

An efficient microwave-assisted enzymatic resolution of alcohols using a lipase immobilised on Supported Ionic Liquid-like Phases (SILLPs).

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1. General Procedures

Candida antarctica lipase type B (CALB, Novozyme 435, 7300 PLU/g) was a gift from Novozymes. All other reagents were obtained from Sigma-Aldrich and used without further purification. Gases Chromatography (GC) analysis were carried out in a Varian 3900 using a CyclodexB column (length 30m, I.D. 0.25mm, film 0.25 μ m). The Microwave irradiation experiments were performed with Discover System Model 908010 from CEM Corporation using custom-made high purity quarts vials (capacity 10mL).

The initial SILLPs prepared were analyzed through IR and Raman spectroscopy (for further information, see ref.1)

2. Lipase immobilization onto SILLPs and Amberlite XAD4

Firstly, the commercial enzyme preparation was ultrafiltered, to eliminate all the low molecular weight additives, as follows: 25 mL of Novozym 525L were diluted in 225 mL of water, and the resulting solution was concentrated 10-fold by ultrafiltration at 8°C, using a Vivaflow 50 (Sartorius) system equipped with polysulphone membranes (10 kDa. cut-off), obtaining a 15.1 mg protein/mL lipase solution. Immobilized lipase derivatives were prepared by simply mixing the aqueous solution of lipase (1.5 mL) with the support (Amberlite XAD4, or SILLP) (1 g). The mixture was gently shaken for 5 hours at room temperature for enzyme adsorption. Then the supernatant was recovered, and the support washed with water to remove non-adsorbed enzyme. The immobilized derivative was frozen at -60°C, then lyophilized, and finally stored under controlled a_w (0.11) conditions over LiCl in desiccator at 8°C until use.¹

3. Reaction profiles. Conversion vs. time for the different catalysts evaluated

Kinetic resolution (KR) of (\pm)-1-Phenylethanol under MW heating. A solution 0.1M of (\pm)-1-phenylethanol and 0.21M of vinylpropionate in cyclohexane or 2-methyl-metrahydrofuran as solvent was prepared. The 1.5mL stock solution of (\pm)-1-phenylethanol (18.6 μ L, 0.15mmol) and vinylpropionate (34.3 μ L, 0.315mmol) in cyclohexane was charged with 20mg of biocatalyst (CALB-SILLP-L(Cl), CALB-SILLP-L(NTf₂), CALB-SILLP-H(Cl), CALB-SILLP-H(NTf₂), CALB-SILLP-R(Cl), CALB-SILLP-R(NTf₂), CALB-XAD4 or Novozym 435 were used) into a screw-capped tube. The resultant mixture was heated in a microwave oven (CEM Discover, CEM Microwave Technology Ltd, USA) at 40°C with low stirring and 100Watts. The system was run at constant temperature operation mode by using the air cooling feature of the apparatus. Aliquots of 50 μ L were taken in different time intervals, depending on the biocatalyst activity, and solved in 700 μ L of hexane HPLC grade for GC analysis. One unit of synthetic activity was defined as the amount of immobilized enzyme that produces 1 μ mol of (R)-1-phenylethyl propionate per min. All the experiments were carried out in duplicate.

KR of (±)-1-Phenylethanol under conventional heating. A solution 0.1M of (±)-1-phenylethanol and 0.21M of vinylpropionate in cyclohexane or 2-Methyl-Tetrahydrofuran as solvent was prepared. Using HPLC vials, the 1mL stock solution of (±)-1-phenylethanol (12.36μL, 0.1mmol) and vinylpropionate (23.36μL, 0.21mmol) in cyclohexane was charged with 13.3mg of biocatalyst (proportionally to amount of substrate; CALB-SILLP-L-(Cl), CALB-SILLP-L-(NTf₂), CALB-SILLP-H-(Cl), CALB-SILLP-H-(NTf₂), CALB-SILLP-R-(Cl), CALB-SILLP-R-(NTf₂), CALB-XAD4 or Novozym 435 were used). The resultant mixture was heated at 40°C with low stirred in a carrousel heating plate. Aliquots of 50μL were taken in different time intervals, depending on the biocatalyst activity, and solved in 700μL of hexane HPLC grade for GC analysis. One unit of synthetic activity was defined as the amount of immobilized enzyme that produces 1 μmol of (R)-1-phenylethyl propionate per min. All the experiments were carried out in duplicate

