INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING

Modern methods for assessment of enzyme activity in microcapsules

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Outline

Characterization of encapsulated enzyme

1. Encapsulation efficiency

- Protein amount and location,
- Conformational enzyme changes,
  - Circular Dichroism (CD),
  - Differential Scanning Calorimetry (DSC),
  - Fourier Transform Infrared (FTIR),
  - X-ray diffraction pattern.
- Protein versus enzyme activity
- Enzyme activity,

2. Catalytic performance

- Recycling stability,
- Mass-transfer property,
- Thermal stability,
- Substrate conversion,
- Organic solvent tolerance,
- Storage efficiency and stability,
- Sweeling property,
- Microenvironment and changes with catalysis.

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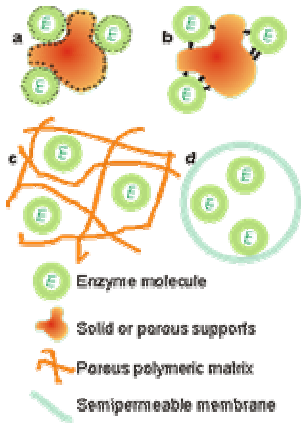
Enzyme and cell immobilization

Methods for immobilizing of enzymes and cells :

- adsorption (a)
- covalent binding (b)
- entrapment (c)
- encapsulated within a semi-permeable membrane (d)



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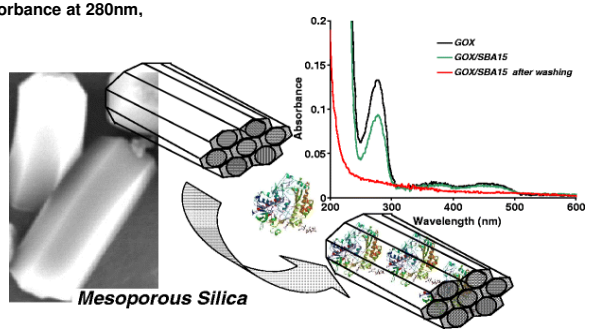
Encapsulation efficiency – Protein amount and location

1) Indirect encapsulated protein or enzyme by mass balance.

Encapsulation efficiency (%) = (1 – $Prot_{supernatant}$) / ($Prot_{dissolved}$) x 100

2) Direct protein assay in microcapsules are:

- Absorbance at 280nm,



Ispas C. et al. Anal Bioanal Chem (2009) 393:543–554

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Encapsulation efficiency – Protein amount and location

- Absorbance at specific wavelength for color proteins such as HRP at 403nm,

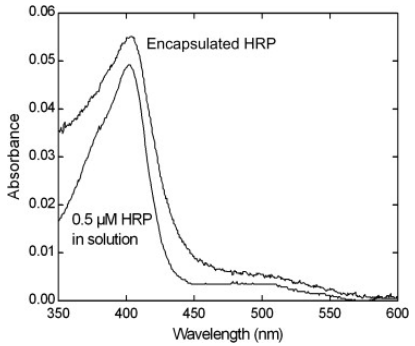


Figure: Absorption spectrum of native horseradish peroxidase entrapped in polyacrylamide nanoparticles.

Poulsen A.K. et al. Anal. Biochem. (2007) 366 (1) 29-36

Encapsulation efficiency – Protein amount and location

- Tagged proteins with colorimetric dyes, fluorescent compounds, and isotopic labeled compounds such as ^{125}I.

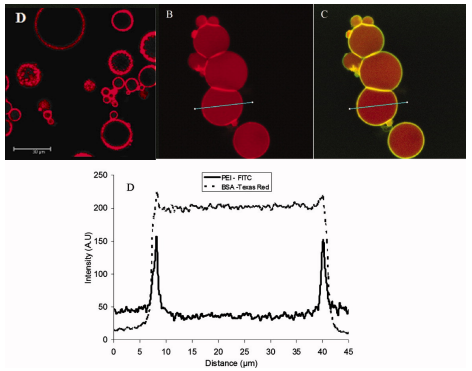


*Figure: Fluorescence signals (red signal) of tagged BSA PEI microcapsules detected by **confocal laser scanning**.*

