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Enzymatic production of alcohols by valorisation of volatile fatty acids embedded in anaerobic digestate

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Introduction

Wastewater valorization

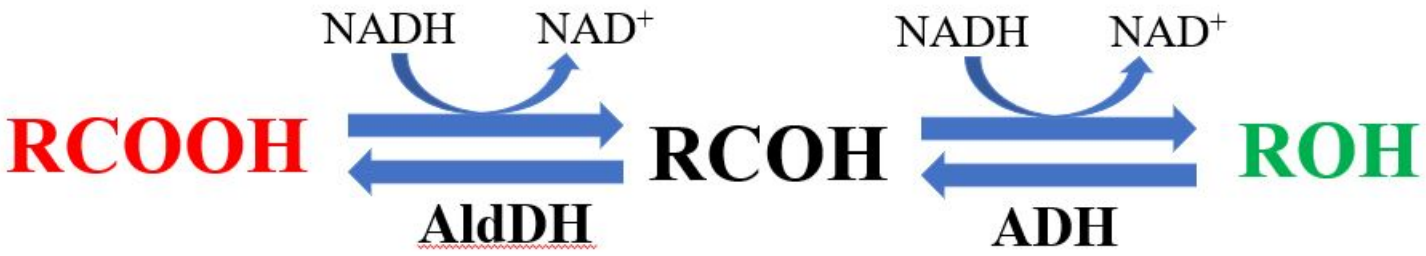
Carboxylic acids embedded in anaerobic digestate → Bio-alcohols →

- ❑ Fuels;
- ❑ Fragrances;
- ❑ Emollients;
- ❑ Thickeners;
- ❑ Plasticizers.

1. Thermochemical conversion
2. Microbial conversion
3. Enzymatic conversion

- Renewable Catalysts
- Possibility to operate with environmental P and T
- High selectivity
- Cost
- Stability
- Often dependent on a cofactor

Carboxylic acid reduction



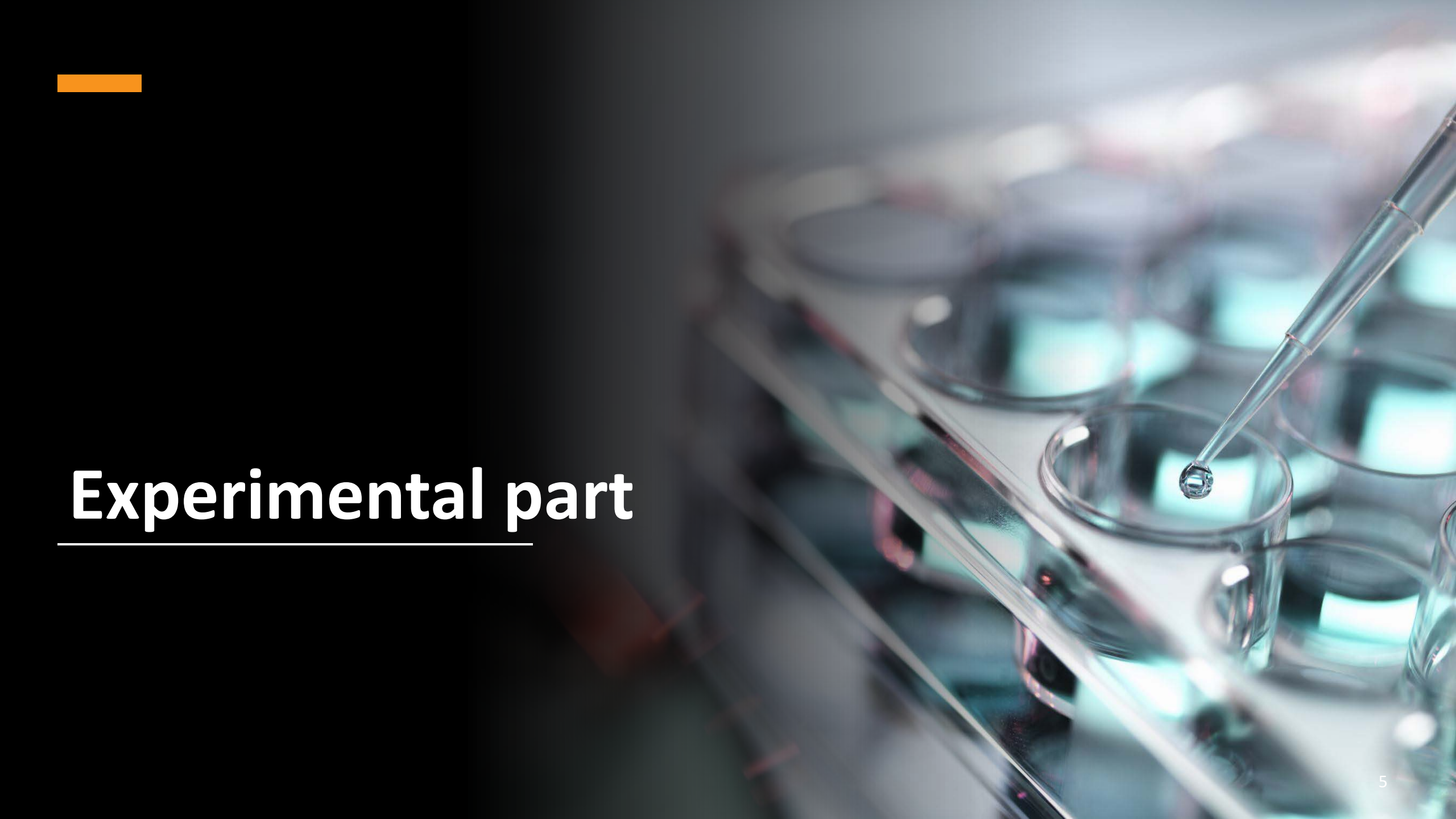
- ❑ Use of two enzymes in series;
- ❑ Use of the NADH cofactor as a reducing agent.