The Conversion was calculated considering the following equation: $C = ee_s/(ee_s+ee_p)*100$;

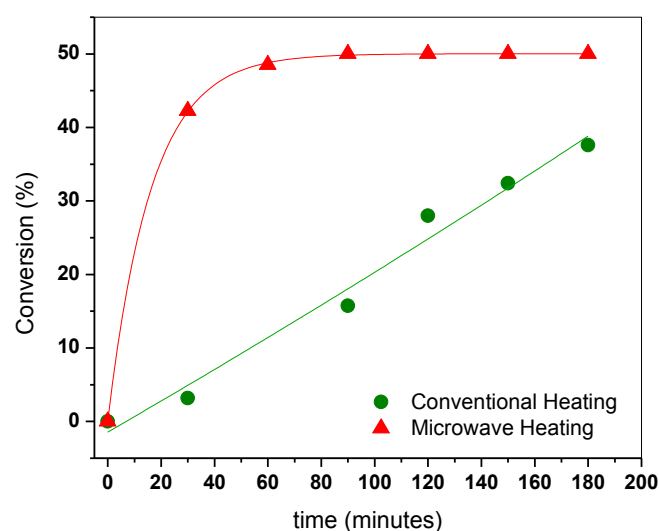


Fig. S-1. Kinetic Resolutions of (±)-1-Phenylethanol catalyzed by CALB-SILLP-L-(Cl).

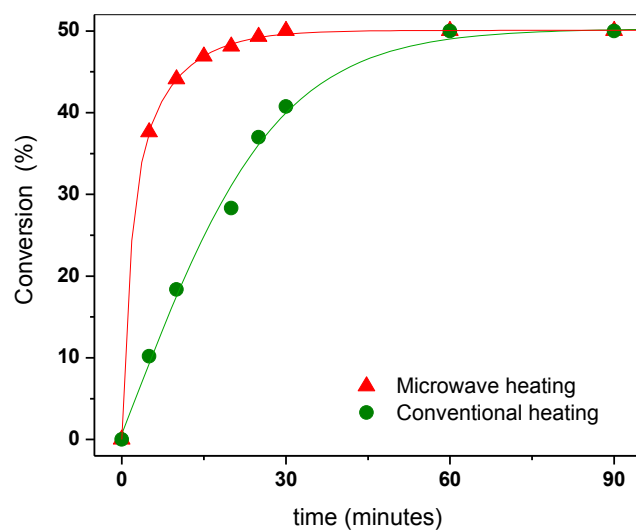


Fig. S-2. Kinetic Resolution of (±)-1-Phenylethanol catalyzed by **CALB-SILLP-L-(NTf₂)**.

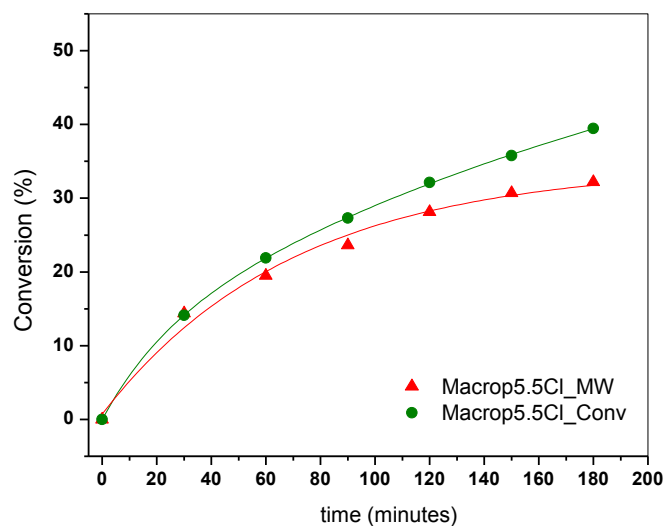


Fig. S-3. Kinetic Resolution of (±)-1-Phenylethanol catalyzed by **CALB-SILLP-H-(Cl)**.

