

Towards the production of an industrial amino acid oxidase: stabilization of a thermophilic L-aspartate oxidase

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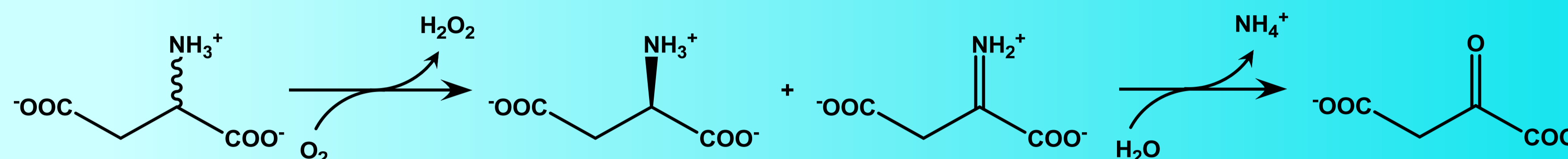
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Introduction

L-Aspartate oxidase is a FAD containing enzyme, catalyzing the stereospecific oxidative deamination of L-aspartic acid to oxalacetate, ammonia and hydrogen peroxide.

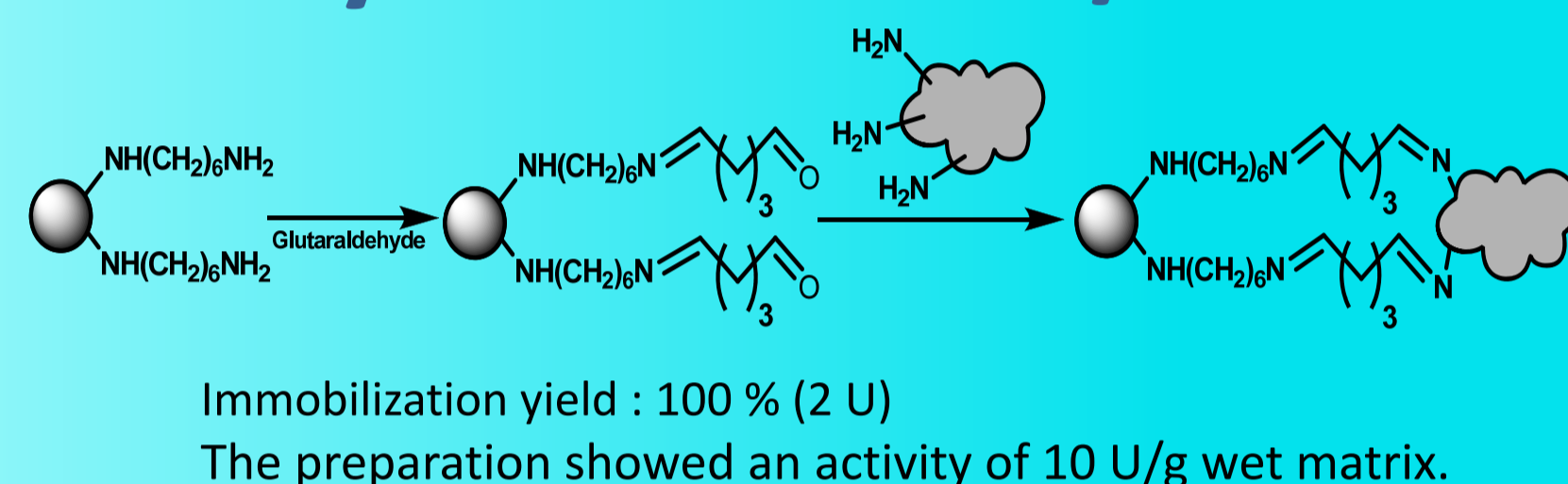


This flavoenzyme from the thermophilic archaea *Sulfolobus tokodaii* (StLASPO) was efficiently produced as a recombinant protein in *E. coli* and has been deeply characterized.^[1] StLASPO presents very distinctive features such as: high thermostability, stable activity over a broad range of pH values, low K_m for dioxygen and tight binding of the FAD cofactor, making it very attractive for biotechnological applications. Immobilization studies of this novel biocatalyst on different solid supports such as resins or in the form of cross-linked enzyme aggregates (CLEA) were performed.^[2]

A recently developed approach is represented by the immobilization of a flavooxidase on magnetic Fe_3O_4 nanoparticles (NP@RgDAAO)^[3], which now paves the way to the immobilization of StLASPO.