Kouisni L and Rochefort D., J. Apllied Polymer Science 111 (2008) 1-10

Encapsulation efficiency – Protein vs Enzyme activity

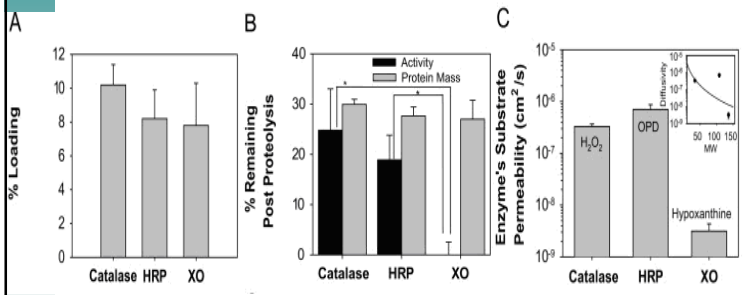


Figure: (A) ^{125}I-labeled encapsulated enzymes into PNC, (B) Residual enzymatic activity and load of radiolabeled enzyme into PNC, (C) Analysis of substrate permeability (a product of partitioning and diffusivity).

Dziubla T.D. et al., Biomaterials 29 (2008) 215-227

Encapsulation efficiency – Conformational changes

- Circular Dichroism (CD)

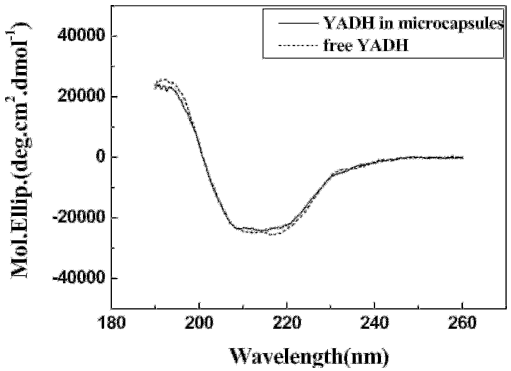


Figure: CD spectra of free and encapsulated YADH in calcium phosphate-mineralized chitosan/alginate microcapsules.

Jiang Y et al., Ind. Eng. Chem Res. 47 (2008) 2495-2501

Encapsulation efficiency – Conformational changes

- Differential Scanning Calorimetry (DSC),

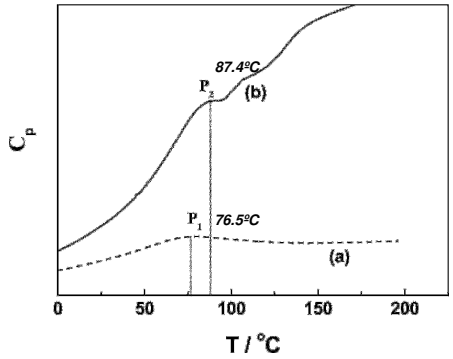


Figure: DSC thermograms (unfolding temperature) of free and encapsulated YADH in calcium phosphate-mineralized chitosan/alginate microcapsules.

Jiang Y et al., Ind. Eng. Chem Res. 47 (2008) 2495-2501

Encapsulation efficiency – Conformational changes

- X-ray diffraction pattern,

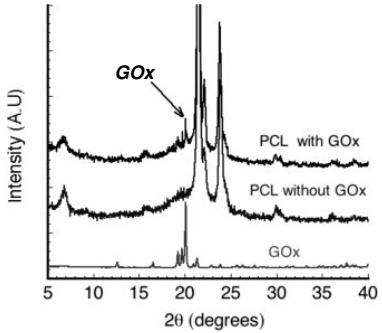
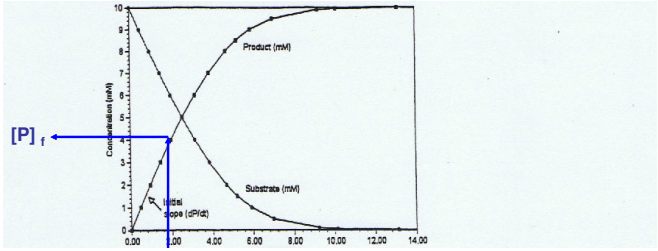


Figure: X-ray diffraction pattern of PCL microparticles with and without GOx encapsulated inside and X-ray diffraction pattern from pure crystalline GOx.