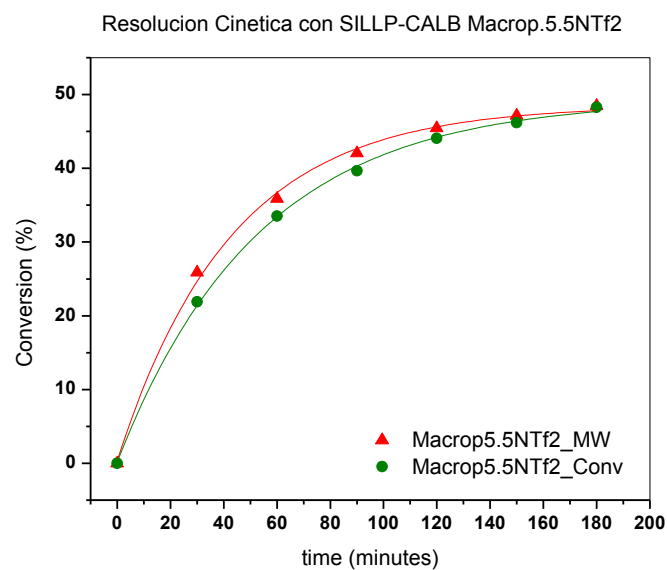


Fig. S-4. Kinetic Resolution of (\pm)-1-Phenylethanol catalyzed by **CALB-SILLP-H-(NTf₂)**.

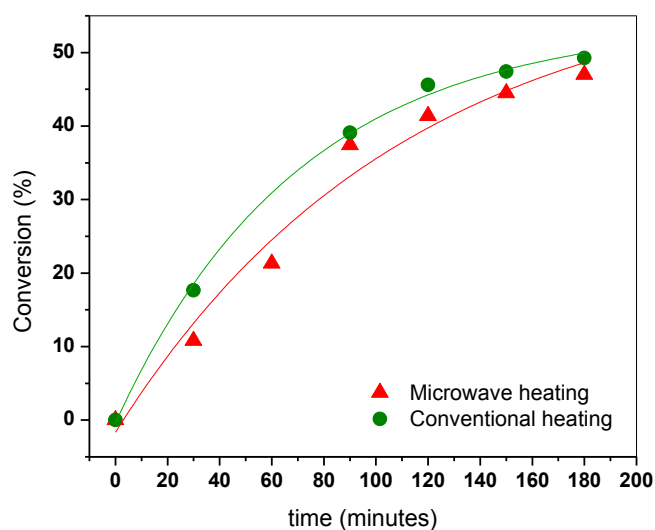


Fig. S-5. Kinetic Resolution of (\pm)-1-Phenylethanol catalyzed by **CALB-SILLP-R-(Cl)**.

