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Supplementary Information

Characterization of lipase from *Candida rugosa* entrapped in alginate beads to enhance its thermal stability and recyclability

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ALGINATE CHARACTERIZATION

M/G ratio

The determination of the M/G ratio of sodium alginate was performed using two spectroscopic methods: FTIR-ATR and NMR. The IR spectrum of the sodium alginate were acquired using the FTIR Spectrum Two (Perkin-Elmer) equipped with a UATR module at room temperature in a scan range between 4000 and 450 cm^{-1}, with 4 accumulations and at a resolution of 4 cm^{-1}. The mannuronate to guluronate ratio was firstly calculated according to Gómez-Ordóñez and co-workers through the ratio between the maximum intensity of the absorption bands centred at 1030 and 1080 cm^{-1} (A_{1030}/A_{1080}); these two bands are typical of the mannuronate (O-H bending) and guluronate (C-O-C stretching) subunit respectively [1,2].

The M/G ratio was also determined through NMR spectroscopy by applying the experimental procedure already reported in the literature [3]. An alginate solution 100 mg/ml were prepared in D_2O at pD=7 and placed in an NMR tube; the NMR spectrum has been acquired at the frequency of 50 MHz with pulse duration of 0.8 s and 40000 scans. Moreover, in order to minimize the drawbacks related to the viscosity of the alginate sample, the temperature of the probe has been set at 90 °C. The M/G value was obtained from the average between the integral ratios of all the individual carbon peaks of the mannuronate subunit and the guluronate ones.

Molecular weight

The determination of the average molecular weight of sodium alginate was obtained adopting the viscosimetric approach based on the Mark-Houwink-Sakurada relationship reported below:

$$[\eta] = K \cdot \overline{MW}^{a}$$

The values of K and a used for the determination of the MW of alginate are 7.3×10^{-5} dL/g and 0.92 for the measurements carried out in a 0.1 M sodium chloride solution and 1.23×10^{-4} dL/g and 0.96 in distilled water [4]. The viscosity of alginate solutions, measured by ranging the biopolymer concentration from 0.1 to 7 g/dL, increases exponentially increasing its concentration; therefore, in order to extrapolate the intrinsic viscosity only the first stroke of the curve, comprise between 0.1 and 1.5 g/dL was considered.

Table S1 The maximum intensity of IR absorption bands centred at 1030 (A_{1030}) and 1080 cm^{-1} (A_{1080}), corresponding to O-H bending of mannuronate and C-O-C stretching of guluronate respectively, have been used to calculate M/G ratio of alginate through their ratio (A_{1030}/A_{1080}).

A_{1030}	A_{1080}	A_{1030}/A_{1080}
1.27	0.67	1.90
0.97	0.57	1.70
0.89	0.51	1.75
0.96	0.50	1.92
Average M/G ratio		**1.82 ± 0.11**

