LACCASES IMMOBILIZED ON MAGNETIC PARTICLES AND THEIR APPLICATION IN A BIOREACTOR FOR THE ELIMINATION OF ENDOCRINE DISRUPTING CHEMICALS

P. Demarche, C. Junghanns, R. R. Nair and S. N. Agathos
Earth & Life Institute - Lab of Bioengineering, University of Louvain, Croix du Sud 2/19, 1348 Louvain-la-Neuve, Belgium
E-mail: philippe.demarche@uclouvain.be

Introduction Laccases are oxidative enzymes capable of degrading a variety of xenobiotics, e.g. phenols, dyes or endocrine disrupters, thereby often reducing their toxicity. Despite their biotechnological potential, laccases are still restricted to laboratory scale due to their high production costs. Immobilization allows the biocatalyst recovery and recycling, together with improving stability. Nowadays, there is a growing interest in using magnetic materials for the for protein immobilization in biotechnology, due to simplified downstream processing, exploiting paramagnetic properties of the support material. In this study, different commercial paramagnetic microparticles were tested for effective laccase binding. The biocatalysts' stability against temperature was evaluated. One biocatalyst was selected for an application in a fluidized bed reactor with a magnetic retention principle for anthraquinonic dye decolourization.

Laccase immobilization

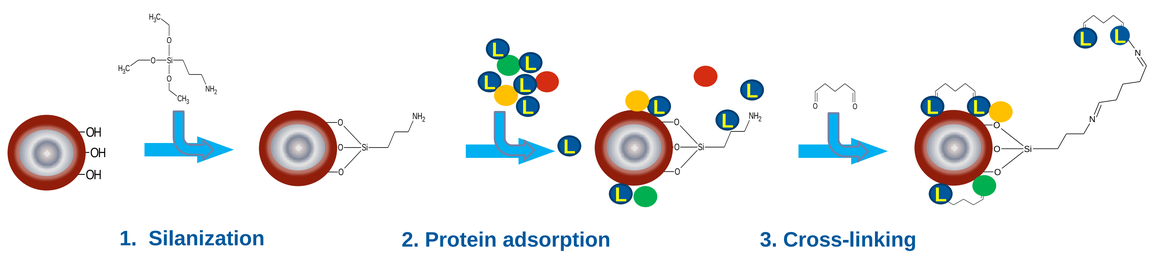

Figure 1 Immobilization procedure. Particle concentration was about 50 mg/L. 3-aminopropyltriethoxysilane 10 % (v/v) was used for silanization. C*oriolopsis polyzona* culture supernatants (10 kDa filtrated) was used as laccases source (= laccase). Glutaraldehyde 5 % (v/v) served as cross-linker.

A laccase preparation from *Coriolopis polyzona* (MUCL38443) was immobilized on paramagnetic supports. These non-porous composite particles were made of a maghemite core covered by silica (15 to 25 % iron oxide). The immobilization procedure was simplified using magnets instead of centrifugation to recover particles from the bulk. The protein preparation was absorbed prior to cross-linking with glutaraldehyde, promoting inter-protein reticulation (Figure 1).

Table 1 Physical characteristics of paramagnetic particles and their activity after immobilization. [a]SiMag1 was provided functionalized (Chemicell, GmbH, Germany). [b]Hydrodynamic diameter. [c]Particles were provided at 50 % particle concentration compared to other particles. SiMag2,4,7 were purchased from Microparticles GmbH, Germany.

Particles	Diameter (µm)	Specific surface area (m²/kg)	Activity (U/g)
[a]SiMag1	[b]1.0 ± n.c	3000 ± n.a.	594 ± 9
SiMag2	2.47 ± 0.07	1500 ± 43	166 ± 23
[c]SiMag4	4.26 ± 0.17	880 ± 70	160 ± 6
SiMag7	7.52 ± 0.26	499 ± 35	124 ± 2

The specific activities achieved are presented in Table 1. Immobilization efficiency decreased with the particle diameter (Fig. 2), SiMag1 was most effective for laccase binding, with 50 % expressed activity and 30 % immobilization yield. Except SiMag1, all biocatalysts showed higher thermal long-term stability compared to free enzyme (Fig. 4).

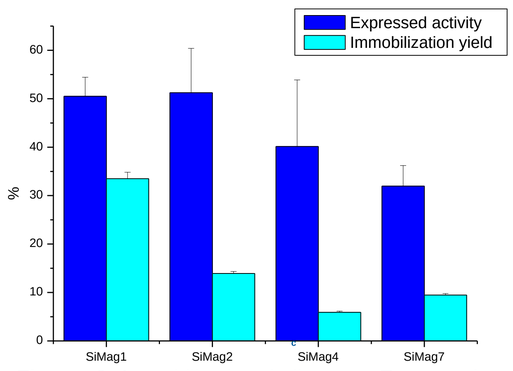

Figure 2 Immobilization indicators. Expressed activity [%] = theoretically bound activity / biocatalyst activity. Immobilization yield [%] = biocatalyst activity / initial activity. For abbreviations refer to in Table 1. Given are means (n=3) and S.D.