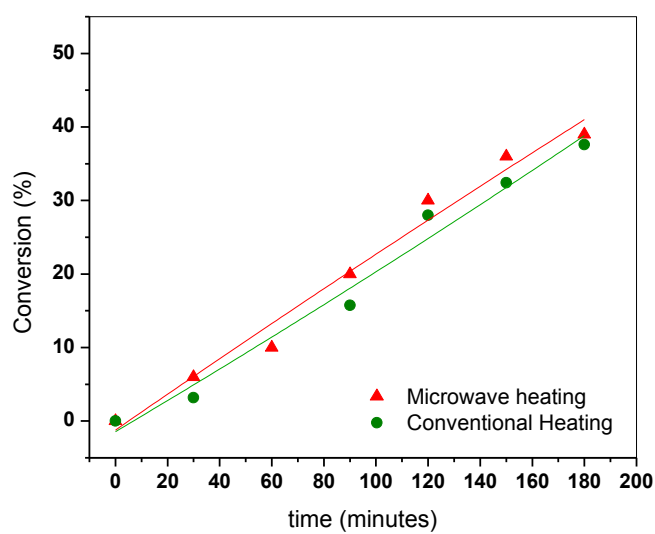


Fig. S-6. Kinetic Resolution of (±)-1-Phenylethanol catalyzed by **CALB-SILLP-R-(NTf₂)**.

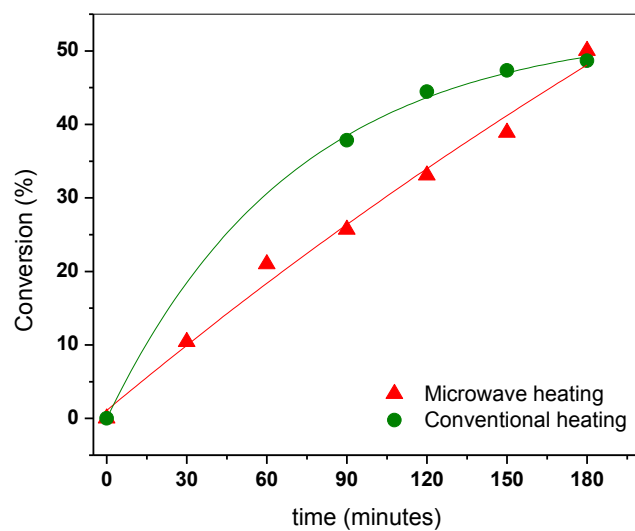


Fig. S-7. Kinetic Resolution of (±)-1-Phenylethanol catalyzed by **CALB-XAD4**.

4. Initial Heating profiles

The initial heating studies were done by taking the direct temperature (each 30-60 seconds) of the solution described in part 3, for the case of conventional heating. The initial heating profiles under MW irradiation were carried out by taking the database from Discover program.

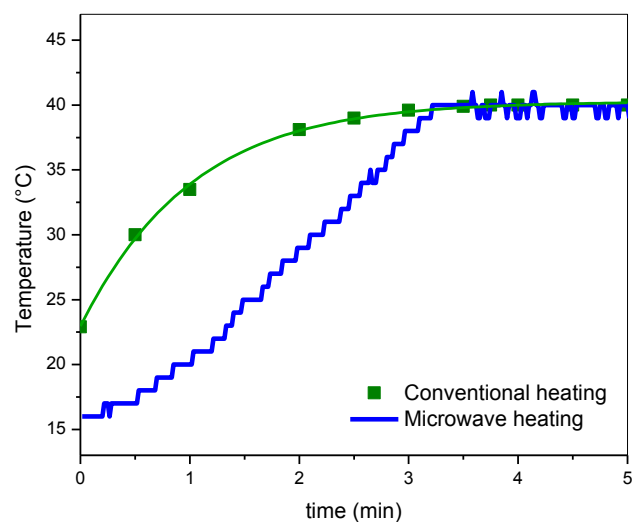


Fig. S-8. Heating profile Conventional vs. Microwave with CALB-XAD4.