Immobilization on Relizyme™ HA403/SR

- 200 mg of resin HA 403/ S R
- 10 mL of 0.125% (v/v) glutaraldehyde solution in water
- 1 mL StLASPO solution (2 U)
- 0.1 M phosphate buffer, pH 7.5



Best Result:

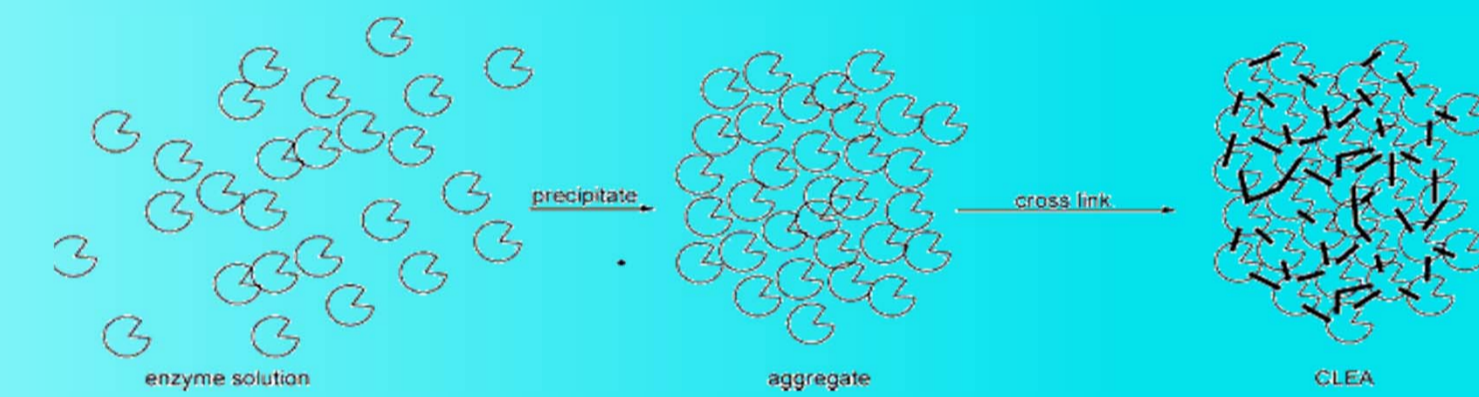
Resolution of 50 mM D,L-Aspartate at pH 10:

- 0.5 mL of D,L-Aspartate solution at pH 10
- 2 U of immobilized StLASPO
- 70 °C in a thermomixer (600 rpm)

Cycle number	Time (h)	e.e. (%)
1	1	> 99.5
2	1	> 99.5
3	1	> 99.5
4	1	87
	2	> 99.5
5	1	77
	2	> 99.5

Immobilization as CLEA

- 4.5 mL $(NH_4)_2SO_4$ precipitating solution are added to 0.5 mL of enzyme solution
- 0.4 % (v/v) glutaraldehyde solution/total precipitation solution)
- The mixture was incubated on a rotary shaker for 2.5 h at room temperature



Best Result:

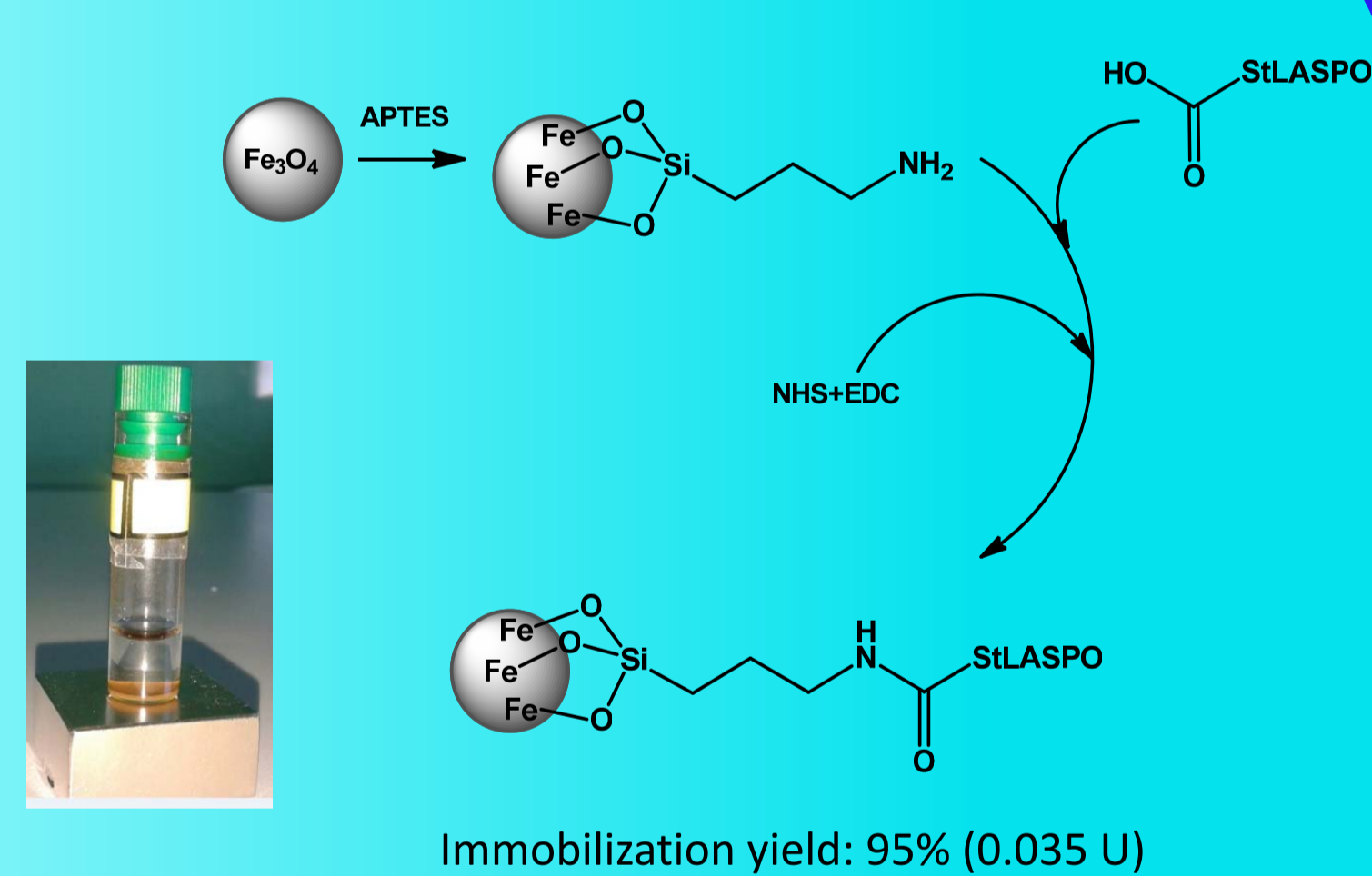
Resolution of 50 mM D,L-Aspartate at pH 10:

- 0.5 mL of D,L-Aspartate solution at pH 10
- CLEA-StLASPO preparation (1.1U)
- 70 °C in a thermomixer (600 rpm)

