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Importantíssimo em imobilização de enzimas:

1) A enzima continua ativa?
2) Quanto de enzima existe na enzima imobilizada? (nano ou não)

<u>Métodos para a quantificação</u>: *massa ou no. mol/massa de suporte ou NP*

- Reagente/ Método de Bradford
- Outros para quantificar proteínas
- Hidrólise total seguida análise de aminoácidos do hidrolisado
- Análise elementar

Biocatalysis 2015; 1: 166–177

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Review

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Susana Velasco-Lozano*, Fernando López-Gallego, Juan C. Mateos-Díaz, Ernesto Favela-Torres

Cross-linked enzyme aggregates (CLEA) in enzyme improvement – a review

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Abstract: Structural and functional catalytic characteristics of cross-linked enzyme aggregates (CLEA) are reviewed. Firstly, advantages of enzyme immobilization and existing types of immobilization are described. Then, a wide description of the factors that modify CLEA activity, selectivity and stability is presented. Nowadays CLEA offers an economic, simple and easy tool to reuse biocatalysts, improving their catalytic properties and stability. This immobilization

industries [1-3]. However, to be profitable at the industrial level, industrial enzymes must allow easy handling and operation procedures, stability and reuse. For this purpose, enzyme immobilization has emerged as a tool to produce robust industrial biocatalysts; and it has been employed over the last forty years for the successful utilization of enzymes in industrial processes [4].

Immobilization is defined as the confinement of enzymes in a defined space, retaining their catalytic activity and allowing their repeated and continuous use [5]. Furthermore, immobilization of enzymes must

Chamados:
carrier-free immobilized enzymes

This immobilization methodology has been widely and satisfactorily tested with a great variety of enzymes and has demonstrated its potential as a future tool to optimize biocatalytic processes.

Como fazer experimentalmente?