Rubio-Retama J. et al., J. Biomed. Mat. Res. partB: Appl. Biomat. 83B (2007) 145-152

Encapsulation efficiency – Encapsulated enzyme activity at end-point assay



T_f end time of reaction

Activity (μmol/(ml.min)) = Δ[P] / Δt = $[P]_f$ / t_f

Encapsulated enzyme activity at end-point assay is commonly used for conventional analytical approaches characterized by long analysis time such as:
- Gas chromatography (GC),
- High-performance liquid chromatography (HPLC),
- Mass spectrometry or NMR.

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Encapsulation efficiency – Encapsulated enzyme activity at end-point assay

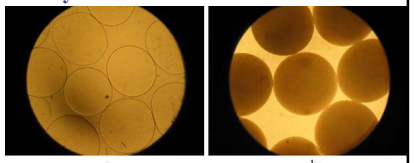

*Figure: Photomicrographs of APA microcapsules: **a** control (empty) and **b** encapsulating feruloyl-esterase-producing L. fermentum 11976 cells*

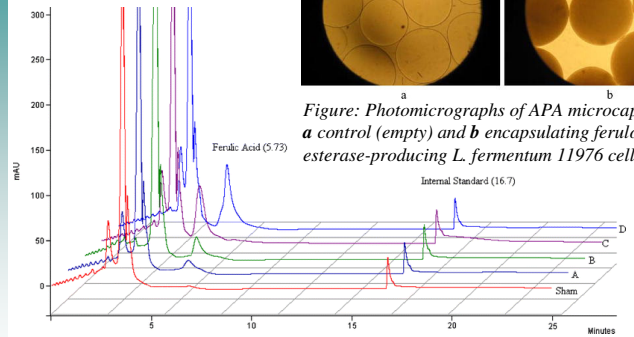

Figure: Overlaid HPLC chromatograms of the samples (10 h) the peak areas of ferulic acid indicate feruloyl esterase activity of different encapsulated Lactobacillus cells in alginate poly-L-lysine-alginate .

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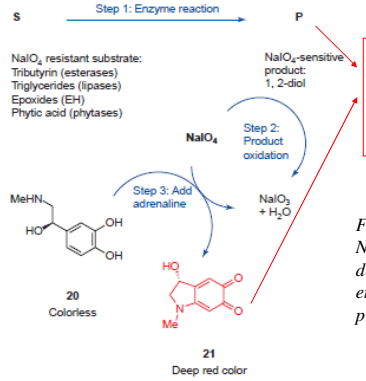
Bhathena J. et al. Appl. Microbiol. Biotechnol. 75 (2007) 1023-1029

Encapsulation efficiency – Encapsulated enzyme activity at end-point assay by spectrometric methods

Indirect product detection as an end-point is also possible by the principle of **back-titration**. It can be realized by applying a reagent that specifically detects a functional group that is formed in the reaction.



The product in the sample collected from reaction media is proportional to the decrease in adrenochrome formation.

Figure: Back-titration of the unreacted NaIO4 with adrenaline (20) to form the deeply colored adrenochrome (21) enables quantification of the extent of product formation.

Wahler D. et al., Tetrahedron, 3 (2004) 703-710

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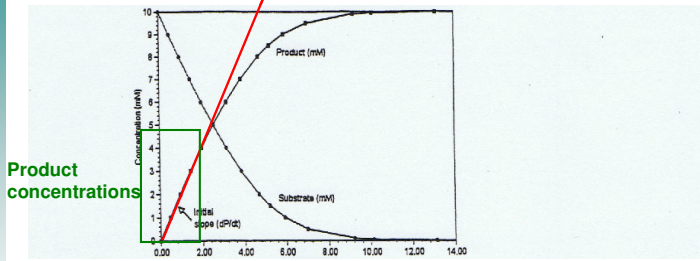
Encapsulation efficiency – Encapsulated enzyme activity at real-time assay

Reaction velocity for t ~ 0



Product concentrations

Sampling period

aA + bB → cC $\quad v_o = \frac{1}{c}\frac{d[C]}{dt} = -\frac{1}{a}\frac{d[A]}{dt} = -\frac{1}{b}\frac{d[B]}{dt}$

