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Development and Optimization of Efficient Biocatalysts from the Perspective of Green Chemistry

Zhuo Chen

The University of New SouthWales, Sydney, Australia

ABSTRACT

The purpose of this study is to investigate the innovative design and efficiency improvement strategies of biomass catalysts from the perspective of green chemistry. In view of the increasing global awareness of environmental protection and the keen demand for sustainable development models, green chemistry has become an indispensable evolution direction in the field of chemical engineering. Biomass catalysts, with their excellent efficiency, excellent selectivity, and low impact on the environment, have shown great potential applications in the territory of green chemistry. This paper describes how to select specific microbial populations, use gene manipulation technology to breed high-performance recombinant enzymes, and use immobilization technology to enhance their durability and reuse. Experimental experience confirms that the improved biomass catalyst exhibits outstanding catalytic activity and stability, significantly reducing energy consumption while minimizing by-product output. Through the characterization of the catalyst, the catalytic mechanism of the catalyst is discussed and evaluated from the standpoint of green chemistry. These findings not only lay the theoretical foundation for the construction of innovative biomass catalysts, but also light the green light for technical support to further promote more eco-friendly chemical processes. Future work will examine more closely the potential of such biocatalysts in complex industrial ecosystems.

KEYWORDS

Green Chemistry; Biocatalyst; Enzyme engineering; Immobilization technology; Catalytic activity; Optimization Strategy

1. INTRODUCTION

As industrialization accelerates and ecological stress intensifies, traditional chemical industry's environmental burden and energy usage are notably rising. To achieve sustainability, green chemistry emerged, an interdisciplinary field addressing contemporary needs. It fosters the development of eco-friendly processes and products, minimizing harm to ecosystems and human health. The fundamental concept of this discipline advocates both economic benefits and environmental protection, and achieves dual goals through innovative chemical technology. Its signature programme, the 12 Principles of Green Chemistry, emphasises the role of catalysts, and in particular the importance of high performance, non-toxic and renewable catalysts in this field. Biocatalysts, especially enzymes, have become the focus of green chemistry research because of their high specificity, mild reaction conditions and environmental affinity. Enzymatic reactions not only increase selectivity and yield, but also reduce by-product generation, while reducing energy consumption and environmental load. However, natural enzymes, due to their inherent instability and unrecyclability in industrial operations, pose obstacles to their widespread application [1]. Therefore, the development of efficient and stable biocatalysts and the improvement of their performance have a profound impact on the progress of green chemistry. With the transformation of genetic engineering, protein engineering

technology, and the strategy of physicochemical fixation, the efficiency and industrial practicability of biocatalysts have been significantly enhanced. Recent breakthroughs in biocatalyst research have attracted attention, and genetic engineering has enhanced the heat resistance of enzymes and substrate selection. Immobilization technology enhances the enzyme's durability and ability to be reused. However, there are still limitations in the existing research, and it is an urgent challenge to further improve the long-term stability of enzymes and how to maintain catalytic efficiency in a complex industrial context.

This research will enable the exploration of innovative and efficient biocatalyst design and optimization, with a focus on improving catalytic performance, which will be achieved with the help of gene manipulation and solid phase technologies. Specifically, we plan to select microstrains with specific catalytic properties and construct recombinant enzymes with enhanced expression properties by gene splicing. Further, we use physical and chemical methods to implement the immobilization of microorganisms. The superior properties of the modified biocatalysts in terms of activity, durability and renewable utilization have been confirmed through a detailed set of experiments, and a comprehensive evaluation has been carried out in the light of green chemistry [2]. In the end, this study is expected to contribute to this branch of green chemistry, promote biocatalysts with higher potency, and further stimulate the practice and promotion of green chemistry technology.

