Secteur des Sciences et Technologies

Invitation à la soutenance publique de thèse de

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Master bioingénieur : chimie et bio-industries

Pour l'obtention du grade de Docteur en sciences agronomiques et ingénierie biologique

« Design of heterogeneous biocatalysts for flow chemical processes- towards greener transamination reactions»

qui se déroulera

le vendredi 19 octobre 2018 à 16h00

Salle Jean-Baptiste Carnoy

Place Croix du Sud, 4-5

1348 Louvain-la-Neuve

Membres du jury :

Prof. Damien Debecker (UCLouvain), supervisor
Prof. Patrice Soumillion (UCLouvain), supervisor
Prof. Yann Garcia (UCLouvain), chairperson
Prof. Eric Gaigneaux (UCLouvain), secretary
Prof. Patricia Luis Alconero (UCLouvain)
Prof. Francesca Paradisi (University of Nottingham, UK)
Dr. Cédric Boissière (Université Pierre et Marie Curie, France)

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Since industrial era has begun, chemical industries have released large amount of hazardous compounds in the environment. They produced tons of toxic, polluting and non-recoverable by-products, using large quantities of reactants, solvent and energy. In particular, pharmaceutical industries are largely responsible for that issue: drugs must be produced in large amount, with high purity, and quickly to meet the consumer demand.

Transaminases (TA) have been used for the green production of chiral amines (precursors of many drugs). There are two main limitations to the use of TA: the price of enzymes, and the unfavourable thermodynamics. Immobilizing TA allows to recover the biocatalyst and to reuse them in further transformations. Working in flow mode allows to increase the overall process productivity. Strategies to displace equilibrium allow performing the asymmetric synthesis (*i.e.* the production of chiral amine of industrial interest).

The main objectives are (i) developing suitable carriers for enzyme immobilization; (ii) finding an optimal method for enzyme immobilization while retaining activity; (iii) assessing biocatalysts efficiency in flow mode; (iv) the development of strategies to displace equilibrium towards the asymmetric synthesis. At the end, a standard method (for flow reactions using immobilized enzymes) is developed, and is applied to any enzyme or reaction.

Several immobilization methods are investigated (simple adsorption, covalent grafting, His-tag immobilization, etc.). Porous silica monoliths -Si(HIPE)- are employed as solid carriers suitable for flow transformations. Carriers are deeply characterized. Biocatalysts are systematically tested in flow mode to assess enzyme activity.

We developed a suitable flow device for flow transformations. The enzyme is studied in batch and flow modes after several optimizations. Transaminases are active when immobilized onto silica monoliths through covalent grafting (monoliths functionalized with APTES (3-aminopropyltriethoxysilane) and pre-activated with glutaraldehyde). Enantioselectivity is retained, and enzymes are stable over long period of time.

APTES functionalization was optimized: a better dispersion, higher repeatability and activity (when used as enzyme carrier) were obtained when using water saturated toluene and dry monoliths. The influence of temperature on enzyme impregnation was highlighted. Imine reduction was studied to get even more stable biocatalysts over time.

Two methods of synthesis of hydrophobic monoliths were studied: the one-pot method led to brittle monoliths; the post-grafting method led to robust hydrophobic monoliths. However, hydrophobicity did not influence the flow biocatalysts activity.

A His-tagged transaminase (His-TA) was produced with high purity. Immobilization through covalent grafting and His-tag were studied. Best results (maximal yield in flow mode) were obtained when His-TA was covalently immobilized onto APTES grafted monoliths.

An equilibrium displacement strategy was selected for implementation in flow mode. Satisfying activity in flow was obtained (using His-TA covalently immobilized).

Finally, we applied our strategy to another transformation (the tyrosine deamination) catalysed by another enzyme, the tyrosine ammonia-lyase (TAL). Using the same device and the best carrier developed for TA, immobilized TAL were highly active in flow mode.