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NATHALIA SARAIVA RIOS

DEVELOPMENT OF STRATEGIES FOR THE PRODUCTION OF BIOCATALYSTS THROUGH IMMOBILIZATION / CO-IMMOBILIZATION OF LIPASE FROM *Pseudomonas fluorescens*

FORTALEZA
2019

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Tese apresentada ao Programa de Pós-Graduação em Engenharia Química da Universidade Federal do Ceará, como requisito parcial à obtenção do título de Doutora em Engenharia Química. Área de concentração: Processos Químicos e Bioquímicos

Orientadora: Profa. Dra. Luciana Rocha Barros Gonçalves.
Coorientador: Prof. Dr. Roberto Fernández Lafuente.

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To God.

To my Family, especially to my parents, Ilzanir and Antonio, my brother, Victor, and my husband, Candido; People who I love that are always by my side.

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"Those who feel satisfied sit and do nothing. The dissatisfied are the only benefactors in the world".

Walter S. Landor

RESUMO

Neste estudo, lipase de *Pseudomonas fluorescens* (PFL) foi imobilizada e co-imobilizada por diferentes estratégias, produzindo uma biblioteca de biocatalisadores capazes de catalisar reações de interesse industrial em diferentescondições operacionais. Os suportes baseados em agarose e nanopartículas magnéticas foram utilizados para a imobilização e co-imobilização de lipases. Para produzir biocatalisadores altamente ativos, a estratégia de imobilização da lipase na sua na forma aberta foi conduzida por adsorção em suportes hidrofóbicos (octil-agarose e octil-nanopartículas), imobilização em suportes heterofuncionais contendo grupos hidrofóbicos (glioxil-octil-agarose) e ligação covalente no suporte ativado na presença de surfactantes (TEOS-nanopartículas). As estratégias de co-imobilização foram derivadas de algumas estratégias de imobilização: multicamadas de PFL foram derivadas da imobilização de PFL por adsorção interfacial em octil-agarose, cuja camada de PFL é imobilizada sobre a anterior para multiplicar a capacidade de carga final do suporte; PFL também foi co-imobilizado com outras lipases (RML ou LU) usando suporte herofuncional (Glioxil-octil-agarose) para reutilizar a lipase mais estável (PFL) após inativação, dessorção e imobilização da lipase menos estável. Esses biocatalisadores co-imobilizados podem catalisar reações enzimáticas em cascata ou catalisar reações envolvendo substratos heterogêneos, como a modificação de óleos e gorduras. Por outro lado, biocatalisadores produzidos por imobilização em suportes à base de agarose geralmente são aplicados para catalisar substratos solúveis (na qual o substrato pode facilmente penetrar nos poros do suporte) e biocatalisadores produzidos por imobilização em suportes baseados em nanopartículas magnéticas geralmente são aplicados na catalise de substratos grandes ou insolúveis, no qual a enzima é imobilizada na superfície do suporte, permitindo o contato da lipase com o substrato.

Palavras-chave: Imobilização. Co-imobilização. Lipase de *Pseudomonas fluorescens*

ABSTRACT

In this study, lipase from *Pseudomonas fluorescens* (PFL) was immobilized and co-immobilized by different strategies, producing a biocatalyst library able to catalyze reactions of industrial interest in some operational conditions. Agarose and magnetic nanoparticles based supports were used as support for lipase immobilization and co-immobilization. In order to produce highly active biocatalysts, the strategy of immobilization in the open-form of lipase was maintained through adsorption on hydrophobic supports (Octyl-agarose and Octyl-nanoparticles), immobilization on heterofunctional supports containing hydrophobic groups (Glyoxyl-octyl-agarose) and covalent attachment on activated support in presence of surfactants (TEOS-nanoparticles). The strategies of co-immobilization were derived of some immobilization strategies: Multilayers of PFL were derived from the immobilization of PFL by interfacial adsorption on Octyl-agarose, which one layer of PFL is immobilized over the previous to multiply the final loading capacity of the support; PFL also was co-immobilized with other lipases (RML or LU) using the hererofunctional support (Glyoxyl-octyl-agarose) to reuse the more stable lipase (PFL) after inactivation, desorption and immobilization of the least stable lipase. These co-immobilized biocatalysts catalyze enzymatic cascade reactions or catalyze reactions involving heterogeneous substrates, such as modification of oils and fats. On the other hand, biocatalysts produced by immobilization on agarose-based supports generaly are applied to catalyze soluble substrates (which the substrate can easily penetrate into the pores of the support) and biocatalysts produced by immobilization on magnetic nanoparticles-based supports generaly are applied to catalyze insoluble or large substrates, which the enzyme is immobilized on the surface of the support, enabling the contact of the lipase with the substrate.

Keywords: Immobilization. Co-immobilization. Lipase from *Pseudomonas fluorescens*

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LIST OF ABBREVIATIONS

BQ	*p*-Benzoquinone
CTAB	Cetyltrimethylammonium Bromide
DVS	Divinylsulfone
FTIR	Fourier-Transform Infrared spectroscopy
GA	Glutaraldehyde
LU	Lecitase Ultra
OA	Octyl-agarose
OCTYL-NANO	Nanoparticles coated with octyltriethoxysilane
OTES	Octyltriethoxysilane
*p*NPB	*p*-Nitrophenyl Butyrate
PFL	Lipase from *Pseudomonas fluorescens*
RML	Lipase from *Rhizomucor miehei*
SDS	Sodium dodecyl sulfate
TEOS	Tetraethoxysilane
TEOS-NANO	Nanoparticles coated with tetraethoxysilane
VSM	Vibrating Sample Magnetometry

SUMMARY