論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Geotrichum candidum acetophenone reductase immobilization on novel support materials
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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12212号, 授与年月日:2022年9月22日, 学位の種別:課程博士, 審査員:松田 知子,福居 俊昭,三重 正和,八波 利恵,秦 猛志
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第12212号, Conferred date:2022/9/22, 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

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	Life Science and Technology 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Engineering)
学生氏名： Student's Name	Kotchakorn T.sriwong	指導教員（主）： Academic Supervisor(main)	Assoc. Prof. Tomoko Matsuda
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要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

Biocatalysis has been extensively engaged in various research and industrial areas for over a century. Its attractive features, such as eco-friendliness, nonhazardous chemical requirement, non-toxic byproduct production, operation under mild conditions, and high selectivity, make it an approach toward green and sustainable industries. However, free enzymes are susceptible to harsh environments in general. Therefore, to improve the feasibility of using enzymes in industrial applications, many attempts have focused on improving enzymes' strength.

Enzyme immobilization is a strategy that has been employed to improve enzyme stability and allow enzyme recycling. Nowadays, the advent of nanotechnology and material science have availed in the success of enzyme immobilization. In the meantime, 3D-printer technologies have been greatly developed due to their advantages, such as being versatile, convenient, time-saving, and inexpensive. Therefore, this research utilized both nanotechnology and 3D-printer technology to develop new approaches for enzyme immobilization and used them for beneficial compounds production. This research has used a novel and robust alcohol dehydrogenase (ADH), acetophenone reductase from *Geotrichum candidum* (*Gc*APRD), as an enzyme model. *Gc*APRD could reduce ketones to their corresponding (*S*)-alcohols with excellent enantioselectivity. Asymmetric reduction of ketones by biocatalyst could be beneficial for chiral alcohols production and be widely utilized in pharmaceutical and agrichemical areas. *Gc*APRD was immobilized in/on novel support materials; 1) organic-inorganic nanocrystal, 2) graphene-based nanomaterials, and 3) 3D-printed reactors. All enzyme immobilization strategies are described here in detail.

Organic-inorganic nanocrystal formation is an enzyme immobilization strategy that is simple and effective. This research successfully immobilized *Gc*APRD by this method to yield *Gc*APRD nanocrystal. The enzyme was immobilized *via* the bonding between Co^{2+} and his-tagged on *Gc*APRD. It was found that *Gc*APRD nanocrystal performed superior properties to the free enzyme, such as improved temperature profile, pH profile, and stability. Besides, *Gc*APRD nanocrystal was able to be recycled to reduce acetophenone up to 7 times with a noticeable high yield (>99%) and excellent enantioselectivity (>99%(*S*)). In addition, this research synthesized beneficial compounds by *Gc*APRD nanocrystal such as (*S*)-1-(3',4'-dichlorophenyl)ethanol with >99% yield and >99% *ee*. Meanwhile, morphology studies such as scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and thermal gravimetric analysis (TGA) were performed to confirm the presence of *Gc*APRD within the nanocrystal.

Graphene oxide (GO) and reduced graphene oxide (rGO) have been attractive carriers for enzyme immobilization because of their unique characteristics, for examples, strong mechanical strength and large specific area. Thus, this work immobilized *Gc*APRD on GO and rGO *via* physical adsorption to achieve GO-*Gc*APRD and rGO-*Gc*APRD. It appeared that rGO-*Gc*APRD exhibited high activity, 104% compared with that of free form, which was greater than GO-*Gc*APRD (65%). Moreover, rGO loaded *Gc*APRD with a high immobilization yield (maximum ~3 mg protein/mg rGO). This approach solved the trade-off relationship problems between immobilization yield and the retained enzyme activity found in general immobilization methods as a consequence of the large specific area of GO and rGO. Additionally, rGO-*Gc*APRD was able to be recycled up to 7 times to produce an enantiopure alcohol with a high yield (>99%) and excellent enantioselectivity (>99% (*S*)), as well as produced drug intermediate such as (S)-1-(3′,4′ - dichlorophenyl)ethanol with 80% isolated yield and > 99% *ee*. Morphology of the nanomaterials such as SEM, EDX, and Fourier-transform infrared spectroscopy (FTIR) were conducted to confirm the presence of *Gc*APRD on GO-*Gc*APRD and rGO-*Gc*APRD.

Bioreactors were designed to immobilize *Gc*APRD by computer-aided design (CAD) programs and fabricated with a 3D-printer using polypropylene as a material. Then, the surfaces of the 3D-printed reactors were functionalized by mussel-inspired polydopamine, glutaraldehyde, and polyethylenimine for *Gc*APRD immobilization. It was found that *Gc*APRD immobilized on glutaraldehyde *via* covalent attachment performed sufficient activity, excellent enantioselectivity, and recyclability in batch bioreactors. The immobilized enzyme per bioreactor area was 6.9 ± 1.0 μg/cm^2. Consequently, this study immobilized *Gc*APRD onto 3D-printed microfluidic reactors and found that greater immobilized enzyme per area of the bioreactor was achieved in microfluidic reactors (13.3 μg/cm^2). Moreover, this research successfully established continuous flow processes and realized that the systems could produce an enantiopure alcohol, and be operated up to 117-144 h. In total, 12.5 units of *Gc*APRD immobilized on 3D-printed microfluidic bioreactors in this study could produce 43.2 mg of (*S*)-1-phenylethanol (32.5 mg isolated yield) with excellent enantioselectivity (>99% *ee*), which was greater than a millimole scale reaction of free *Gc*APRD reported before (50-250 units of free *Gc*APRD produced 98 mg of (*S*)-1-phenylethanol).

Thanks to the applications and techniques from the various research areas, this study successfully developed novel systems to immobilize an ADH and, therefore effectively used them repeatedly to produce various intermediates. This achievement is very promising for green chemistry.