1. Enzymatic stability
2. Reuse of enzymes

→ Immobilization process

Vidal et al., 2018, *BBA - Proteins and Proteomics*, **1866**, 327-347.

Experimental part



Specific enzyme activity

IU (international activity unit): amount of enzyme necessary to catalyze the transformation of a µmol of substrate per minute in conditions of:

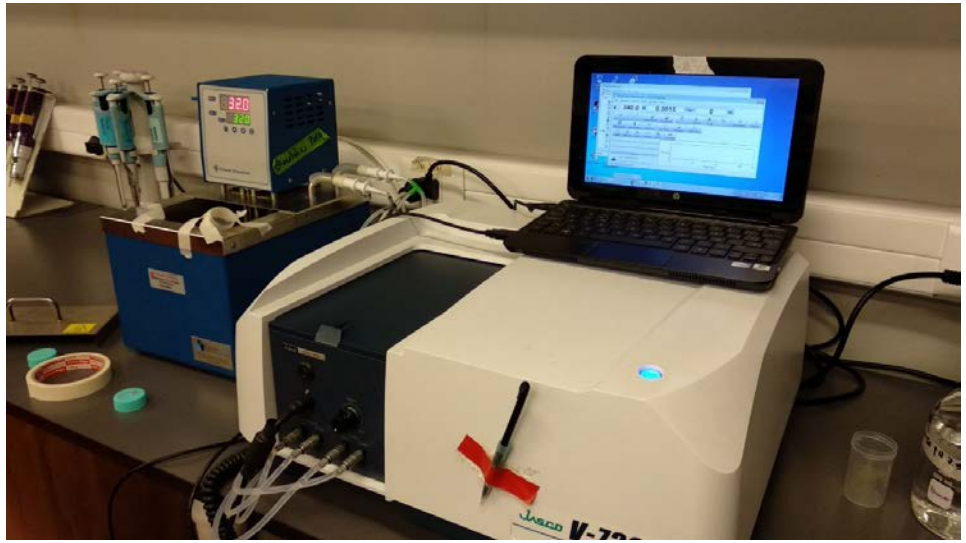
- ❑ Concentration of saturating substrate;
- ❑ pH = 7;
- ❑ T = 30 °C.

For the activity of the free and immobilized enzyme the change in the absorbance of NADH at 340nm is measured, under stirring

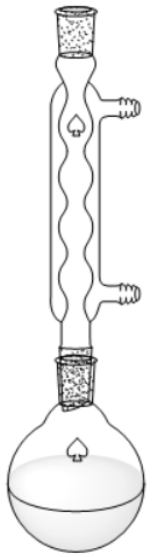
Specific activity →

$$\frac{UI_{FE}}{mg_{enz}} = \frac{\Delta A}{\Delta t} \cdot \frac{1}{\varepsilon} \cdot \frac{V_{tot}}{mg_{enz}}$$

$$\frac{UI_{IE}}{mg_{supp}} = \frac{\Delta A}{\Delta t} \cdot \frac{1}{\varepsilon} \cdot \frac{V_{tot}}{mg_{supp}}$$



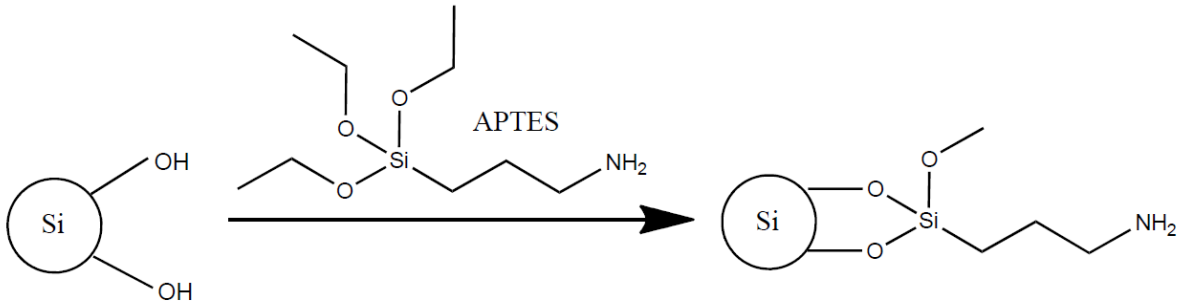
Functionalization with amino groups



The reaction is carried out in a boiling flask with refrigeration column

Amino groups formations:

- ❑ APTES 1% v/v in Toluene
- ❑ 105°C, 5h.