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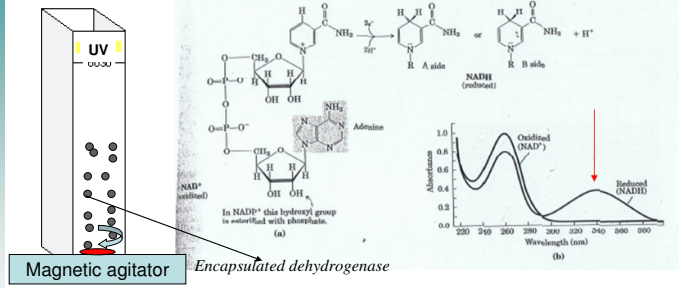
Batch activity assay for nano- and micro-beads based on natural, unmodified substrates,

$$CH_3CHOHCOOH + NAD^+ \overset{LDH}{\rightleftharpoons} CH_3COCOOH + NADH + H^+$$



LDH Activity (µmol NADH/(ml.min)) = d [NADH] / d t

The absorbance particles was subtracted by placing particles devoid of dehydrogenase in the reference cuvette.

Ma L. et al., Enz. Microb. Technol., 42 (2008) 235-241

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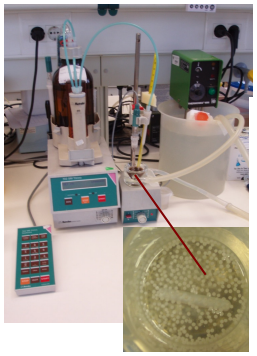
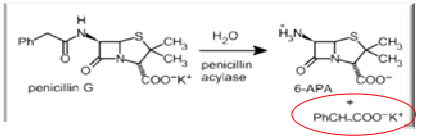
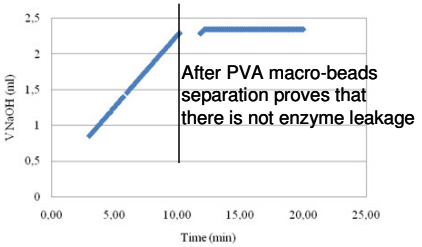
Batch activity assay for nano, micro-and macro-beads based on natural, unmodified substrates,

Real time titration with a standard alkaline solution allows to keep a constant value of pH in reaction media and quantify the H^+ formation resulting from hydrolysis of penicillin G.







After PVA macro-beads separation proves that there is not enzyme leakage

Maduro, F. (not published data) adapted from Fonseca L.P. et al. J. Chem. Tech. Biotechnol. 58 (1993) 27-37

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Batch activity assay for nano- and micro-beads based on synthetic labeled substrates,

Synthetic labeled substrates produce a color or a fluorescent change with a direct connection between enzymatic activity and the optical signal.

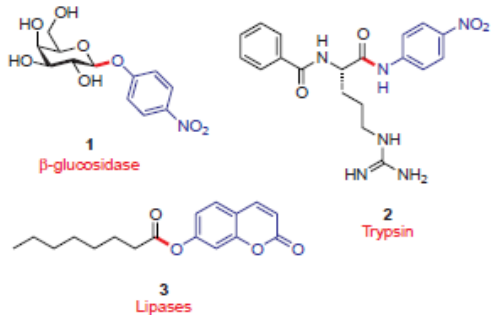
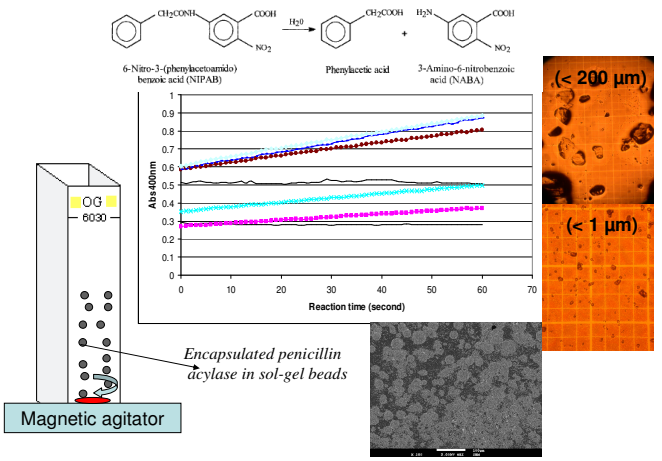


Figure: Chromogenic and fluorogenic substrates with activated leaving groups. Substrates are shown for glycosidases (1), proteases (2) and lipases and esterases (3).