2. THEORETICAL BASIS

2.1. Section Headings

Eco-friendly chemistry Thus avoids or minimizes the potential adverse effects of its products and processes on human physiology and the Earth's ecosystems. At the initiative of the U.S. Environmental Protection Agency (EPA) and the American Chemical Society (ACS), a set of 12 guidelines for green chemistry emerged, which has had a profound impact on the sustainable evolution of chemical engineering. Here is a brief overview of the principles. First, nip it in the bud. Manage waste generation at source to improve economic efficiency and environmental protection. Maximization of atomic utility. The optimal synthesis pathway seeks to maximize the conversion rate so that all reactants can be fully converted to the final product. Toxicological considerations. In the construction of chemical synthesis pathways, harmful raw materials and solvents should be avoided. Fourth, environmentally friendly product design. Chemical products need to be designed with full consideration of toxic effects throughout their life cycle in order to minimize ecological and biological risks. 5. Selective solvents and auxiliaries. Advocate the use of non-toxic or slightly toxic solvents and additives. 6. Improvement of energy efficiency. Increase the energy efficiency of chemical processes to reduce consumption. Sustainable use of resources. Promote renewable resources to replace the use of non-renewable fossil fuels. 8. Prudent operation of derivatives. Use derivatives only when necessary and ensure that they are de-derivable or harmless in the subsequent process. 9. Catalyze innovation. Catalytic action is used instead of chemometry to optimize reaction performance. 10. Biodegradable design. Construct products that are easy to decompose to ensure that they do not last long after being discarded. 11. Real-time monitoring of pollution. Develop real-time detection technology to dynamically monitor pollution sources and block the emission of harmful substances. 12. Full security guarantee. Ensure the safety of chemicals and their configuration throughout.

2.2. Overview of Biocatalysts

Biological catalysts, such as enzymes, intact microbial cells, or biological tissue extracts, are representative of biological forces that exhibit unique efficacy in facilitating the transformation of compounds. Enzymes, exemplified in this category, are known for their unrivalled specificity and decisiveness, directing reactions to operate at mild conditions, often in the environment that

organisms tolerate - that is, in aqueous systems at near-neutral pH and room temperature and pressure. Compared to catalysts of abiotic origin, enzymes can accurately identify and select closely related molecules, ensuring excellent spatial and regional selectivity, and further achieving unprecedented precision in the manipulation of molecular construction. The byproducts accompanying its catalytic process are minimal, which significantly reduces the ecological load and is conducive to environmental protection [4].

Many enzymes can be produced on a large scale by microbial fermentation and are reproducible.

The utility of biocatalysis has penetrated into many industries such as pharmaceutical, food manufacturing, textile and paper, showing its broad application potential. The role of enzyme catalysis in the pharmaceutical field lies in the precise construction of chiral drug molecules. In the food industry, enzyme intervention improves the quality and nutritional properties of products. The textile industry has witnessed the innovative use of enzymes in refining and coloring processes. With the evolution of science and technology, the field capacity of biocatalysts continues to expand. In order to exploit its potential, researchers have adopted various strategies to modify and enhance the function of the enzyme. These include the use of gene manipulation techniques to adjust the amino acid arrangement of enzymes to enhance their heat resistance and selectively target specific substrates. Directed evolutionary strategies have also been used to discover highly efficient enzyme variants to optimize catalytic activity. In addition, the immobilization technology, that is, the enzyme is firmly attached to the supporting medium by physical or chemical means, to ensure that the stability and reuse of the enzyme can be significantly enhanced [5]. The comprehensive application of various technologies has systematically optimized the overall performance of biocatalysts.

3. EXPERIMENT AND RESULT

The aim of this research is to select microbial strains with special catalytic properties from the environment. Through gene manipulation techniques, we successfully constructed an engineered strain that can express the target enzymes at a high level. The recombinant enzymes were purified by precise separation methods. To boost stability and reusability, the enzyme is securely attached to the support via physical and chemical methods. This entails evaluating its catalytic efficiency, longevity, and reusability. Biochemical analysis revealed the enzymatic reaction's background and catalytic characteristics. The same search extends to the selection of microbial strains with unique catalytic activity from soils of different geographical origins. In the experimental design, we tend to choose suitable support materials, such as porous glass beads and polyacrylamide gel, for the enzyme immobilization process. In the selection of reagents, we insist on the use of analytical purity grades of chemicals, and even purer grades, all purchased from well-known suppliers Sigma-Aldrich. The inorganic salts and other compounds used in the buffer solution were purchased from China National Pharm Group. The facility's advanced tools, such as HPLC, UV-visible photometers, electrophoresis, PCR machines, ultra-centrifuges, and freeze dryers, are crucial for experimental operations.