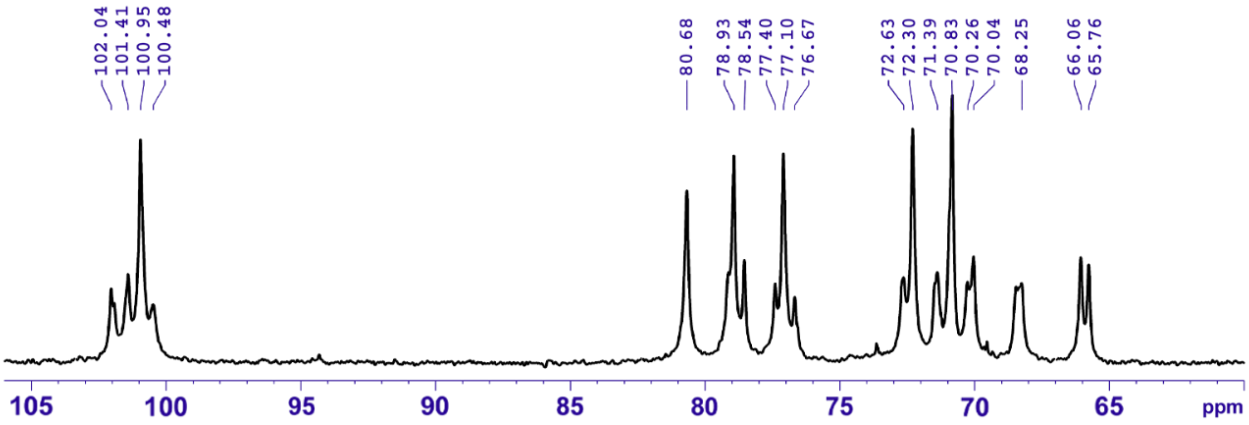


Fig. S1 ^{13}C-NMR spectrum of an alginate solution 100 mg/ml prepared in D_2O at pD = 7.

Table S2 Carbon integrals values of mannuronate and guluronate subunits, obtained from ^{13}C NMR analysis and the M/G ratios obtained. The average M/G ratio is also reported with its associated standard deviation.

	C1	C2	C3	C4	C5
M	2629.99	3416.12	2508.90	2519.38	2444.70
G	1488.36	1232.44	1711.23	1439.38	1364.14
M/G	1.77	1.71	1.82	1.75	1.79
Average M/G ratio				**1.77±0.04**	

Table S3 Enzyme concentration measured, by means of the Bradford method, in the storage solution for all six bead formulations

	BEADS 1	BEADS 2	BEADS 3	BEADS 4	BEADS 5	BEADS 6
	Enzyme loss, μg/ml [a]					
preparation solution	0	0	0	0	0	0
washing solution	0	0	0	0	0	10
after 2h	0	7	0	7	0	0
after 24h	0	10	0	0	0	0
after 48h	30	14	12	15	0	0
after 96h	30	12	0	0	0	0
after 7d	27	13	0	0	0	0
after 14d	31	15	11	9	0	0
after 18d	38	22	12	8	14	14
after 30d	35	22	11	0	12	0

[a] The sensitivity of the detection method is 22 μg/ml [5]

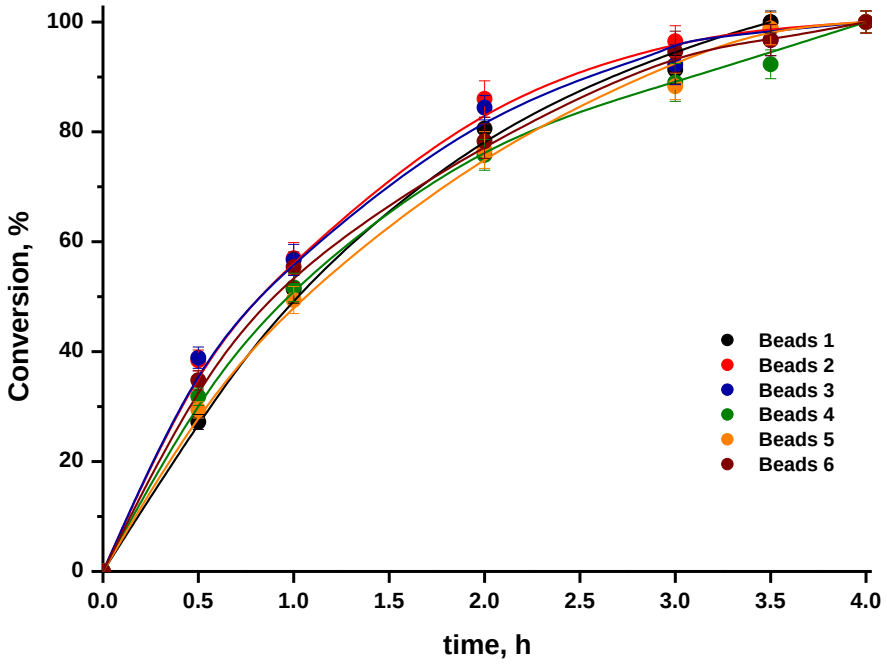


Fig. S2 Substrate conversion percentages for all types of beads in distilled water as a function of time after one month of storage in distilled water at 4 °C; [*p*-NPA] = 10 mM, T = 25 °C.

