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Novel continuous carboxylation using pressurized carbon dioxide by immobilized decarboxylase

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ABSTRACT

Novel continuous flow carboxylation system was constructed using pressurized CO_2 at 6.5 MPa and immobilized enzyme, *Bacillus megaterium* PYR 2910 decarboxylase. The decarboxylase catalyzed the backward reaction, carboxylation, to convert pyrrole to pyrrole-2-carboxylate at the improved space-time yield by 25 times compared to the corresponding batch reaction.

There is a pressing need to develop a method for organic synthesis using CO_2 as a carbon source to promote green chemistry for the protection of the global environment. Both chemical catalyst[1] and enzyme[2,3] have played an important role in the reaction that uses CO_2 as a substrate. Although chemical catalyst has been more extensively examined than biocatalyst, investigation on biocatalyst is also very important due to the advantages of enzyme being natural, reproducible, and having high chemo-, regio- and enantioselectivity. Among enzymes, decarboxylase catalyzing decarboxylation under the natural conditions is one of the attractive kinds of enzymes because it was shown to catalyze CO_2 fixation, backward reaction, recently.[2] For example, decarboxylase from *Bacillus megaterium* PYR2910 catalyzes the carboxylation of pyrrole to produce pyrrole-2-carboxylate,[2a–d] and *Clostridium hydroxybenzoicum* decarboxylase catalyzes the carboxylation of phenol to produce 4-hydroxybenzoate.[2e,f]

B. megaterium decarboxylase has an additional advantage when used as a catalyst for carboxylation; it can catalyze the reaction with supercritical CO_2 using a batch-type reactor.[2d] To shift the equilibrium between decarboxylation and carboxylation toward the carboxylation, high density of CO_2 (pressurized CO_2) plays an important role.

On the other hand, for the reaction using pressurized or supercritical CO_2, construction of flow process is very important from the practical point of view because for any reaction, the reactor size can be decreased by changing batch process to flow to produce a comparable amount of product.[4] In other words, flow-type reactor can increase the productivity, namely space-time yield, and reduce both the cost and the safety problems.[4] Therefore, continuous phase, high pressure supercritical fluid reactor for both pilot and commercial scale production was launched in 2001.[5] Continuous hydrogenation of organic compounds in supercritical CO_2 was conducted using poly-siloxane-supported noble metal catalysts.[4] Continuous chemoselective methylation of functionalized amines and diols with supercritical methanol over solid acid and acid–base bifunctional catalysts was also conducted.[6] Concerning the enzymatic process in supercritical CO_2,[7,8] by changing the batch process to a continuous-flow process for kinetic resolution of racemic alcohols to produce optically active compounds with an immobilized lipase, the space-time yield was improved significantly.[7a] Semi-continuous flow process for alcohol dehydrogenase-catalyzed asymmetric reduction has also been reported.[8] However, the kind of enzymes used in the flow process is very limited to stable and easy-to-use enzymes, such as lipase.[7] Decarboxylase has not been used in flow process although decarboxylase is indispensable for carboxylation. Therefore, in this study, the continuous-flow process for the carboxylation using *B. megaterium* PYR2910 decarboxylase was constructed, and as a result, the space-time yield was improved by 25 times compared to the corresponding batch reaction using pressurized CO_2. This is the first flow process using pressurized CO_2 and immobilized decarboxylase for the carboxylation as to the best of our knowledge.

First, the whole cell of *B. megaterium* PYR2910 (6.5 g wet wt) was cultivated[2a] and immobilized onto ion exchange resin with polyallylamine polymer[9] to produce 13.5 g dry wt of immobilized enzyme. This immobilization method was chosen because whole cell instead of isolated enzyme can be used, and moreover, it has

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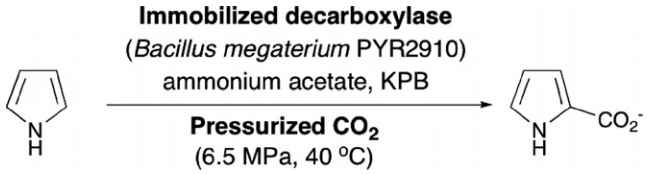

Scheme 1.

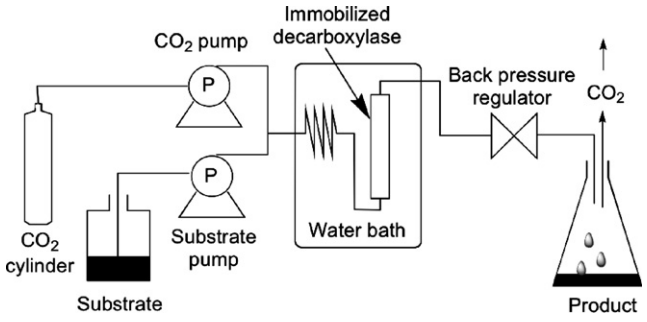

Figure 1. Flow-type reactor for carboxylation using immobilized decarboxylase.

the advantage of low diffusion barrier inside the gel layer around the enzyme, and long time and stable operation result achieved for the L-asparagic acid synthesis.[9]

Then, the reaction shown in Scheme 1 was conducted using batch-type reactor by adding CO_2 at 6.5 MPa to the mixture of pyrrole (0.10 M), the immobilized enzyme (0.20 g), and ammonium acetate (0.14 M) in potassium phosphate buffer (0.10 M, pH 8.1, 1.0 mL) in a steel pressure-resistant vessel (Taiatsu Techno, Co., Osaka, TVS-N2 type, 10 mL) equipped with a stop valve (Whitey Co. SS3NBS4), manometer (Taiatsu Techno, Co., Osaka, 15 MPa) and a magnetic stirrer in the water bath at 40 °C. As a CO_2 source, $KHCO_3$ was not used to confirm that CO_2 was actually used as a substrate. As a result, after 3 h, the yield of pyrrole-2-carboxylate was determined to be 2.7 μmol by HPLC.[2a]

Next, to improve the space-time yield, the flow-type reactor[7a] shown in Figure 1 was constructed. CO_2 was pumped (ISCO Syringe Pump 260D and Pump Controller Series D) at the rate of 1.5 mL/min, and pyrrole solution (pyrrole: 0.10 M, ammonium acetate: 0.14 M, and potassium phosphate buffer: 0.10 M, pH 8.1) was pumped (JASCO, PU-2085) at the rate of 0.15 mL/min. The immobilized enzyme (2.0 g) was packed in an up-flow tubular reactor (1/2 inch × 10 mm × 135 mm).[6] The pressure was set to be 6.5 MPa by the back-pressure regulator (JASCO, SCF-Bpg). The system was at equilibrium after 2 h at 40 °C, and the desired product, pyrrole-2-carboxylase, was obtained successfully at the rate of 24 ± 7 μmol/h continuously for 4 h. The space-time yield was improved by 25 times compared to the corresponding batch reaction.

In conclusion, the immobilized decarboxylase was used in a continuous flow system using CO_2 for carboxylation. The decarboxylase catalyzed the carboxylation of pyrrole successfully to produce pyrrole-2-carboxylate with higher space-time yield than the corresponding batch system. The ability of this first biocatalytic-flow-carboxylation process using CO_2 will be indispensable as a seminal step toward further developments.

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