The experiment was carried out from soil samples with abundant organic matter. According to the unique properties of the target enzyme, a suitable screening culture medium for microbial multiplication was configured. The bacteria with target enzyme activity were selected by solid and liquid culture mode. Then all the DNA was extracted from the selected strains. The coding gene of the target enzyme was amplified by polymerase chain reaction (PCR), then cloned into the expression vector, and successfully transformed into the host bacteria of Escherichia coli BL21(DE3). Further studies were conducted to refine and optimize the expression level of target enzymes by adjusting the induction temperature control, induction duration and IPTG concentration. In the experiment, E. coli was broken by ultrasonic cracking technology, and the target enzyme was purified by ammonium sulfate salting out method. In order to achieve higher purity, the chromatography techniques of ion exchange and affinity chromatography have been developed. Based on the specific properties of the enzyme, the matching support material is selected. The enzyme was stably fixed to the supporting

medium by covalent bonding or physical adsorption. Through the experimental optimization of the optimal immobilization conditions (such as temperature, pH, etc.), the activity and efficiency of the enzyme are ensured. All purification and mounting steps are performed in a low temperature environment of 4°C. According to the optimal pH value of the enzyme, the corresponding buffer solution was prepared, and the appropriate reaction medium was selected to adapt to different experimental stages. The enzyme activity was estimated by UV-visible light spectrophotometry, and the catalytic efficiency of the enzyme was determined by standard curve. In addition, the stability of the immobilized enzyme was verified by repeated use tests, and the evolution of catalytic activity after repeated use was recorded in detail. Finally, with the help of high performance liquid chromatography and other advanced analytical techniques, the product was identified and the catalytic mechanism was gained insight.

Through careful selection of multiple soil samples, we have effectively extracted microbial strains with specific enzymatic response capabilities. After a two-stage screening process, an outstanding strain (codenamed X) was identified that demonstrated outstanding biocatalyst potential on a specially designed screening substrate. The target gene fragment multiplied by polymerase chain reaction (PCR) is then safely spliced into the expression vector system. Next, we investigated the synthesis of target proteins in the host system of E. coli BL21(DE3). The production efficiency of the target enzyme is maximized by finely tuned induction parameters, including isopropyl-1-phospho-N '-trifluoroacetate, reaction temperature and duration. Blot analysis of the antigen promotion display showed a distinct band that matched the predicted molecular weight, confirming the correct folding and translation of the target protein. After purification by ammonium sulphate and ion exchange chromatography, the activity and yield of the enzyme were greatly increased. The analysis of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed high purity of the enzyme preparation. By means of covalent bonding strategy, the enzyme was bonded to porous glass microbead media, and the optimal fixation conditions were discovered. The immobilized enzyme exhibits a high degree of catalytic efficiency in a specified biochemical transition, showing a significant increase in stability compared to the enzyme in the free state. Especially under the most favorable pH conditions, the durability and catalytic efficiency of the immobilized enzyme are greatly increased, even after several rounds of operation, its activity degradation is minimal. Further immobilized enzymes maintain robust thermal stability over a wide temperature span (25 ° C to 60 ° C). At the most efficient temperature point, the catalytic decoupling efficiency of the immobilized enzyme reaches its highest point. Heat shock tolerance tests confirm that this enzyme remains active even when subjected to intense heat for a short period of time. Even after ten consecutive applications, the catalytic activity of the immobilized enzyme still exceeded 80% of the initial level, which strongly demonstrated that the immobilization strategy played a key role in improving the reuse of the enzyme while significantly reducing the production cost.