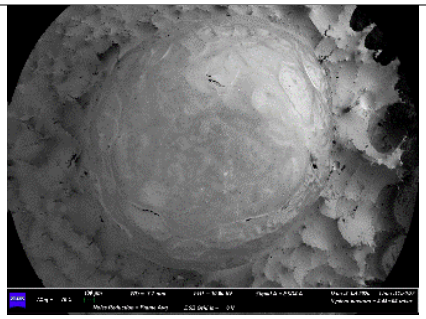
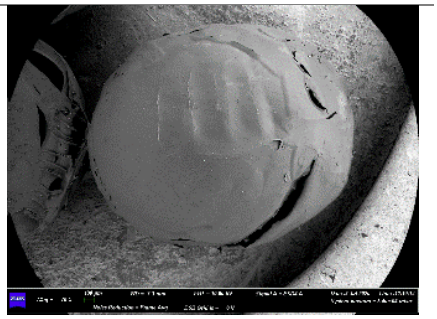
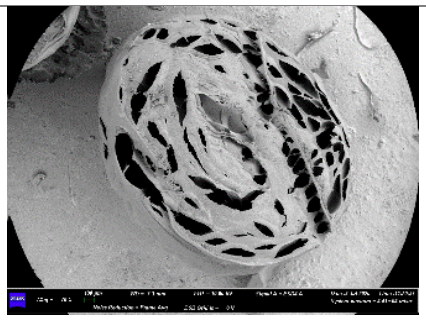
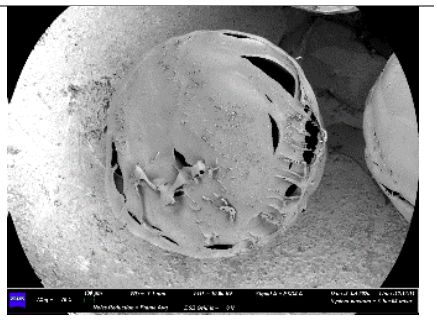
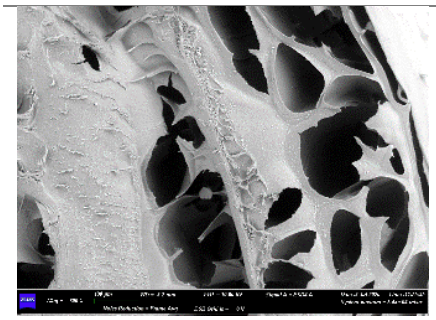
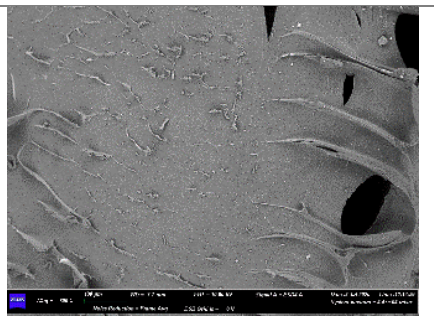
	Beads 2	Beads 5
External surface **70 x**		
70 x		
Internal structure **300 x**		

Fig. S3 SEM images at 70x magnification of the external structure and at 70x and 300x magnifications of the internal structure of **Beads 2** and **Beads 5.**

