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Biocatalytic reduction of ketones by a semi-continuous flow process using supercritical carbon dioxide

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The immobilized resting-cell of *Geotrichum candidum* was used as a catalyst for the reduction of a ketone in a semi-continuous flow process using supercritical carbon dioxide for the first time; it was also applied for the asymmetric reduction of a ketone and resulted in excellent enantioselectivity (ee >99%) and a higher space-time yield than that of the corresponding batch process.

Supercritical carbon dioxide ($scCO_2$) has been used as a solvent for organic synthesis, extraction and chromatography due to its environmentally benign nature as well as its unique characteristics and high functionalities, such as high diffusivity and solubilizing power.[1] Enzymes such as lipases have been applied to $scCO_2$ reactions since the first reports in 1985.[2] Both batch- and flow-type reactors have been used. With flow reactors, the addition of a substrate to the column with a catalyst yields the product and CO_2, which is a gas at ambient pressure, whereas, with the batch reactor, extraction of the product from the biocatalyst is necessary after depressurization, and an organic solvent may be used. Therefore, the flow type is superior to the batch type for achieving virtually no solvent reaction. Moreover, the size of the reactors using the flow process to generate an amount of product comparable with the corresponding batch reactors is smaller, which is particularly attractive for a supercritical fluid system.[3]

Compared to the studies using hydrolytic enzymes in $scCO_2$, dehydrogenase-catalyzed reactions in $scCO_2$ have not yet been developed. Only two reports have been found for biocatalytic reduction in $scCO_2$, and neither of these used flow reactors: asymmetric reduction using *Geotrichum candidum*,[4] and reduction of butyroaldehyde by horse liver alcohol dehydrogenase (HLADH) with a fluorinated coenzyme.[5] In this communication, we report the first use of the immobilized cell of *G. candidum* as a catalyst for the reduction of ketones with a semi-continuous flow process using $scCO_2$. The reduction of cyclohexanone was successful, and the biocatalyst was recycled up to four times with only a slight loss in activity. Recycling was not possible using the corresponding batch system because the biocatalysts can not tolerate repeated depressurization at a very low temperature and separation of the product from the biocatalysts using organic solvents. This method was also applied for the asymmetric reduction of *o*-fluoroacetophenone, achieving excellent enantioselectivity (ee >99%) and a higher space-time yield than the corresponding batch process.

The reduction of cyclohexanone was examined first as a model reaction to test the viability of the process (Fig. 1(a)). The apparatus[6] is shown in Fig. 2. The typical experiment is as follows. The resting cell of *G. candidum* IFO 5767 was immobilized on a water-absorbing polymer (cell (wet wt) : H_2O : water-absorbing polymer (Osaka Yuki Kagaku Kogyo Co., BL-100) = 4 : 3 : 1), as reported previously.[7] The immobilized cell was added to a glass test tube (6.0 mL) and placed in a stainless reactor, and a stainless pipe was inserted in the bottom of the test tube. The temperature was set to 35 °C. The pressure was increased to 9.0 MPa at 10 mL min^{-1} (volume of liquid CO_2 at −10 °C per min) and to 10 MPa at 2.0 mL min^{-1}. The pressure and the flow rate were then kept constant. The

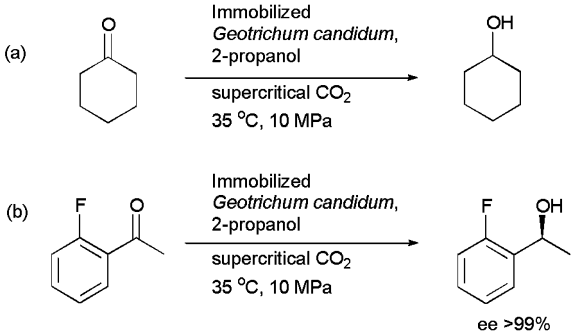

Fig. 1 Reduction of ketones by a semi-continuous flow process using $scCO_2$.

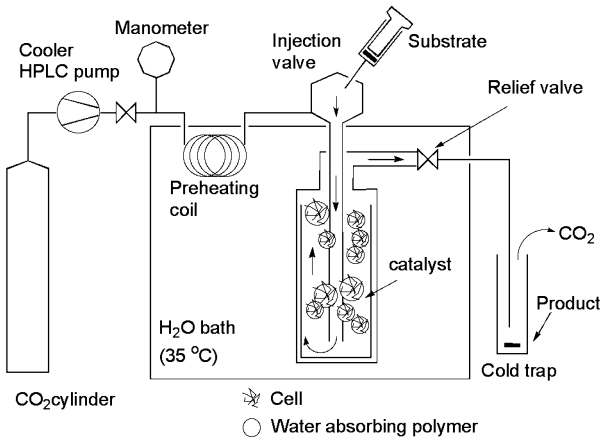

Fig. 2 Apparatus for biocatalytic reduction with a flow process using $scCO_2$.

substrate, cyclohexanone (19 μmol) dissolved in 2-propanol (499 μmol), which is necessary as a hydrogen donor, was injected through an HPLC injection valve. The product was trapped at −78 °C (injection/trap 1 in Table 1) for 5 min. The injection of the substrates was repeated four times to assess reusability (injection/trap 2–5). The contents in the traps were examined by GC analysis using dodecane as an internal standard.

Table 1 Reduction of cyclohexanone with the semi-continuous flow process

Injection/trap	Conversion[a] (%)
1	12
2	21
3	36
4	36
5	30

[a] Conversion to cyclohexanol based on the injected substrate, determined by GC analysis.

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The results are shown in Table 1. Cyclohexanol was obtained successfully. The conversions to cyclohexanol in the first and second trap were smaller than that of the third trap because some of the product remained in the column. The conversions in the third and fourth were constant, and that in the fifth decreased slightly. This may be due to the change in the water content in the water-absorbing polymer on which the cell was immobilized. Viability of the cell was not examined, but this may not effect enzyme activity.

Encouraged by this promising result, reduction of *o*-fluoroacetophenone was conducted by the same method (Fig. 1 (b)), using cyclooctane as a GC internal standard. (*S*)-*o*-Fluorophenylethanol was obtained successfully with a conversion of 8%. The enantioselectivity was determined to be > 99% ee by GC analysis using a chiral column (Chirasil-DEX CB; 25 m; He 2 mL min^{-1}, 130 °C). The absolute configuration was determined by comparing the GC retention times with those of the authentic samples. Similar results were obtained with repeated-use of the biocatalyst.

The space-time yield of this flow system was compared with that of the corresponding batch system.[4] That of the flow system (0.24 μmol min^{-1}) was almost twice as much as that of the corresponding batch system (0.13 μmol min^{-1}) at 35 °C and 10 MPa using a pressure-resistant vessel (Taiatsu Techno Co., Osaka, TVS-N2 type, 10 mL) for the reduction of *o*-fluoroacetophenone. Therefore, the flow-type reactor is more efficient than the corresponding batch system.

In conclusion, the immobilized resting cell of *G. candidum* was used in a semi-continuous flow system using $scCO_2$ for the first time. The alcohol dehydrogenase in the cell reduced the cyclohexanone successfully, and when *o*-fluoroacetophenone was used as a substrate, excellent enantioselectivity and a higher space–time yield than the corresponding batch system were obtained. The ability of this first biocatalytic-flow-reduction process using $scCO_2$ to solve the product-extraction problem in the dehydrogenase reactions will be indispensable as a seminal step toward further developments in the field.

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