In order to further evaluate the utility of the immobilized enzyme, we extended a comparative analysis to include references to the performance of the immobilized enzyme against conventional chemical catalysts and free enzymes reported in the literature. The multi-dimensional comparison highlighted the outstanding performance of the enzyme in catalytic performance, durability and repeatability. In particular, in the field of substrate specificity and ecological compatibility, the implantable enzymes show self-evident superiority. By means of HPLC product analysis, we have proposed a preliminary rational hypothesis for the unique mechanism of action of the immobilized enzyme. These results indicate that the specific toughness of the immobilized enzyme in the catalytic reaction significantly enhances the formation of the target transformation while significantly suppressing the formation of non-target by-products. The further immobilization process consolidates the structural stability of the enzyme and ensures its lasting catalytic performance. The green chemistry perspective emphasizes that our development of immobilized enzymes is compatible with the principles of sustainable chemistry. First, it allows catalysis under mild environmental parameters, further saving energy consumption. Secondly, it induces fewer by-products, which contributes to environmental protection and reduces the potential pressure on the ecological environment. In addition, the technology of

ballast ensures the reuse of enzymes, reduces the loss of enzyme preparations, and reduces the production cost at the economic level.

4. GREEN CHEMISTRY EVALUATION

Green chemistry strives to safeguard the environment and human health by minimizing or eliminating hazardous substances via designing eco-friendly chemicals and processes. The efficient biocatalysts crafted in this study adhered to green chemistry principles, ensuring environmental friendliness and sustainability.

The strains used in this study were screened from nature, and their genetic resources were abundant and easy to obtain. In addition, the recombinant enzymes obtained through genetic engineering use conventional bacterial expression systems that are mature and reliable. Therefore, from a raw material point of view, the strains and expression systems used are highly reproducible. The reagents, though potentially environmentally impactful, are used in pure forms and controlled strictly, minimizing harm. Optimized reactions reduce reagent usage, lessening the environmental load. The immobilized enzyme efficiently catalyzes under mild conditions, conserving energy. It displays high activity at room temp, emphasizing energy savings. Biocatalyst production is eco-friendly, energy-efficient, and simple, involving culture, expression, purification, and immobilization. Compared to chemical catalysts, biocatalysts are greener, reusable, and cost-effective.

The immobilized enzyme minimizes byproduct formation during catalysis, lowering waste treatment costs and environmental pollution. Optimizing reaction conditions further curbs byproduct generation. The waste generated during the experiment was mainly liquid waste and discarded immobilized carrier. The waste liquid can reach the discharge standard after neutralization treatment. Waste carriers can be incinerated or biodegraded for eco-friendliness. The immobilized enzyme ensures stability, reduces leakage, and enhances safety. Mild reaction conditions minimize harm to operators. The enzyme is non-toxic, posing no health risk. Rigorous QC/SE ensures biocatalyst safety. These biocatalysts have broad industrial potential, spanning pharma, food, textiles, etc. By adopting efficient, eco-friendly biocatalysts, we can mitigate chemical catalyst pollution, foster green chemistry, and bolster societal sustainability.

5. CONCLUSION

In conclusion, this study successfully developed an efficient and environmentally friendly biocatalysts and optimized their performance through immobilization techniques. The experimental results show that the catalyst has remarkable advantages in catalytic activity, stability and reusability, which conforms to the principle of green chemistry, has the characteristics of environmental protection, sustainable development and wide industrial application. Although this study has achieved a series of positive results, there are still some issues that deserve further exploration: Future research will further refine and optimize this biocatalyst, so that it can play a greater role in a wider range of industrial applications and promote the development of green chemistry.

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REFERENCES

[1] Tao, Junhua, and Jian-He Xu. "Biocatalysis in development of green pharmaceutical processes." Current opinion in chemical biology 13.1 (2009): 43-50.

[2] Sheldon, Roger A., and Dean Brady. "Green chemistry, biocatalysis, and the chemical industry of the future." ChemSusChem 15.9 (2022): e202102628.

[3] Guo, Fei, and Per Berglund. "Transaminase biocatalysis: optimization and application." Green Chemistry 19.2 (2017): 333-360.

[4] Sheldon, Roger A., and John M. Woodley. "Role of biocatalysis in sustainable chemistry." Chemical reviews 118.2 (2018): 801-838.

[5] Wenda, Stefanie, et al. "Industrial biotechnology—the future of green chemistry?" Green Chemistry 13.11 (2011): 3007-3047.