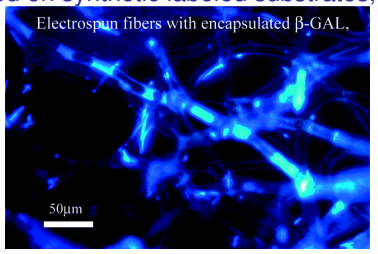
Goddard J-P and Reymond J-L Trends in Biotechnol. 22 (7) (2004) 363-370.

Batch activity assay for nano- and micro-beads based on synthetic labeled substrates,



Fonseca, L.P. (not published data) adapted from Azevedo A. J. Chem. Tech. Biotechnol. 74 (1999) 1110-1116

Batch activity assay for nano- and micro-beads based on synthetic labeled substrates,



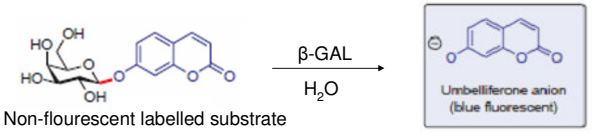
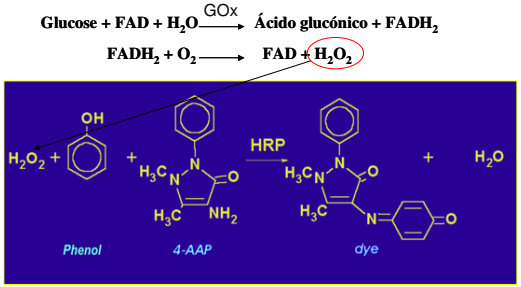


Figure: The imaging of fluorescent microtubes fabricated by co-electrospinning filled with a fluorescent product (derivative UMBELLIFERONE) resulting from encapsulated β-galactosidase hydrolysis of derivative UMBELLIFERYL-β-d-galactoside.

Dror Y. et al., Macromolecules, 41 (2008) 4187-4192.

Enzyme activity assay by sensing reactions using combining multi-enzyme reactions

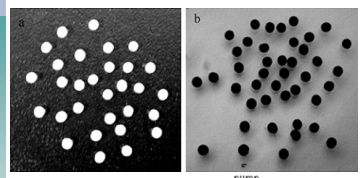
Oxidase activity assay by H_2O_2 formation and combining with HRP which oxidases phenolic compounds and dye precursors as shown in Trinder Reaction (Quinoneimine λ_{max} = 490-520nm) with ε_{490nm} = 5.56 mM^{-1} cm^{-1}.



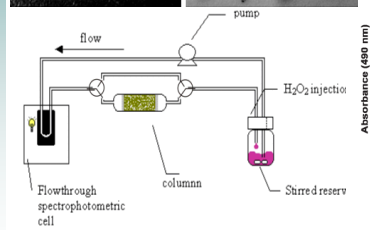
Oxidase activity (µmol/(ml.min)) = $\frac{dc_{H_2O_2}}{dt} = \frac{1}{\varepsilon * l} * \frac{dA}{dt}$

Vojinovic, V. et al., Biosens. Bioelectron., 20 (2005) 1955-1961.

Batch activity assay methods for macro-beads by sensing reactions using combining multi-enzyme reactions



The reaction activity assay media flow through micro-reactor column packed with macro-beads (> 1000 µm) *before flow again through continuous optical cell.*



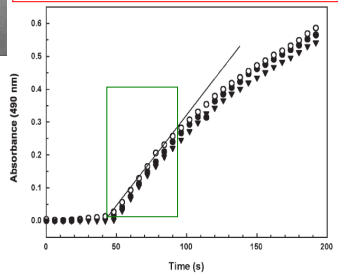


Figure: Time course of reaction for encapsulated HRP assay by Trinder Reaction Method.

Lourenço, N. (not published data) adapted from Vojinovic, V. et al., Biosens. Bioelectron., 20 (2005) 1955-1961.