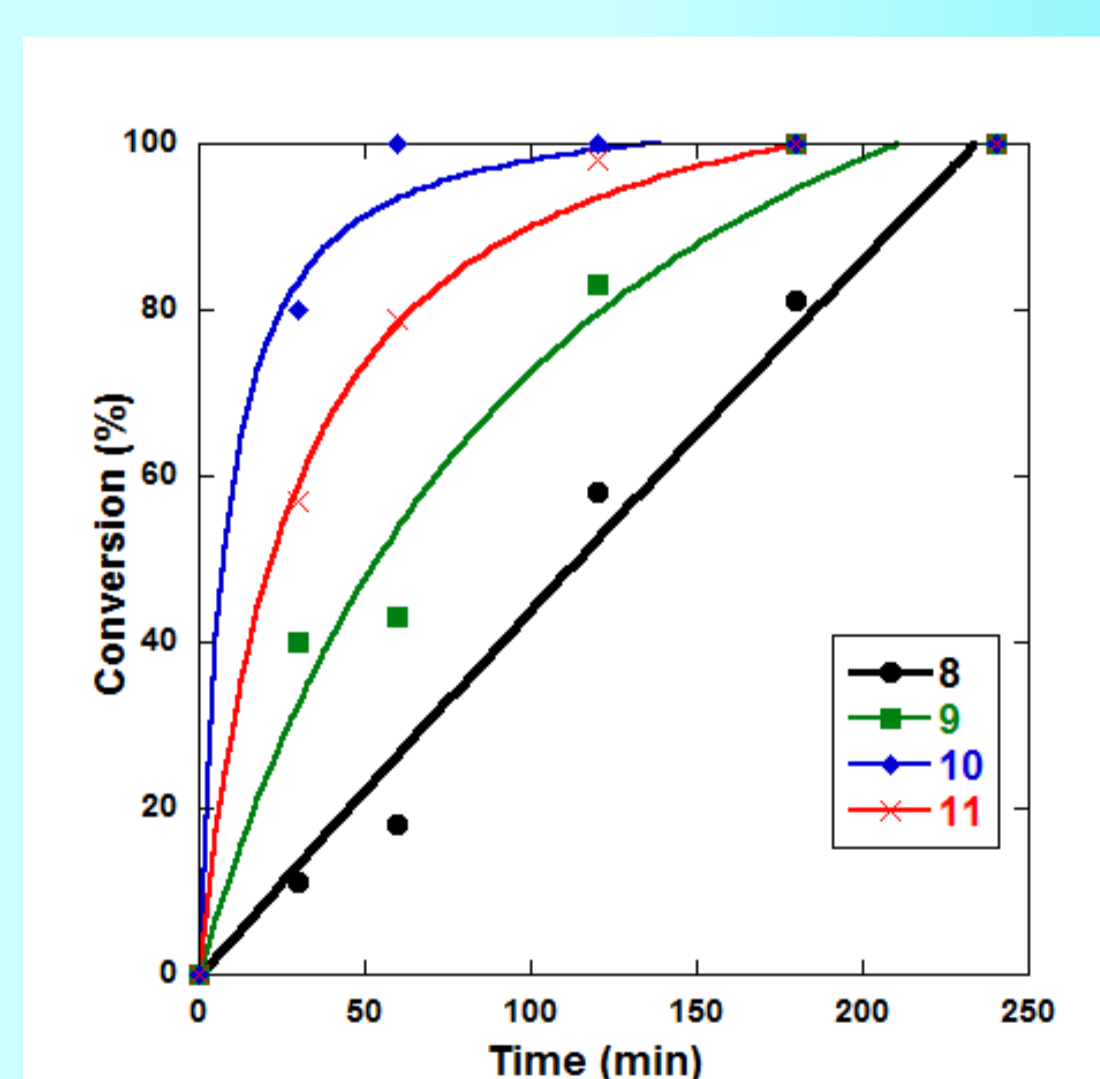
Cycle number	Time (h)	e.e. (%)
1	4	> 99.5
2	4	> 99.5
3	4	> 99.5
4	4	68
	6	> 99.5
5	4	69
	6	95

