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論 文 内 容 要 旨

In recent years, new sustainable energy, especially bioethanol, has received wide attention. As a “green” approach, the preparation of bioethanol from lignocellulosic biomass by enzymatic hydrolysis and bio-fermentation is very popular, due to the environmentally friendly and low production cost. Cellulase is an important role in the biodegradation of cellulose to glucose. Because the enzymatic hydrolysis could be effectively and specifically conducted under mild conditions. However, some factors limit the application of free cellulase, such as changes in pH, temperature and ionic strength, product inhibition, and difficulty in recovering from the reaction medium. Therefore, it is meaningful to improve the stability and reusability of cellulase. The use of immobilization technology can improve the stability of the enzyme and enable it to be recycled and reused. The key point of this technology is the choice of the immobilization method and support. In this study, porous biochar (obtained from bagasse) combined with chitosan and magnetic particles to prepare several functional carriers for cellulase immobilization via covalent bonding or

adsorption. The effects of chemical characteristics and morphology of the support on the cellulase activity recover and recycle were discussed.

In chapter 1, the research background and the construction of this thesis are described. The objectives of the research are to study the preparation of functional porous biochar and its application for cellulase.

In chapter 2, the properties of materials used in this thesis, experimental methods, and characterization are presented.

In chapter 3, porous biochar derived from sugarcane bagasse was prepared and then coated with different amounts of chitosan (C@CS) for cellulase immobilization. Cellulase was covalently immobilized on the support by using glutaraldehyde as a linker. The chemical characteristics and morphology of the samples were determined by SEM, BET, FT-IR and XPS. The properties of immobilized enzyme were evaluated by activity recovery, optimum pH value and temperature, and recyclability. The results showed that all the three kinds of immobilized cellulase did not change the optimum pH value of 4 and temperature of 60 °C, and they also exhibited good activity and reusability. Especially for C@CS25 (the feeding ratio of porous biochar to chitosan was 0.5 g : 25 mg), the support retained the morphology of porous biochar well. The corresponding immobilized cellulase kept 67% activity of free cellulase at pH = 4 and 60 °C, and showed a glucose productivity of 90.8% even after 10 cycles.

In chapter 4, because of the high specific surface area, polyporous structure and ease of preparation, porous biochar from lignocellulosic biomass is popular for being used as support for enzyme immobilization. In this work, polyporous biochar combined with magnetic particle γ-Fe_2O_3 was prepared by calcination and then used as support for cellulase adsorption. The effects of calcination temperature and time on the properties of magnetic polyporous biochar were investigated and the optimum preparation condition was obtained. For the cellulase adsorption, the immobilization capacity for the magnetic support reached as high as 266 mg/g with a relative activity of 73.6% compared with free cellulase. The behavior of cellulase adsorption showed that an

endothermic process occurred more easily at high temperatures, which resulted in a high adsorption amount.

In chapter 5, the porous biochar was obtained from sugarcane bagasse by alkali activation and pyrolysis and then magnetized with γ-Fe_2O_3 by calcination. After functionalization with chitosan and activation with glutaraldehyde, the as-prepared chitosan/magnetic porous biochar was served as support to immobilize cellulase by the covalent bonds. The immobilization amount of cellulase was 80.5 mg cellulase/g support at pH=5 and 25 ºC for 12 h immobilization. To determine the enzymatic properties, 1% Carboxymethylcellulose sodium (CMC, dissolved in 0.1 M buffer.) has been considered as a substrate for hydrolysis at different pH values (from 3 to 7) and temperatures (30 to 70 ºC) for 30 min. The results have shown that the optimum pH and temperature of the free and immobilized cellulase did not change, which was pH 4 and 60 ºC, respectively. The immobilized cellulase has a relatively high activity recovery of 73.0%. However, it also exhibited a higher Km value and a slower Vmax value comparing to the free enzyme. In the reusability assay, the immobilized cellulase showed initial glucose productivity of 330.9 mg glucose/g CMC and kept 86.0% after 10 uses. In conclusion, the chitosan/magnetic porous biochar has great potential applications as support for enzyme immobilization.

In chapter 6, general conclusions of the study are made.

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論　文　題　目	Preparation of Functionalized Porous Biochar Supports for Cellulase Immobilization （機能性多孔質バイオチャーの創製および固定化担体への応用に関する研究）
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論文審査結果要旨

本論文は、活性と再利用性が高く、簡単に回収可能な固定化酵素の開発を目的として、機能性多孔質バイオチャーの創製と構造を評価し、その酵素固定化用の担体としての応用性についても検討したものである。本論文は全 6 章で構成されている。

第 1 章は緒論として、固定化酵素に関する既存の研究から本研究の背景を述べ、研究の目的を示している。第 2 章では材料および試験方法として、固定化酵素の作製方法およびそれらの酵素特性の評価方法、担体の内部構造などの分析方法について説明している。第 3 章では、バイオチャーにキトサンを被覆することにより親水性基および共有結合における官能基として、グルタルアルデヒド（GA）により酵素が担体に固定する方法を検討している。作製した固定化酵素は生体適合性と再利用性を向上させており、10 回繰り返し使用後でも、90.8%の活性を保持させている。第 4 章では、固定化酵素が反応系から簡単に分離できるようにするために、焼成法により磁性粒子をバイオチャー内で均一に成長させる方法を検討している。磁性担体の最適焼成条件は 500℃で 60 分であることを明らかにし、作製した磁性担体の酵素吸着量が高く、酵素単体の活性の 73.6%を保持しており、担体の回収も容易にできている。第 5 章では、第 3、4 章の利点を活かし、酵素を担体に共有結合で固定する方法を検討している。作製した固定化酵素の活性は酵素単体の 73%を保持しており、優れた安定性を示し、10 回繰り返し使用後でも、86%の活性を有し、さらに磁力で簡単に回収できる。第 6 章は結論であり、研究結果をまとめている。

本論文の成果は、その学術的な価値だけでなく、実用化における課題を解決している観点から工学的な貢献も大きい。また、研究業績として、査読付国際学術論文 3 編、国際会議 4 件、国内会議 1 件を公表している。

よって、本論文は博士（工学）の学位論文として合格と認める。