Lazghab et al., 2010, *Chemical Engineering Research and Design*, **88**, 686-692 .

Functionalization with glyoxyl groups

Epoxy groups formation:

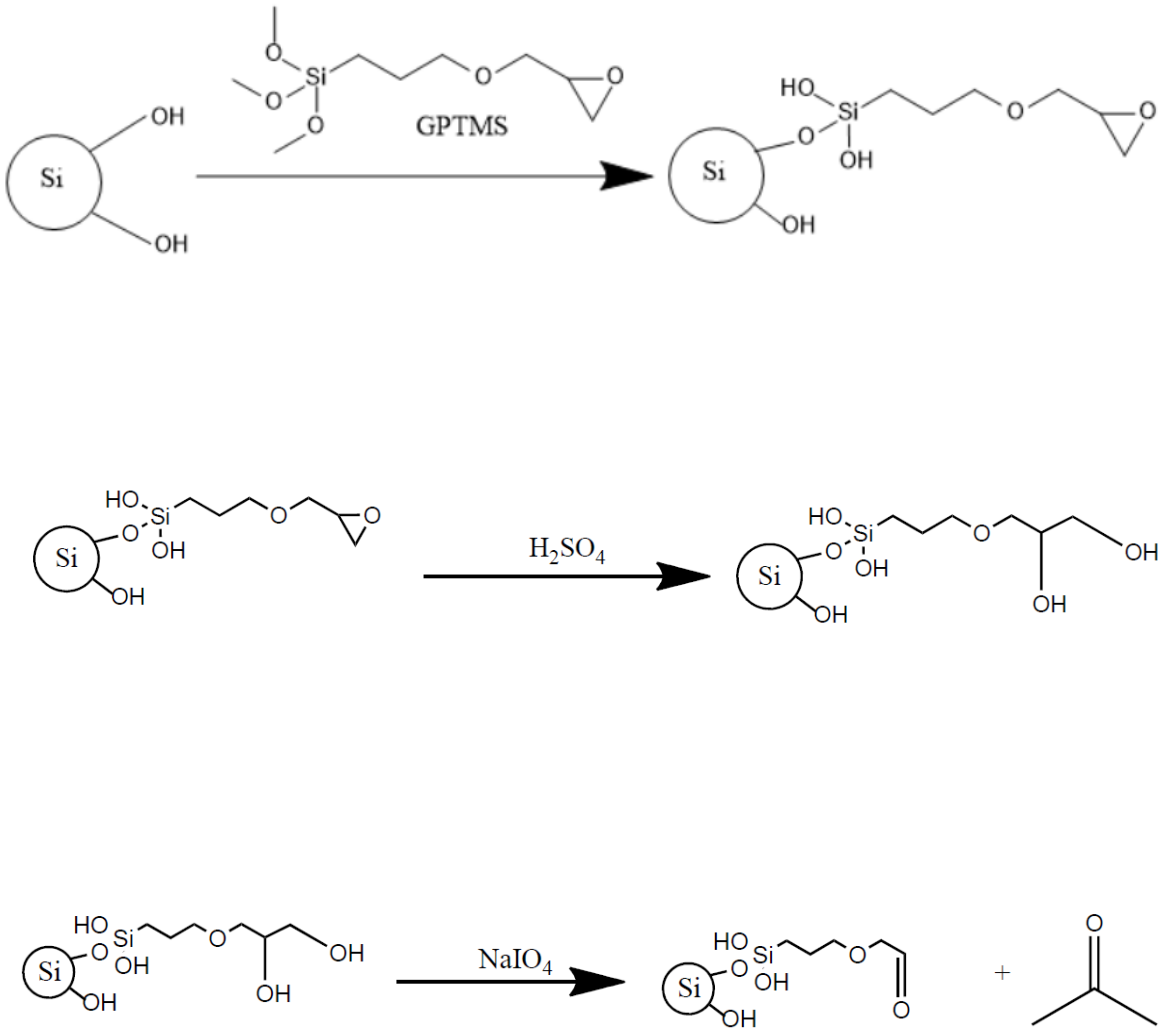
- ❑ GPTMS 1% v/v in Toluene
- ❑ 105°C, 5h.

Hydrolysis of epoxy groups:

- ❑ H_2SO_4 0.1M
- ❑ 85°C, 2h

Formation of glyoxyl groups:

- ❑ $NaIO_4$ 0.1M
- ❑ T_{amb}, 2h



Bernal et al., 2012, *Journal of Molecular Catalysis B: Enzymatic*, **84**, 166-172 .