Immobilization on Fe_3O_4 NPs

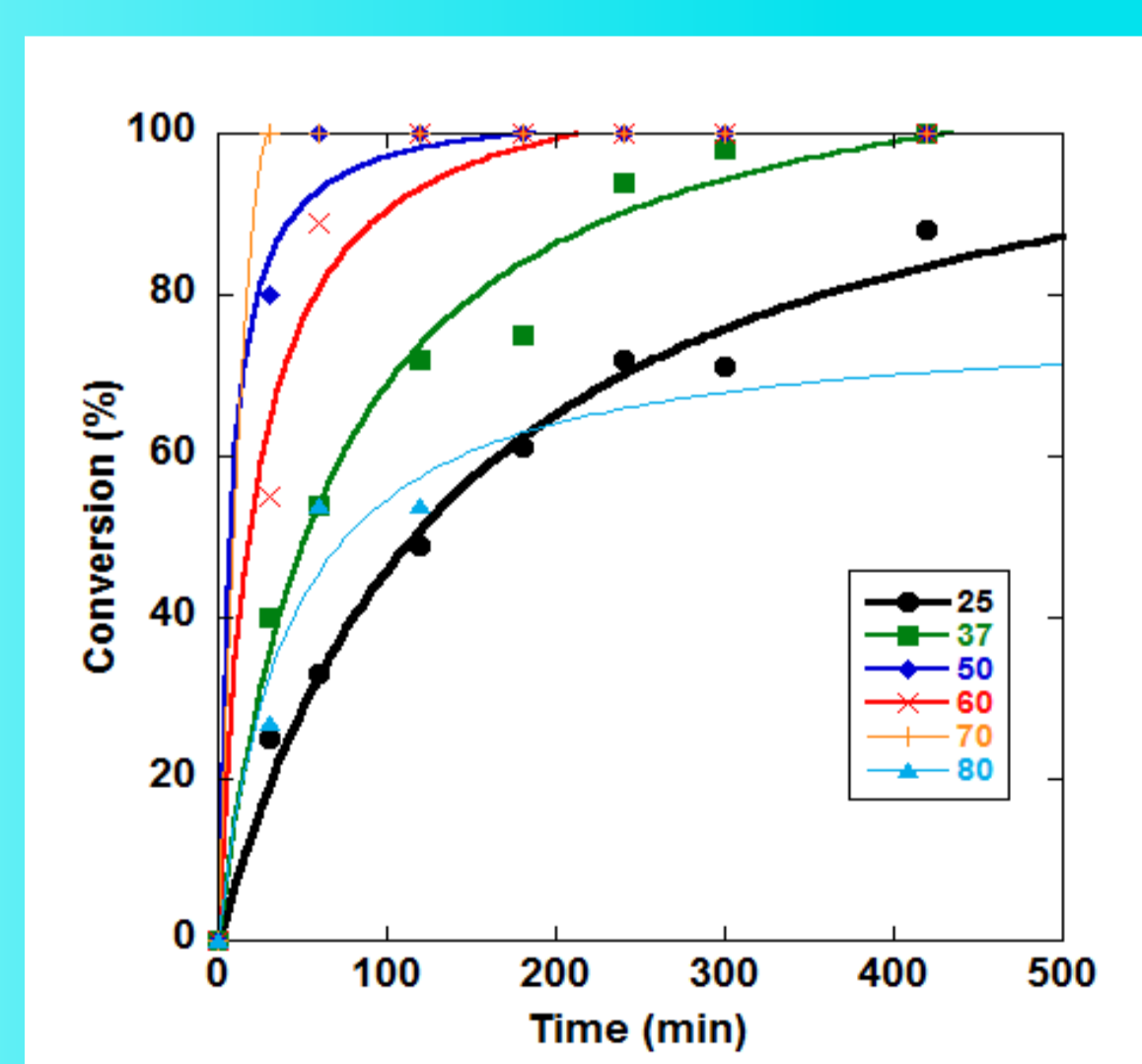
- 150 mg of Fe_3O_4 NP
- 5 mL APTES (2% v/v) in H_2O left under stirring for 5 h at 50 °C.
- Fe_3O_4 NP-APTES were then separated from unbound APTES by using a magnet.
- 4 mg of Fe_3O_4 NP@APTES
- 2 mg of EDC
- 3 mg of NHS
- 0.037 U of StLASPO
- The reaction was carried out for 2 h at r.t.



