

論文 / 著書情報  
Article / Book Information

題目(和文)	高圧二酸化炭素中での生体触媒反応：リパーゼの触媒活性の向上
Title(English)	Pressurized carbon dioxide in biocatalysis: Enhanced catalytic activity of lipase
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出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第10971号, 授与年月日:2018年9月20日, 学位の種別:課程博士, 審査員:松田 知子,中村 聡,小林 雄一,占部 弘和,福居 俊昭
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第10971号, Conferred date:2018/9/20, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

## 論文要旨

THESIS SUMMARY

専攻 : Bioengineering 専攻  
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申請学位 (専攻分野) : 博士 (Engineering)  
Academic Degree Requested Doctor of  
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要旨 (英文 800 語程度)

Thesis Summary (approx.800 English Words )

Solvents, which play an important role in chemical and pharmaceutical industries, have often been claimed as a major source of waste generation and associated environmental and economic burdens. With the eco-environmental urge of developing sustainable processes, academic and industrial intentions have been focused both on minimizing the overall solvent usage and on replacing traditional organic solvents by more environmentally friendly alternative solvents.

On the other hand, the scope of biocatalysis, particularly in asymmetric synthesis, has been extended through the use of enzymes in organic solvents as well as in 'green' anhydrous media, such as ionic liquids and supercritical fluids. Carbon dioxide, which has a number of positive impacts on green chemistry as a nonflammable, nontoxic, abundant, and generally chemically inert source, has been intensively studied in its supercritical phase as an attractive solvent for enzymatic reactions due to its greenness, low viscosity, high diffusivity, and ease of recovery of the reaction products. The aims of the thesis are to utilize pressurized CO<sub>2</sub> solvents as in liquid CO<sub>2</sub> and in CO<sub>2</sub>-expanded bio-based liquids for biocatalysis.

By conducting *Candida antarctica* lipase B (CALB) catalyzed kinetic resolution of *rac*-1-phenylethanol as the model reaction, the feasibility and compatibility of liquid CO<sub>2</sub> as a solvent for biotransformation was investigated, and the reactions were compared with those using conventional organic solvents. The lipase exhibited the highest activity in liquid CO<sub>2</sub> with excellent enantioselectivity. The effects of temperature and pressure on the reactions were also investigated with liquid CO<sub>2</sub>, hexane and solvent free system. The potential applicability of liquid CO<sub>2</sub> fluid for large-scale enantioselective synthesis was also examined with two extreme types of continuous flow bioreactors. The kinetic resolution of *rac*-1-phenylethanol by the immobilized lipase in a continuous packed-column reactor stably afforded corresponding enantiopure products with minimum waste generated.

The alcohol-binding part of CALB contains a stereoselective pocket, which gives this enzyme high selectivity toward various *sec*-alcohols containing one large and one small substituents. However, this stereoselective pocket is well-known to be only large enough to accommodate an ethyl or smaller substituent under standard condition. Investigation of substrate specificity of CAL-B indicated

that the substrate scope of the lipase was expanded toward bulky phenyl alkyl *sec*-alcohols by just using it in liquid CO<sub>2</sub>. Interestingly, by using in liquid CO<sub>2</sub>. The substrate scope CALB was found to be expanded towards sterically bulky *sec*-alcohols such as 1-phenyldodecanol, of which the lipase has been known to have no reactivity in standard conventional solvent.

As emerging alternative solvents, biobased liquids, which can be derived from cheap and renewable feedstock such as biomass, provide environmentally advantages, such as sustainable production, better biodegradability and lower toxicity. The author introduced in this thesis a new concept of using CO<sub>2</sub> gas for volumetric expansion of biobased liquids. CO<sub>2</sub>-expanded biobased liquids inherit the advantages of pressurized CO<sub>2</sub> and of the component biobased solvent in an optimal fashion that pressurized CO<sub>2</sub> enhances the transport properties by increasing diffusivity and by decreasing viscosity of the biobased solvent, while this component biobased solvent improves the solubility power (solubility) of the pressurized CO<sub>2</sub>.

However, some bio-based liquids are not favourable solvents for biocatalysis, especially those with high polarity such as 2-methyltetrahydrofuran (MeTHF), which often witnessed in decreased lipase activity. However, in this thesis, by combining with pressurized CO<sub>2</sub>, CO<sub>2</sub>-expanded MeTHF becomes feasible solvent for lipase-catalyzed reactions. Enhancement of the reaction conversion was observed. Moreover, the biocatalytic activity in a CO<sub>2</sub>-expanded biobased liquid was closely correlated to the physicochemical properties of the solvents, which can be modulated by tuning the CO<sub>2</sub> molar fraction

The underlying mechanism of crucial role of CO<sub>2</sub> to induce increased activity of lipase was studied. Molecular dynamics simulation of CALB showed that pressurized CO<sub>2</sub> can modify the structure flexibility of the active-site entrance, which allows bulkier substrates to reach the active site cavity of CALB. Moreover, the volume of main cavity of CALB was found to be enlarged in pressurized CO<sub>2</sub>, so that the enzyme can comfortably accommodate bulkier substrates.

Herein, pressurized CO<sub>2</sub> (liquid CO<sub>2</sub> and CO<sub>2</sub>-expanded bio-based liquids) was proved as superior media for lipase catalysis. In pressurized CO<sub>2</sub> not only much higher activity were possible, also CALB retained its originally high enantioselectivity towards various sterically bulky *sec*-alcohols. The molecular dynamics simulation of CALB in pressurized CO<sub>2</sub> showed important changes in the lipase conformation that the lipase became more tolerant to bulkier substrates. With these positive findings, I expected that pressurized CO<sub>2</sub> is promising non-aqueous media for other biocatalysis.

備考：論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。

Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).

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