Vejayakumaranet et al., 2008, *Journal of Colloid and Interface Science*, **328,** 81–91.

Immobilization with amino groups

1st step reaction:

- ❑ Amino functionalized support
- ❑ Enzyme
- ❑ Phosphate buffer 5 mM pH 7
- ❑ 4 °C
- ❑ 3 h

Formation of ionic bonds between amino groups on the support and enzyme

2nd step reaction (after filtration)

- ❑ Phosphate buffer 25 mM pH 7
- ❑ Glutaraldehyde 0,1% v/v as linker to stabilize bond
- ❑ 4 °C
- ❑ 30min

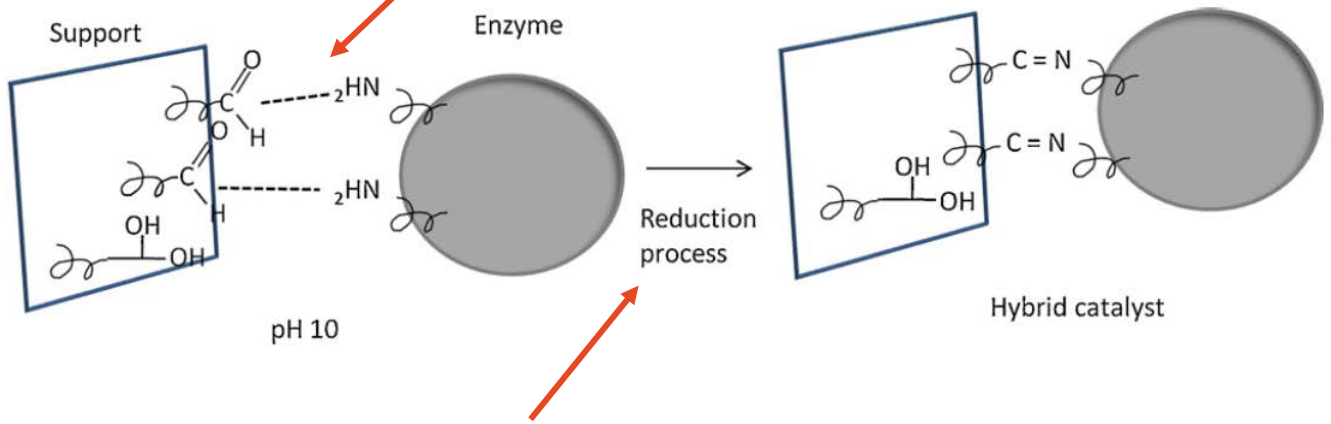
Bolivar et al., 2006, *Enzyme and Microbial Technology, **40***, 540-546

Immobilization with glyoxyl groups

1st step reaction
- ❑ Functionalized support
- ❑ Enzyme
- ❑ 0.1 M carbonate buffer pH 10
- ❑ 4 °C
- ❑ 3 h

2nd step reaction (without filtration)
- ❑ NaBH4;
- ❑ Glycerol.

Formation of weak bonds between support and enzym



$NaBH_4$ as a reducing agent for the formation of the covalent bond, using glycerol as protecting agent

Bernal et al., 2012, *Journal of Molecular Catalysis B: Enzymatic*, **84**, 166-172 .

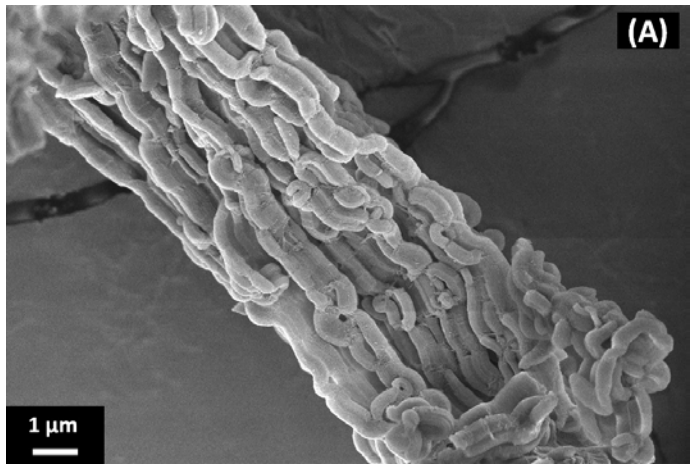
Results

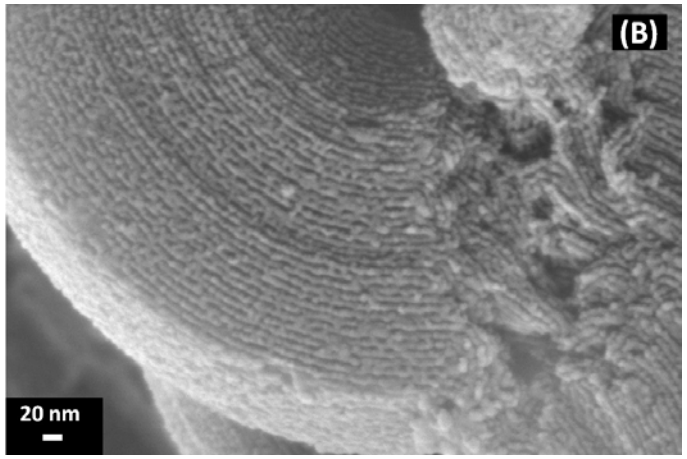
Materials characterization

- Physisorption at -196 °C
  - Specific area greater than 500 m^2/g, in all cases
  - Average pore diameter ranging from 7 to 25 nm
- FESEM analysis
  - MSU-H → Cylindrical shape
  - MSU-F, $MCF_{0,75}$ → Spongy-like structure