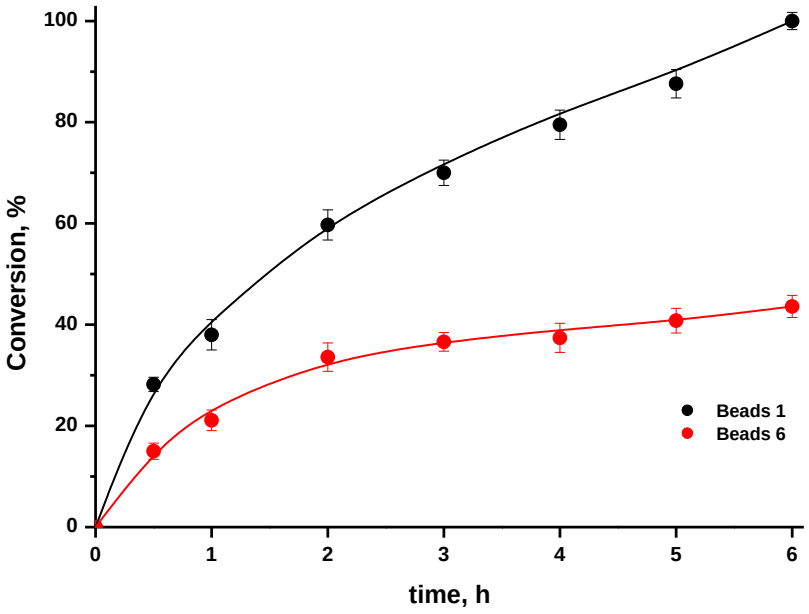


Fig. S4 Substrate conversion percentages over time for **Beads 1** and **6** in *tert*-butyl alcohol as a function of time; [*p*-NPD] = 10 mM, T = 25 °C.

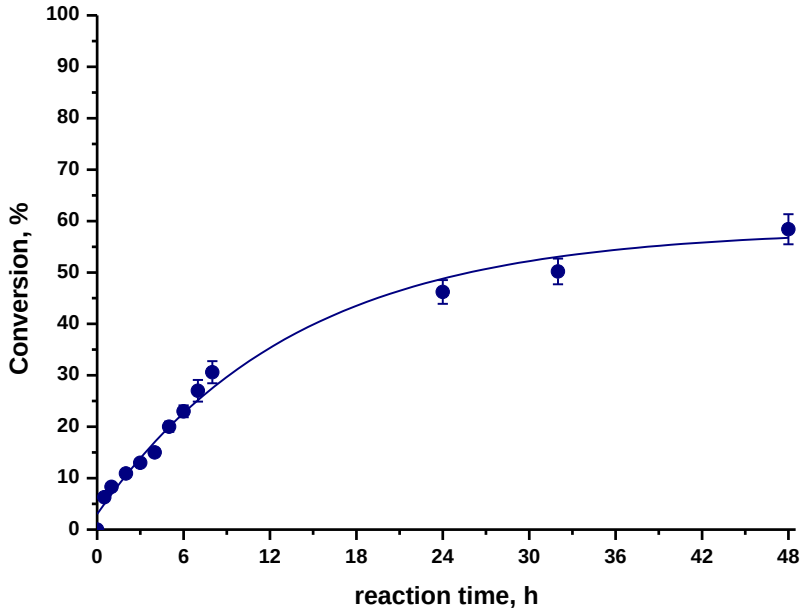


Fig. S5 Substrate conversion percentages over time for the reaction catalyzed by **Beads 1** in *tert*-butyl alcohol; [*p*-NPA] = 10 mM, T = 25 °C.

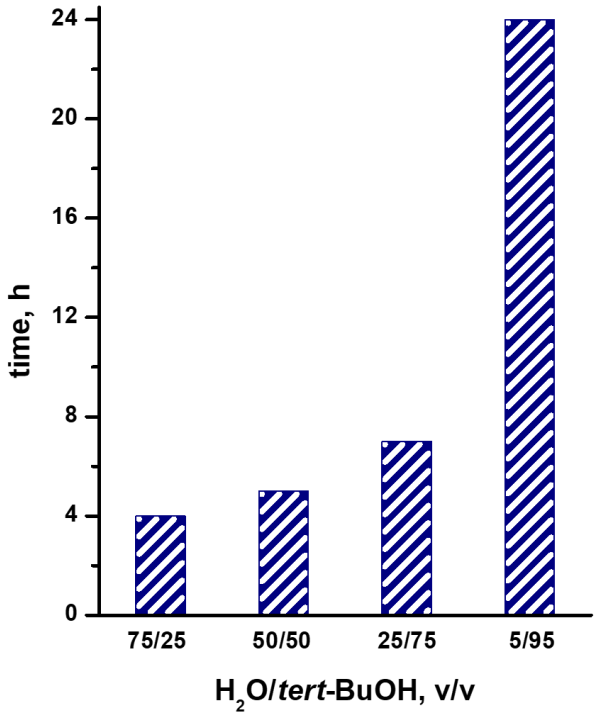


Fig. S6 Effect of the percentage of water added to *tert*-butyl alcohol on time required for complete conversion of *p*-NPA performed by **Beads 1**; [*p*-NPA] = 10 mM, T = 25 °C.