Batch activity assay methods for macro-beads by sensing reactions using flow calorimetry,

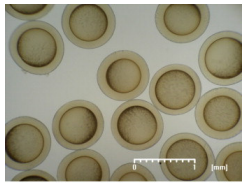


Figure: GOx–SA–CS/PMCG capsules stored in 0.9% (w/v) NaCl solution: Detail of capsules observed by optical microscopy. Length of a scale bar represents 1 mm.

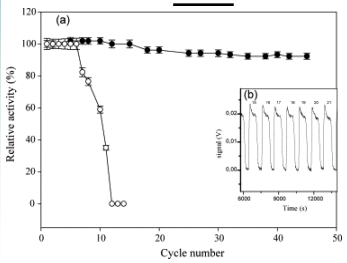
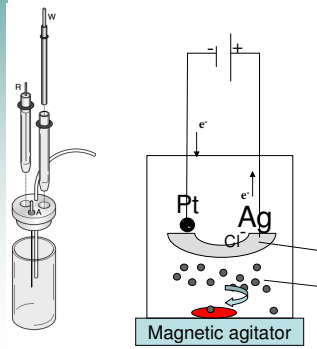


Figure: (a) Operational stability of GOx–SA–CS/PMCG capsules (●) and GOx–CPG beads (○) determined by **flow calorimetry**; (b) **thermograms** (steady states) registered by software-Labtech.

Vikartovsá, A. et al., Enz. Microb. Technol., 41 (2007) 748-755.

Oxidase activity assay by electrochemical methods

The O_2 concentration in the cell is measured by the current generated by the reduction of O_2 at a Pt electrode poised at a potential of -0.6 V (vs. Ag/AgCl).



Electrochemical reactions

Cathode Pt (-600mV) $O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$

Anode $4Ag + 4Cl^- \longrightarrow 4AgCl + 4e^-$

Teflon membrane permeable to O_2

Encapsulated laccase PEI macro-beads

Kouisni L and Rochefort D., J. Apllied Polymer Science 111 (2008) 1-10

Oxidase activity assay by electrochemical reduction of O_2

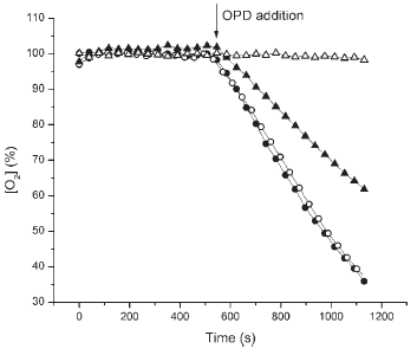
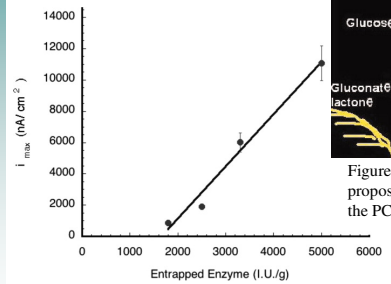


Figure: The enzyme activity of encapsulated laccase was measured by oxidation of o-phenylenediamine (OPD) in oxygen cell, to avoid complications due to the turbidity of the PEI capsule suspensions

Kouisni L and Rochefort D., J. Apllied Polymer Science 111 (2008) 1-10

Oxidase activity assay by electrochemical reduction of H_2O_2



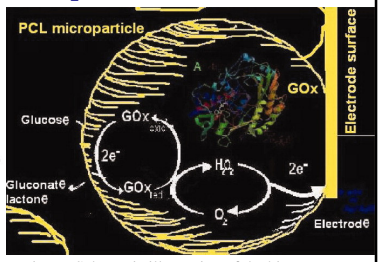
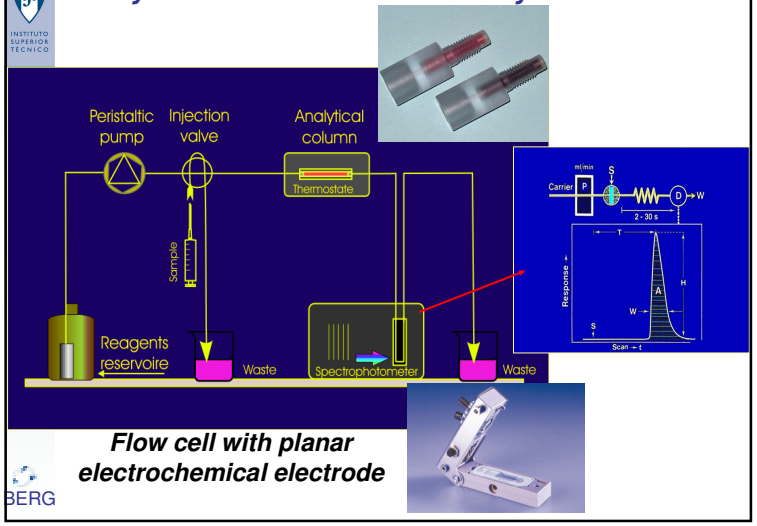