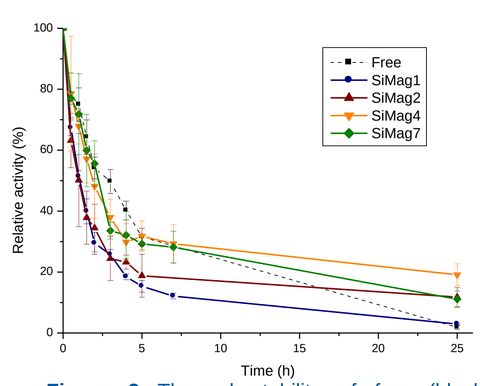

Figure 3 Thermal stability of free (black dashed) and immobilized enzyme (solid line) at 60 ° C, pH 5. Given are means (n=3) ± S.D. For abbreviations refer to Table 1.

Fluidized bed reactor with a magnetic retention principle

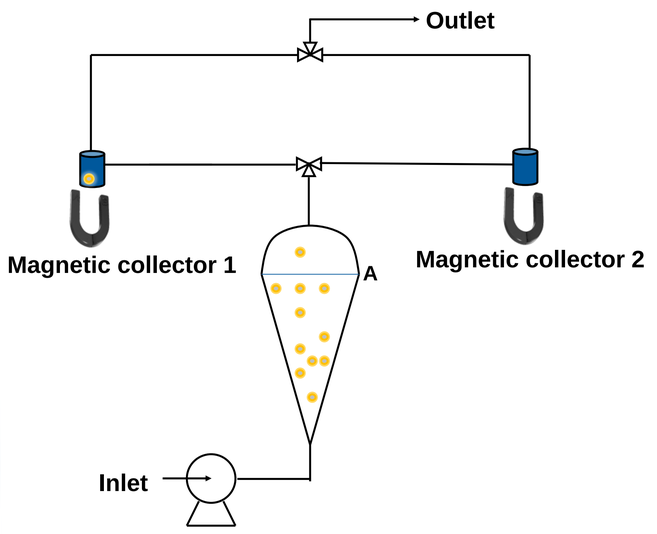

Figure 4 Experimental setup of a lab-scale fluidized bed reactor with a magnetic retention principles.

A fluidized bed reactor, based on a 50 mL separation funnel, was used for the continuous decolourization of Acid Blue 62 (30 mg/L, pH 5.0, 23 ° C) using laccase immobilized on *SiMag4* (20 U/L).
The design of the reactor allowed continuous operation by retaining the biocatalysts in collectors with permanent magnets and subsequently refeeding them in the reactor.

Investigations have been carried out to setup a fluidized bed reactor system with a minimal washout of the biocatalysts. The condition to achieve a stable fluidized bed is summarized in the inequality [3], settling velocity (V_S) and rise velocity of liquid (V_f) are given by equation [1] and [2].

$$V_s = \frac{2}{9}.\frac{(\rho_p - \rho_f)}{\mu}.g.r^2 \quad [1] \qquad V_f = \frac{\dot{V}}{A} \quad [2] \qquad V_s \geq V_f \quad [3]$$

ρ_p = particle density, ρ_f = liquid density, μ = dynamic viscosity, g = standard gravity, r = particle radius, $\dot{V}$ = Volumetric flow, A = the largest cross section area.

Table 2 Estimated settling velocity (V_S)

Particles	V_f (m/s)	V_s (m/s)
SiMag1[a]	4.17*10^{-6}	5.47*10^{-7}
SiMag2		1.99*10^{-6}
SiMag4		**4.95*10^{-6}**
SiMag7		1.85*10^{-6}

For the dimensions of the applied reactor and the operational conditions, (A = 20 cm², flow = 0.5 cm³/min), *SiMag4* was a suitable choice (Table 2).

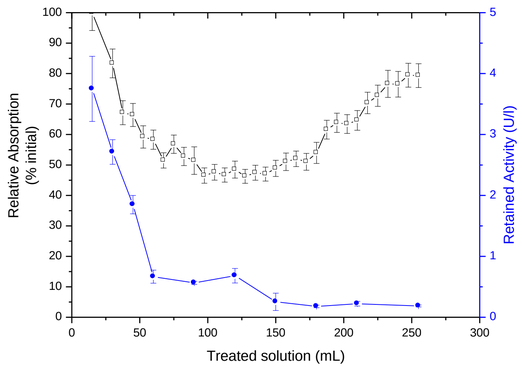

Figure 4 Time course of absorbance and magnetically retained activity during continuous treatment of 30 mg/L ABu62 solution. Reactor volume 99 mL. HRT = 199 min. Initial reactor activity = 20.1 U/L

The reactor was operated over a period of 8 h and no activity was observed in the effluent confirming an **stable laccase immobilization** and demonstrating a **complete capture of washed out catalysts in the magnetic collector.**

Summary & Outlook Laccase preparation from *Coriolopsis polyzona* was immobilized on four commercial paramagnetic particles of different size. Produced biocatalysts had laccase activities up to 594 U/g. The stability study at 60 ° C showed a higher long-term stability compared to free laccase. One selected biocatalyst was used for dye decolourization in a fluidized bed reactor. 100 % of the activity were retained in the magnetic collector, demonstrating the operability of the designed reactor. In the future, reactor setup will be improved and the final purpose of this work will be the long-term continuous degradation of endocrine disruptors, e.g. bisphenol A or nonylphenol, by laccases immobilized on paramagnetic particles.

Abbreviations: ABu 62= Acid Blue 62, HRT= Hydraulic retention time

Acknowledgement
The presented work is funded by the LANCE project (EU and Walloon Region, contract no.917011)