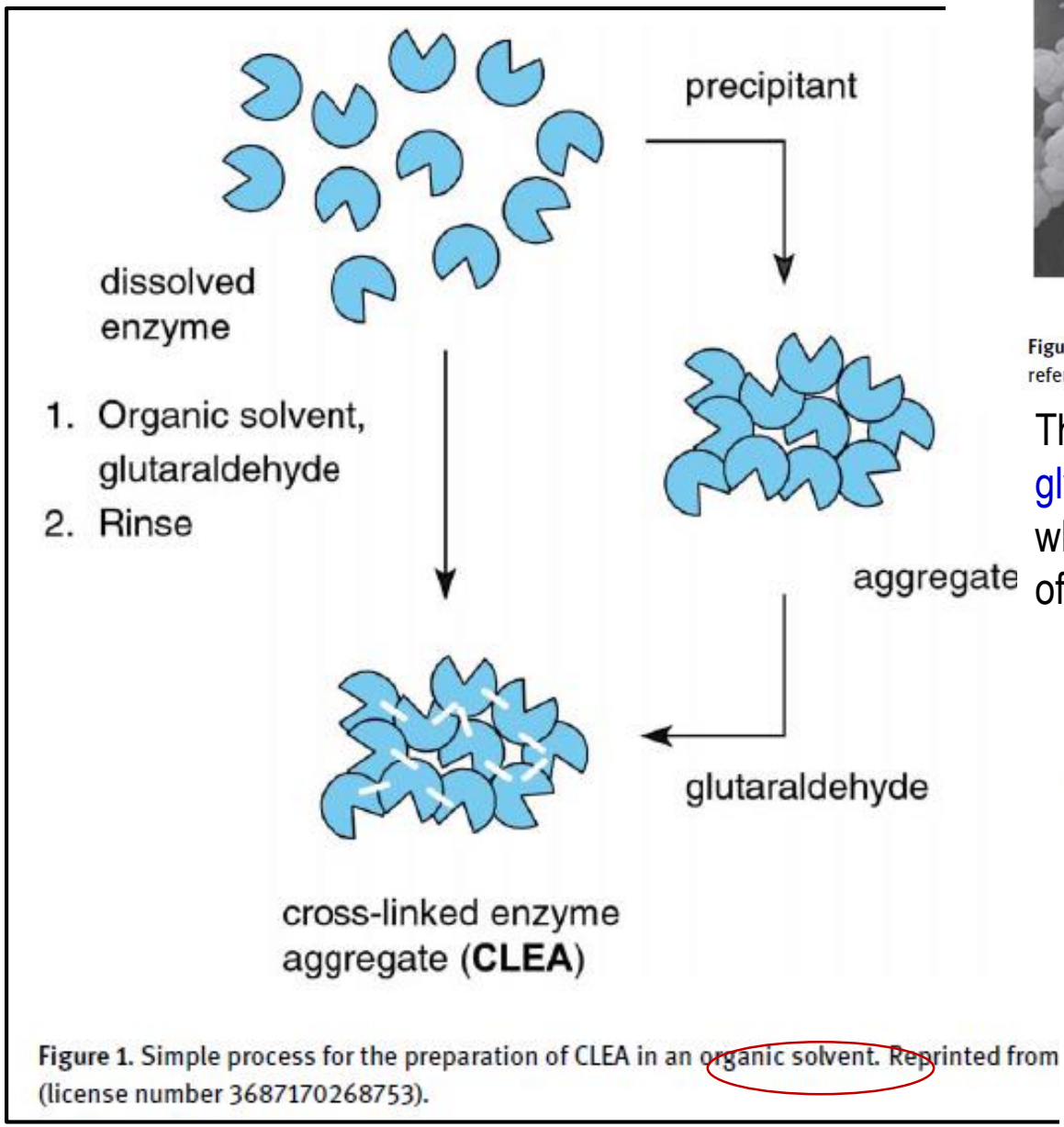


Figure 1. Simple process for the preparation of CLEA in an organic solvent. Reprinted from (license number 3687170268753).

