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Microfluidics-based preparation of nano-sized cross-linked enzyme aggregates

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PURPOSE OF THE ABSTRACT

Enzyme immobilization is a promising strategy for improving enzyme stability, activity, and reusability1. Cross-linked enzyme aggregates (CLEAs) are a popular immobilization approach that offers several advantages, including simple preparation, cost-effectiveness, and high enzyme loading 2. However, the quality and stability of CLEAs can be limited by the batch mode of preparation, resulting in non-uniform and unstable aggregates with low retained activity.

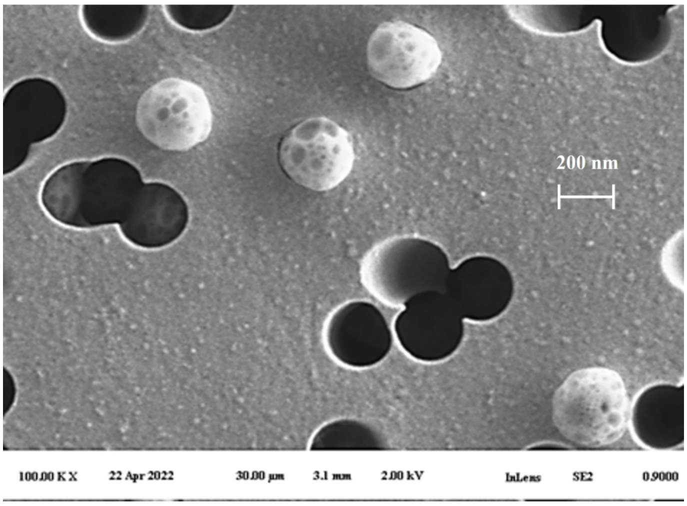
In this study, we present a novel system for preparing uniform and stable CLEAs of nanosize using omega transaminase (ω-TA) as a model enzyme. Our system involves a continuous microflow tubing reactor with controlled flow rates of precipitant and crosslinker that allows for the formation of highly uniform and stable CLEAs. The resulting nanosize CLEAs exhibit enhanced stability compared to conventional CLEAs prepared in batch mode.

The physicochemical properties of the nano-size CLEAs, including size distribution, morphology, and stability, were characterized. The nanosize CLEAs showed a narrow size distribution with an average hydrodynamic radius of about 80 nm and a high uniformity in terms of 0,17 polydispersity index Furthermore, the nanosize CLEAs demonstrated excellent stability under various conditions, including pH, temperature, and storage. Specifically, the nanosize CLEAs retained 87% activity compared to the free enzyme and showed improved stability at higher temperatures.

To further demonstrate the utility of our nano-size CLEAs, we developed a membrane microreactor composed of the CLEAs immobilized onto porous membrane support. Microreactors offer many advantages over traditional batch reactors, such as increased mass and heat transfer rates, improved control over reaction conditions, and decreased enzyme deactivation. The membrane microreactor with immobilized nanoCLEA showed stable and continuous enzymatic activity over a prolonged period of operation, with a significant increase in operational stability compared to the immobilized free enzyme.

Our results demonstrate the potential of our novel system for preparing uniform and stable nano-size CLEAs and subsequent use of these CLEAs in membrane microreactors for improved operational stability. This system can be applied to other enzymes and scaled up for industrial biocatalysis, offering a promising approach for improving the efficiency and sustainability of various biocatalytic processes.

FIGURES



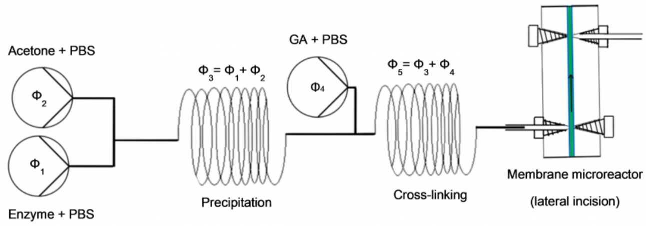


FIGURE 1
SEM image
SEM image of nano-CLEA particles

FIGURE 2
Experimental set-up
The microfluidic system with 3 inlets for ATA-CLEAs generation and their in situ immobilization in a membrane microreactor.

KEYWORDS
Cross-linked enzyme aggregate | enzyme immobilization | flow biocatalysis | membrane microreactor

BIBLIOGRAPHY