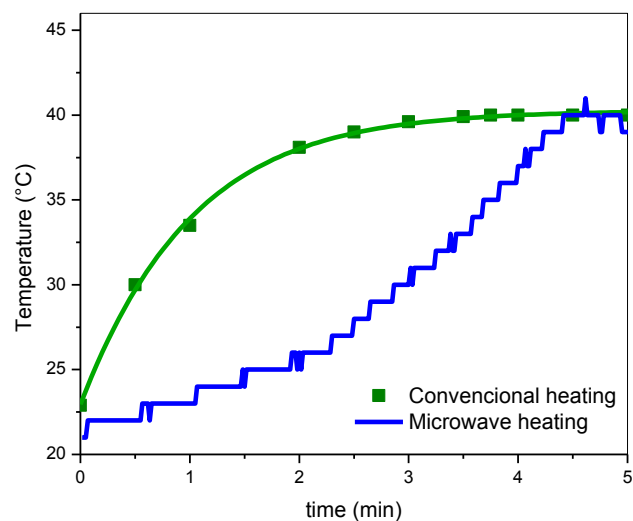


Fig. S-9. Heating profile Conventional vs. Microwave with Novozym 435.

5. Reaction profiles. Conversion vs. time for the different catalysts evaluated at different reaction cycles.

The procedure of the resolution of (\pm)-1-phenylethanol under MW heating was described in part 2. The difference between the first experiments and these, it is the biocatalysts were reused for 3 or more times. To recycle the biocatalyst after using, it was washed with 5mL of hexane and dried under vacuum at room temperature.

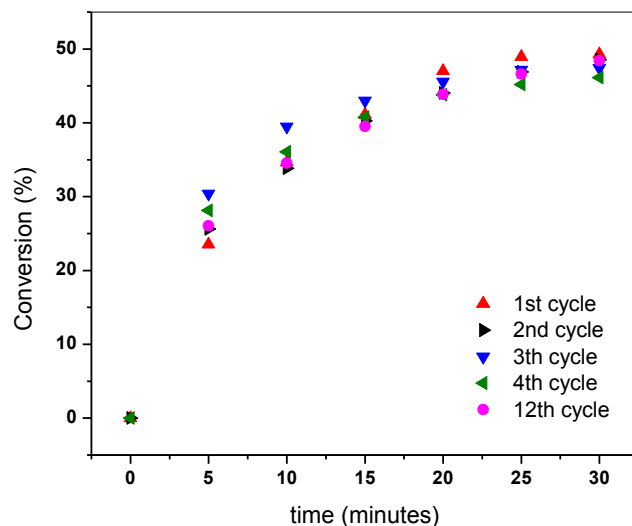


Fig. S-8. Kinetic Resolutions of (\pm)-1-Phenylethanol catalyzed by CALB-SILLP-L-(Cl).

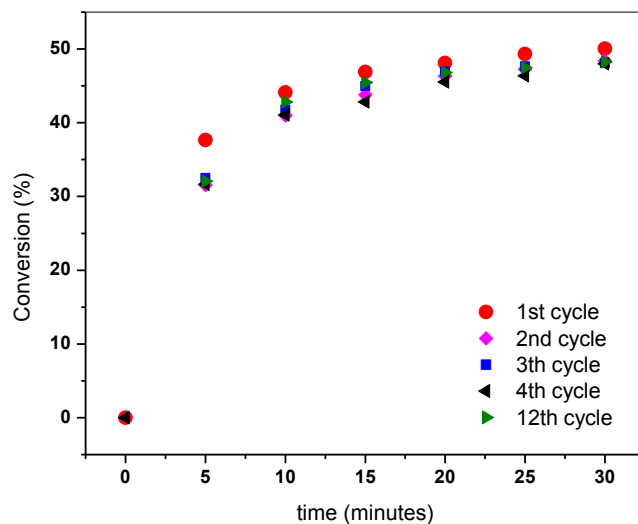


Fig. S-9. Kinetic Resolutions of (\pm)-1-Phenylethanol catalyzed by CALB-SILLP-L-(NTf₂).