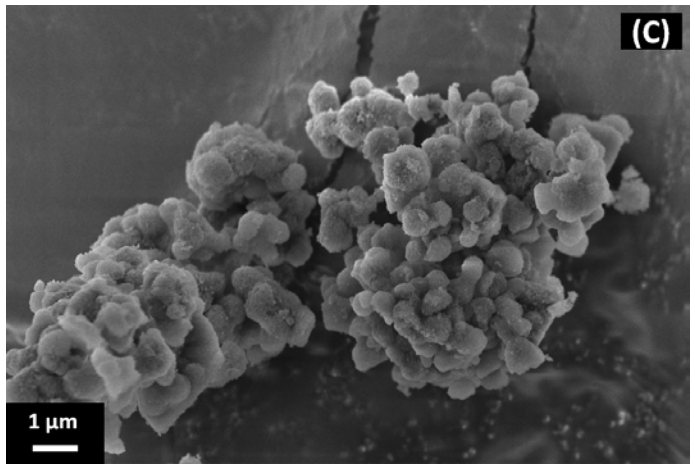
Sample	S_{BET} (m^2/g)	V_p (cm^3/g)	D_p(nm)
MSU-H	750	0.91	7
MSU-F	562	2.31	15
$MCF_{0.75}$	600	1.40	25

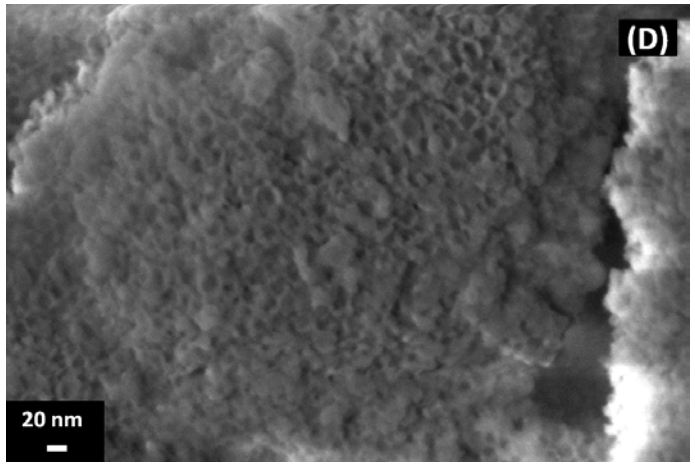
MSU-H



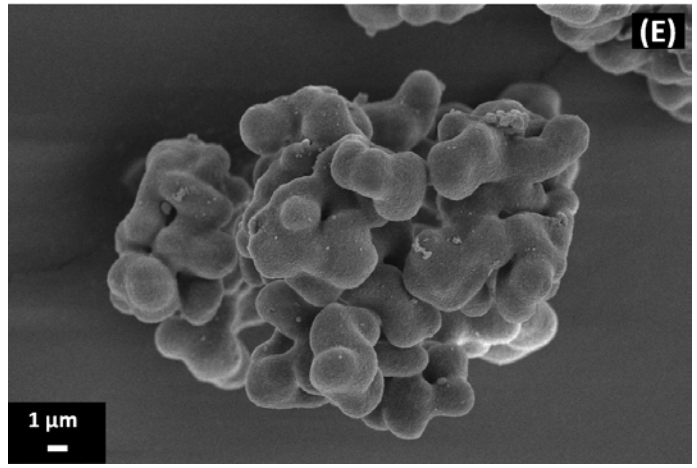


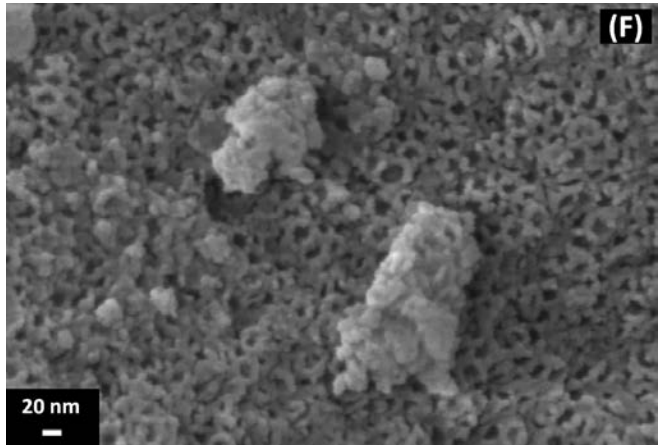
MSU-F





$MCF_{0,75}$





Immobilized enzyme activity and immobilization yield

- ❑ Higher immobilization yields with materials with higher average pore diameter;
- ❑ Higher specific activities with materials with smaller average pore diameters;
- ❑ Enzymatic load: 4mg_{enz}/g_{supp};
- ❑ ADH immobilized on amino support;
- ❑ AldDH immobilized on glyoxyl support.