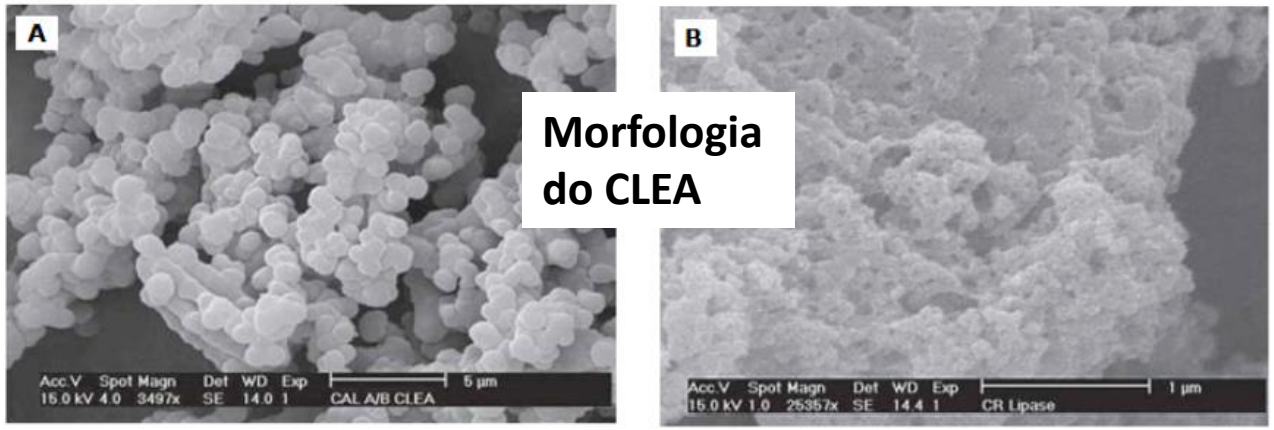


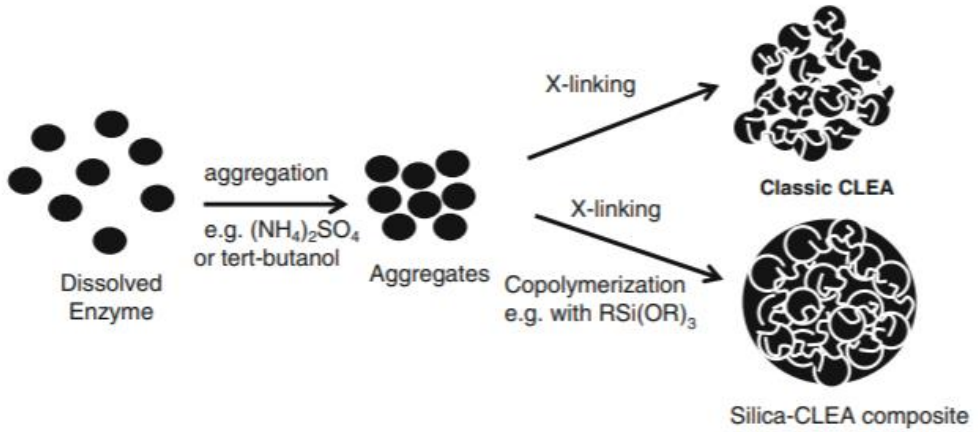
Figure 2. CLEA morphology. A) Type 1, CLEA of CAL-B, amplification X3500; and B) Type 2, CLEA of CRL, amplification X25000. Reprinted from reference [50] with the permission of John Wiley and Sons (license number 3687171223427).

These CLEA measure around 1 µm in diameter and are formed from low glycosylated enzymes with large hydrophobic surfaces such as CAL-B, which contains 8 . 10^8 enzyme molecules per CLEA and forms aggregates of a spherical shape (Fig. 2A)

Grande problema para indústria: instabilidade mecânica

Saída: Coupling CLEA with solid matrixes or nanoparticles

Fig. 1 General scheme for CLEA preparation



Cross-linked Enzyme Crystals - CLECs™

functionally stabilized by
the intermolecular lattice contacts within the crystal and by
chemical cross-links formed with bifunctional reagents such as glutaraldehyde.

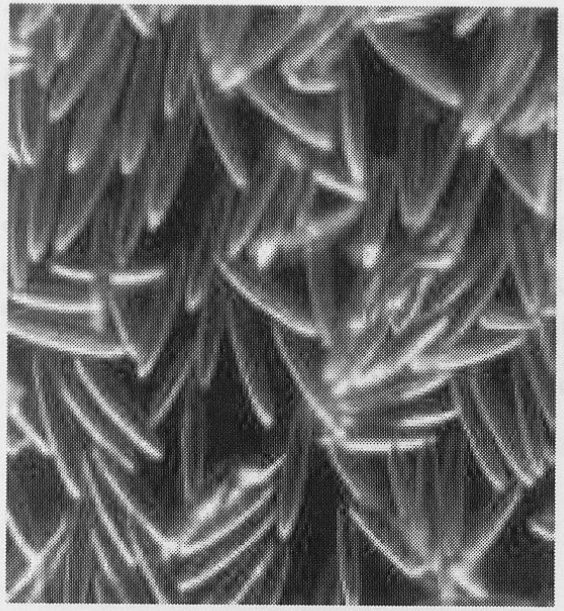


Figure 6-4. Cross-Linked Enzyme Crystals® of Thermolysin, Average Length 40 μm.

CLECs™ of the enzyme used in the manufacture of the artificial sweetener aspartame: *Asp-Phe-OMe*

Remain active in environments that are otherwise incompatible with enzyme function:

- ✓ prolonged exposure to high temperatures,
- ✓ extremes of pH,
- ✓ near-anhydrous organic solvents
- ✓ aqueous-organic solvent mixtures

Remarkably **resistant to autolysis** and **to exogenous protease degradation**.

Crosslinked enzyme crystals (CLECs™) as immobilized enzyme particles. *Studies Org. Chem., 47,1993, 63-73*

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Cross-Linked Enzyme Crystals (CLECs) of Thermolysin in the Synthesis of Peptides

Rose A. Persichetti, Nancy L. S. Clair, James P. Griffith, Manuel A. Navia, and Alexey L. Margolin

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Publication Date: March 1, 1995
https://doi.org/10.1021/ja00115a008

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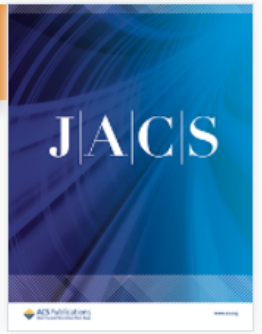

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Strategies in Making Cross-Linked Enzyme Crystals

J. Jegan Roy and T. Emilia Abraham

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