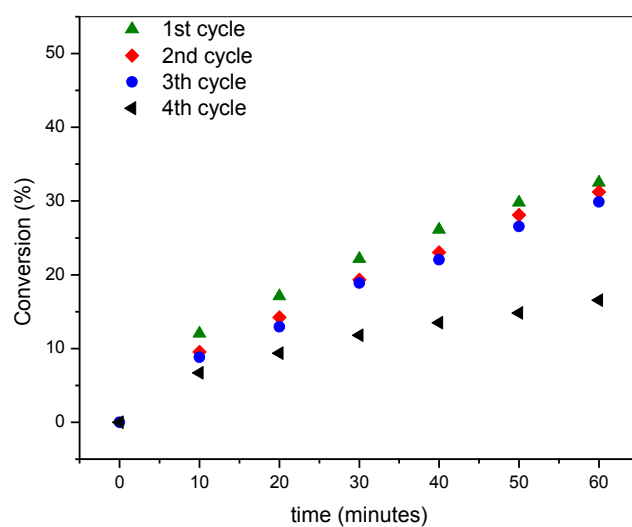


Fig. S-10. Kinetic Resolutions of (±)-1-Phenylethanol catalyzed by **CALB-SILLP-H-(Cl)**.

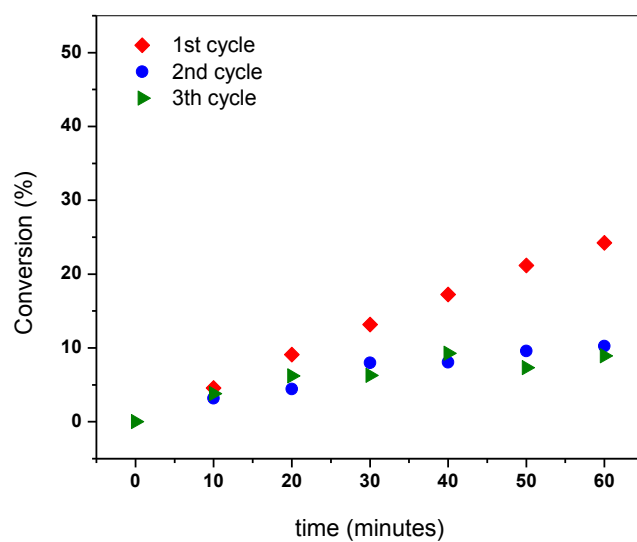


Fig. S-11. Kinetic Resolutions of (±)-1-Phenylethanol catalyzed by **CALB-SILLP-R-(Cl)**.

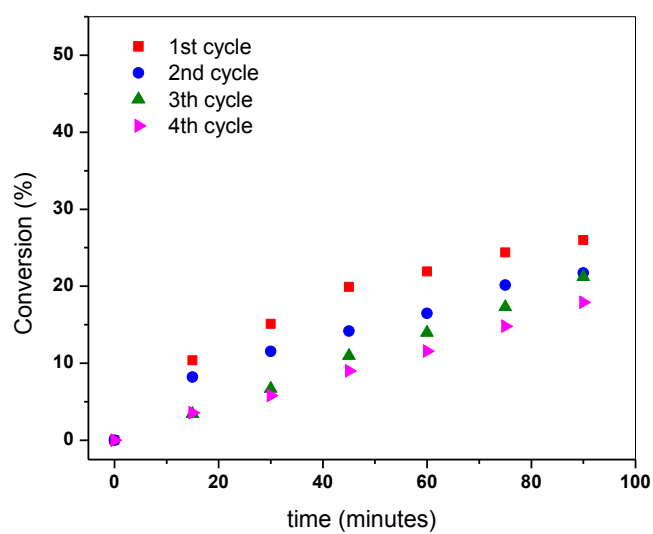


Fig. S-12. Kinetic Resolutions of (\pm)-1-Phenylethanol catalyzed by **CALB-XAD4**.

6. References

- 1 P. Lozano, E. García-Verdugo, J. M. Bernal, D. F. Izquierdo, M.I. Burguete, G. Sanchez-Gomez and S. V. Luis. Immobilised Lipase on Structured Supports Containing Covalently Attached Ionic Liquids for the Continuous Synthesis of Biodiesel in $scCO_2$. *ChemSusChem*, 2012, 5, 790- 798.