Sample	D_p(nm)	IU/g_{supp}	IY (%)
ADH/MSU-H	7	48.6±1.32	82±6.2
ADH/MSU-F	15	12.6±0.89	100±3.1
ADH/$MCF_{0.75}$	25	3.6±0.53	100±4.2
AldDH/MSU-H	7	1.3±0.37	50±5.1
AldDH/MSU-F	15	1.01±0.45	96±4.6
AldDH/$MCF_{0.75}$	25	0.97±0.33	100±3.4

AldDH activity:

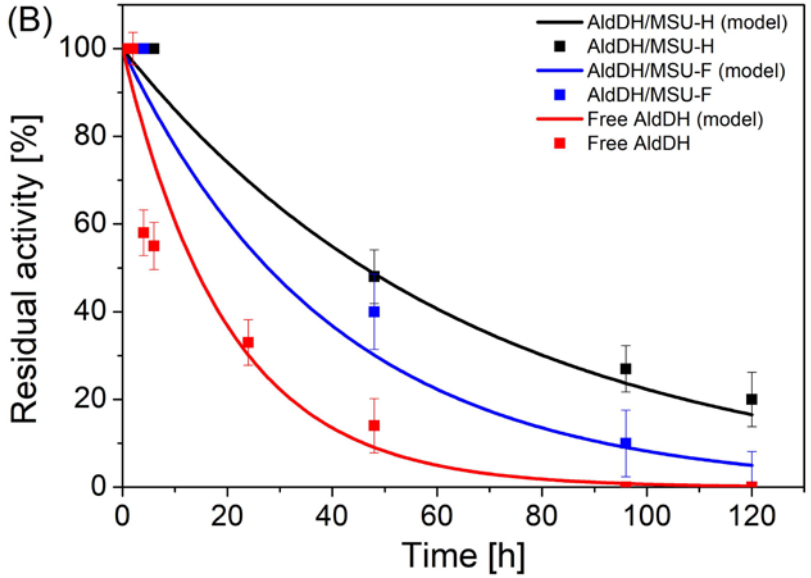
- ❑ 1,8 mL phosphate buffer 100 mM;
- ❑ 0,125 mL propionaldehyde 7,5 mM;
- ❑ 0,125 mL NAD^+ 50 mM;
- ❑ 30°C, pH 7;
- ❑ 5 mg of support or 0,025 mg of AldDH.

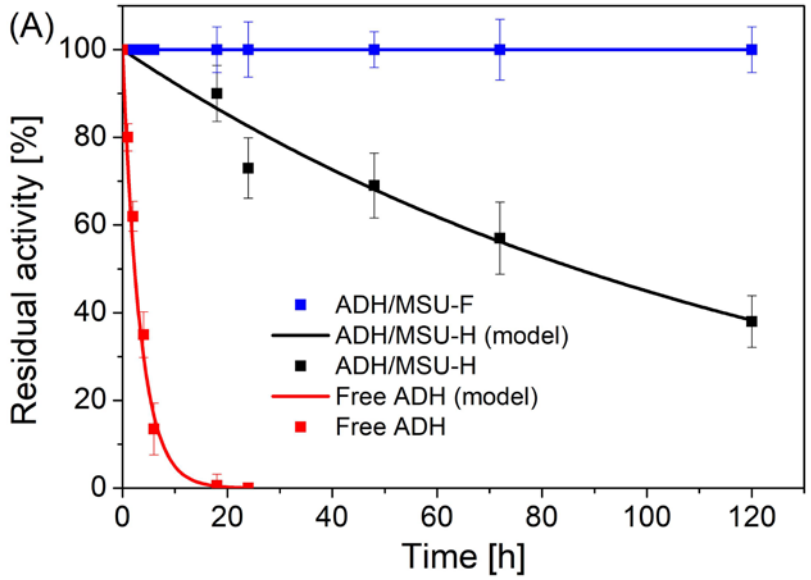
ADH activity:

- ❑ 2 mL ethanol 250 mM in phosphate buffer 100mM;
- ❑ 0,1 mL NAD^+ 50 mM;
- ❑ 30°C, pH 7;
- ❑ 5 mg of support or 0,005 mg of ADH.