Effect of pH



Effect of temperature

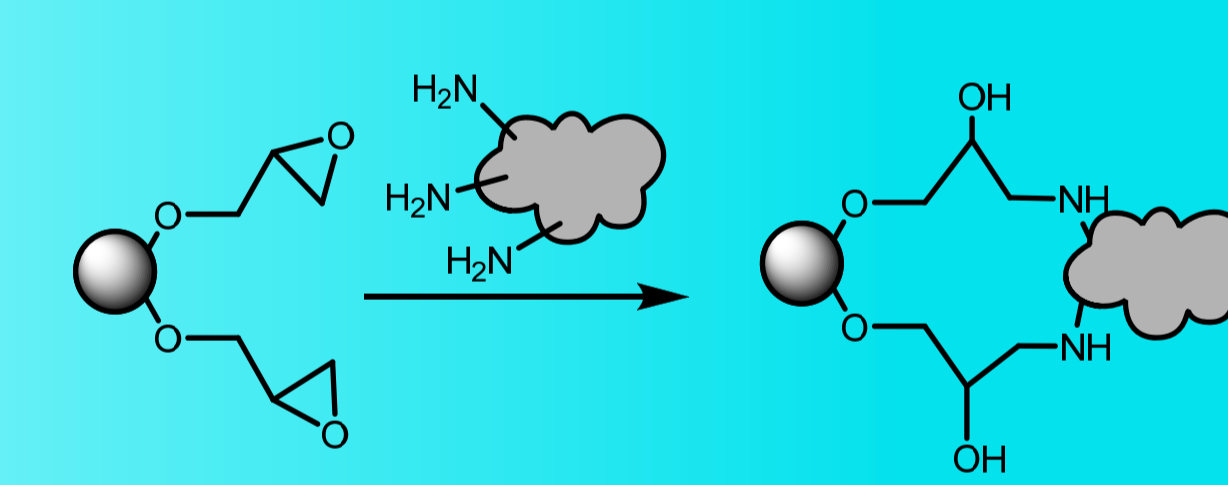


- Fe_3O_4 NP@APTES-StLASPO (0.2699 U on 16 mg NP)
- 100 μ L of 50 mM L-Aspartate in water at different pH values (8, 9, 10, 11)
- 100 μ L of water (adjusted to the desired pH with NaOH 0.5 M)
- 70 °C on a thermomixer (600 rpm)

- Fe_3O_4 NP@APTES-StLASPO (0.322 U on 16 mg NP)
- 100 μ L of a 50 mM L-Aspartate solution at pH 10
- 100 μ L of water (adjusted at pH 10 with NaOH 0.5 M)
- These reactions were conducted at different temperatures (25, 37, 50, 60, 70, 80 °C) on a thermomixer (600 rpm).

Immobilization on SEPABEADS® EC-EP/S

- 0.5 mL (1 U) StLASPO
- 60 mg of SEPABEADS® EC-EP/S
- 0.3 mL 1.25 M potassium phosphate buffer at pH 8
- The mixture was incubated for 18 h at 25 °C



Best Result:

Resolution of 50 mM D,L-Aspartate at pH 10 :

- 0.5 mL of D,L Aspartate solution at pH 10
- 0.5 U of immobilized StLASPO
- 70 °C in a thermomixer (600 rpm)

Cycle number	Time (h)	e.e. (%)
1	4	> 99.5
2	4	> 99.5
3	4	> 99.5
4	4	81
	6	> 99.5
5	4	45
	10	> 95

Conclusions

- Resin HA 403/SR presents a high yield of immobilization; moreover the resulting catalyst can be used for 5 cycles yielding complete conversion in < 2 hours.
- The CLEA-StLASPO preparation maintains its activity for 5 cycles, with complete conversion in < 8 hours.
- The immobilization of StLASPO on magnetic NPs presents very promising results in terms of stability and potential reusability of the biocatalyst. The recovery of the reaction products and of the immobilized enzyme NP@StLASPO is very simple. Indeed, the removal of the catalyst from the reaction medium can be easily performed with a magnet; this is a very attracting aspect helpful for the scaling up of the bioconversion.