore, both the substrate and product solubility and cell permeability of the microorganism were enhanced. This potent process provided an alternative to prepare (R)-3,5-bis(trifluoromethyl)phenyl ethanol with good product yield and excellent e.e. value using a biocatalysis approach.

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REFERENCES