Figure: Schematic illustration of the biosensor proposed for measuring the enzymatic activity of the PCL microparticles with encapsulated GOx.

Figure: Current response produced by polymer microparticles at saturated glucose concentrations as a function of amount of GOx entrapped inside de micropaticles.

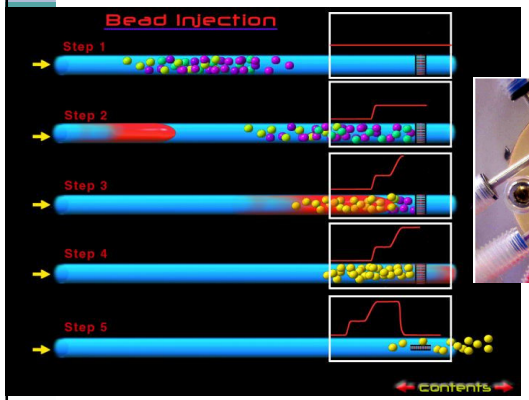
Rubio-Retama J. et al., J. Biomedical Mat. Res. Part B: Appl. Biomaterial., 83B (2007) 145-152.

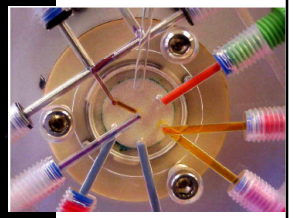
Enzyme micro-reactors in FIA systems



Flow cell with planar electrochemical electrode

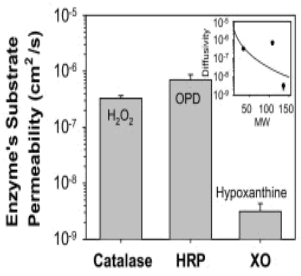
Enzyme activity assay by Beads Injection Analysis (BIA) System





Catalytic performance of encapsulated enzyme preparations

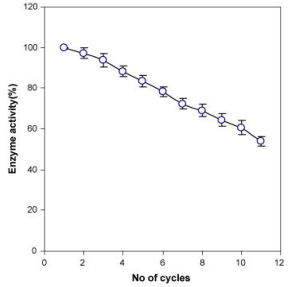
Encapsulated enzymes in PLGA



Mass-transfer resistance

Dziubla T.D. et al., Biomaterials 29 (2008) 215-227

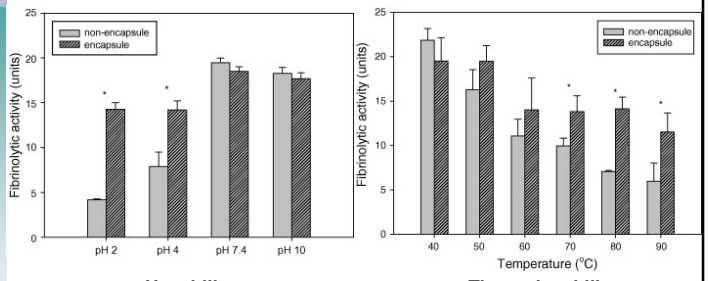
Encapsulated subtilisin in Xerogels



Recycling stability

Sangeetha K. et al., Appl. Cat. A: General, 341 (2008) 168-173.