Comparison of thermal stability

Stability tests carried out at 50 °C for soluble enzyme and immobilized enzyme





Deactivation of the first order:

$$E_{nat} \xrightarrow{k_d} E_{den}$$

Active enzyme concentration:

$$A(t) = A_o\, e^{(-k_d \cdot t)}$$

$$A(t) = A_o[(1-\alpha)e^{(-k_d \cdot t)} + \alpha]$$

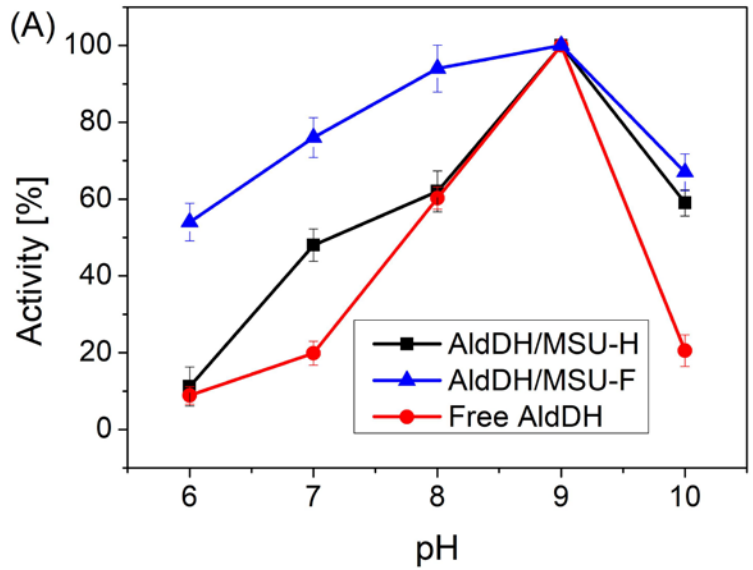
Stability factor:

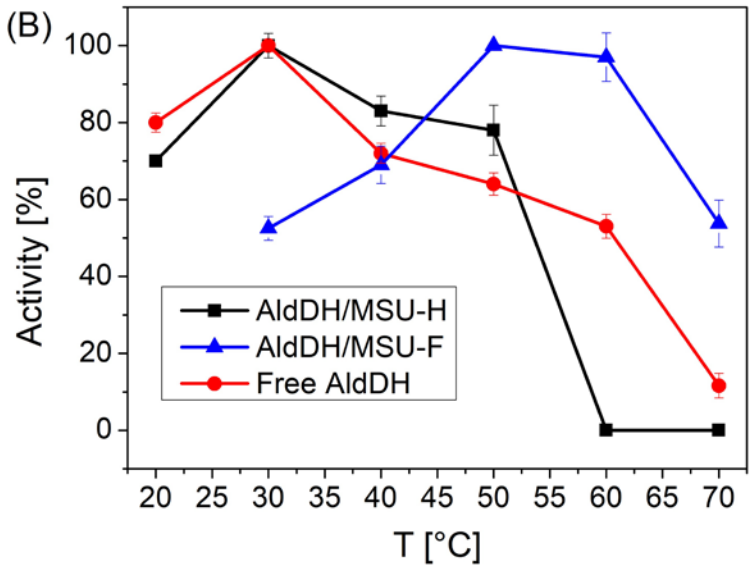
$$F_S = \frac{t_{\frac{1}{2},IE}}{t_{\frac{1}{2},FE}}$$

Sample	k_D (h^{-1})	α (-)	$t_{1/2}$ (h)	F_S (-)	R^2
Free ADH	0.280	-	2.47	-	0.99
ADH/MSU-F	-	-	-	-	-
ADH/MSU-H	0.015	0.3	83.5	33.8	0.95
Free AldDH	0.050	-	13.86	-	0.96
AldDH/MSU-F	0.025	-	27.73	2.0	0.98
AldDH/MSU-H	0.015	-	46.21	3.3	0.98

pH and T profile of AldDH derivates

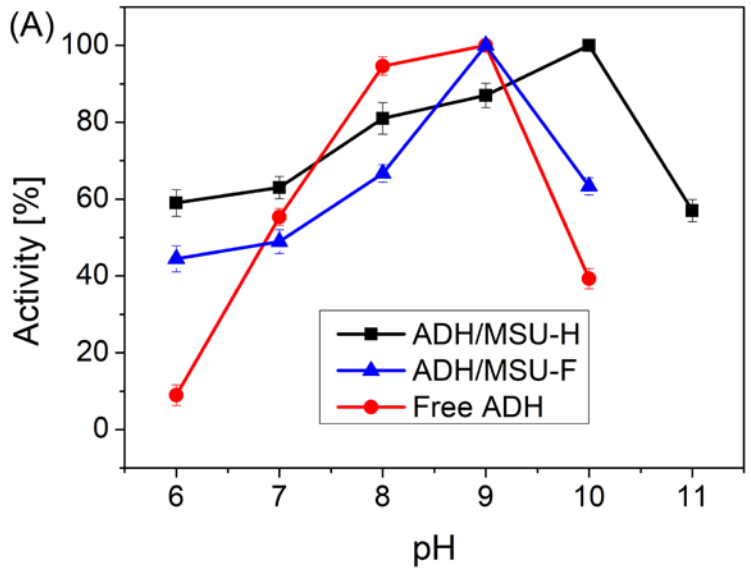
- Activity variation between soluble and immobilized enzyme, with varying pH and T
- No variation of optimal pH
- Good increase in optimal T of AldDH/MSU-F derivates.