Catalytic performance of encapsulated enzyme preparations

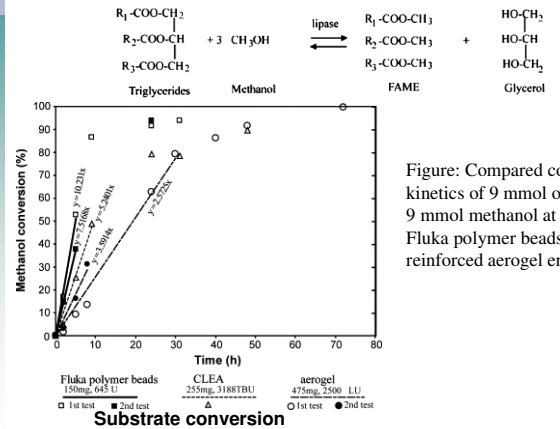


pH stability **Thermal stability**

Figure: The effect of pH and temperature on the encapsulated fibrinolytic activity in alginate microcapsules and free in soybean paste Cheonggukjang (CGJ) extract.

Ko J.A. et al., Food chemistry, 111 (2008) 921-924.

Catalytic performance of encapsulated enzyme preparations



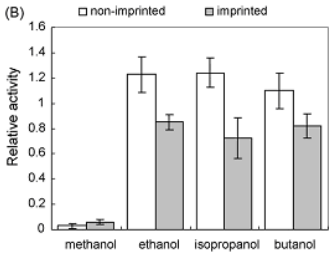
Substrate conversion

Figure: Compared conversion kinetics of 9 mmol oil with 9 mmol methanol at 40 °C with Fluka polymer beads, CLEA and reinforced aerogel encapsulated.

Nassreddine S. et al., Appl. Cat. A: General, 344 (2008) 70-77.

Catalytic performance of encapsulated enzyme preparations

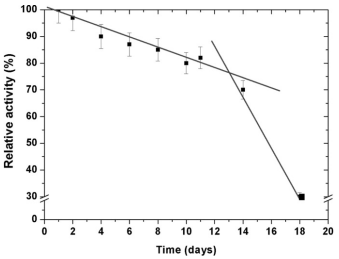
Encapsulated B. cepacia lipase in Sol-gel



Solvent tolerance

Cao X. et al., Proc. Biochem., 44 (2009) 177-182.

Encapsulated GOx in PLC

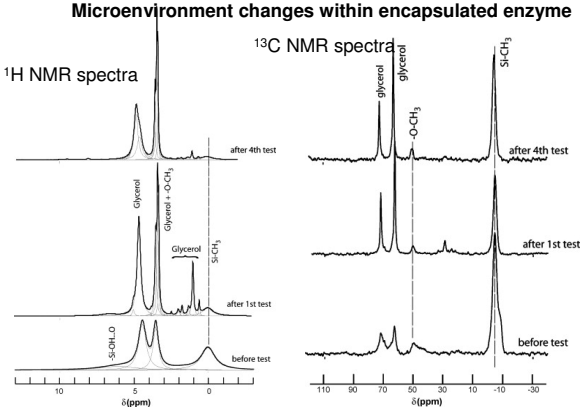


Storage stability

Rubio-Retama J. et al., J. Biomedical Mat. Res. Part B: Appl. Biomaterial., 83B (2007) 145-152.

Catalytic performance of encapsulated enzyme preparations

Microenvironment changes within encapsulated enzyme



Nassreddine S. et al., Appl. Cat. A: General, 344 (2008) 70-77.

Catalytic performance of encapsulated enzyme preparations

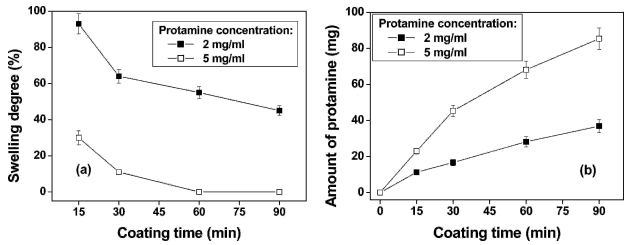


Figure 5. Effect of the coating time and protamine concentration on: (a) the swelling degree of the alginate/protamine/silica (APSi) capsules and (b) the amount of protamine coated on the alginate (Alg) capsules.

Zhang Y. et al., Chem. Mate., 20 (2008) 1041-1048.

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Thanks for your attention

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