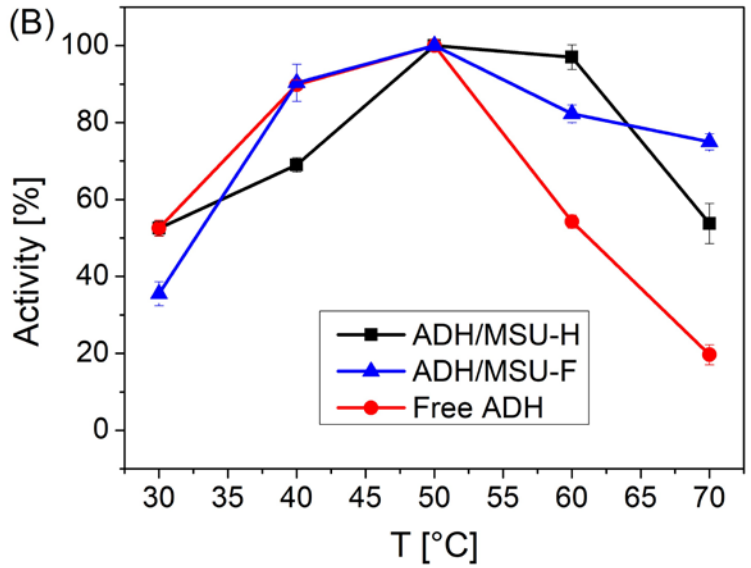




pH and T profile of ADH derivates

- Activity variation between soluble and immobilized enzyme, with varying pH and T
- There is a slight increase in optimal pH of ADH/MSU-H derivates.
- No variation of optimal T





Reduction reaction

1st reaction step: very low conversion, the ATP cofactor is probably also needed.

2nd reaction step: good result obtained with MSU-H; probably with MSU-F the enzyme occludes all the pores and the reagents cannot reach the enzyme site

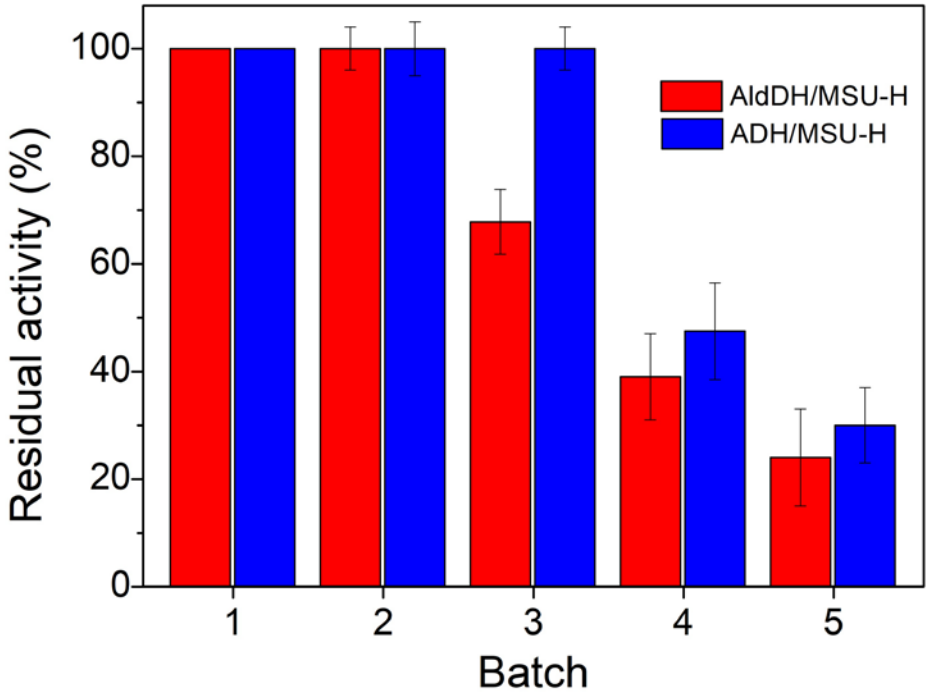
Sample	Conversion yield
AldDH/MSU-H	<0.1%
AldDH/MSU-F	<0.1%
ADH/MSU-H	14%
ADH/MSU-F	<0.1%

Conditions:

- ❑ NADH 50mM;
- ❑ Substrate (propionic acid or propionaldehyde) 50mM;
- ❑ 30°C;
- ❑ pH 7.

Reusability test

- Residual activity is measured after several batch reaction
- In both cases, residual activity greater than 20% is observed after 5 cycles



Conclusions

- Good increase in enzymatic stability after immobilization;
- Residual activity of 20% after 5 batch reactions;
- Need to use another enzyme, more effective in the first reaction step (e.g. CAR enzyme);
- Need to implement cofactor regeneration processes.

Thanks for your attention!

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