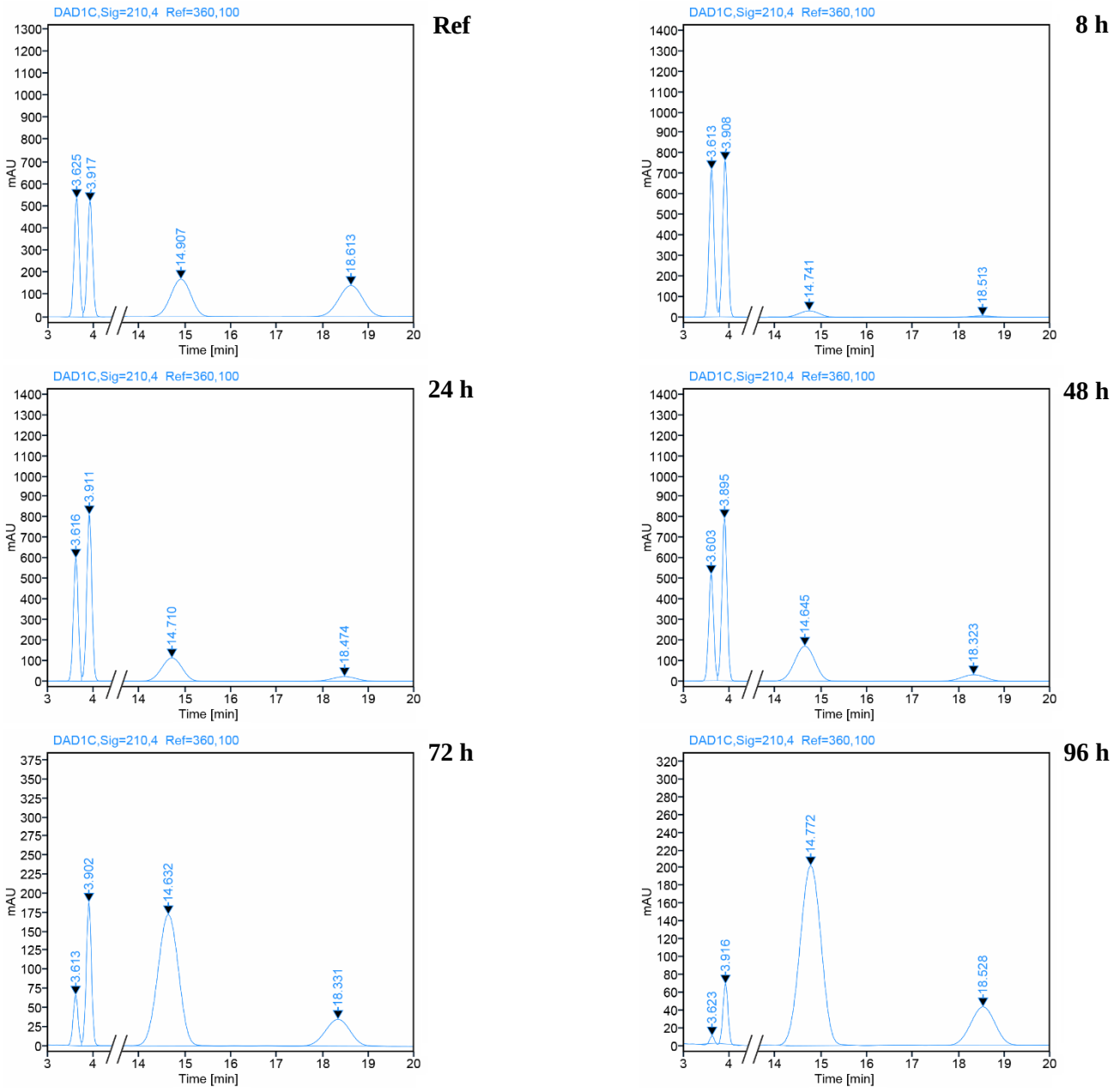


Fig. S7 Different time chromatograms of the kinetic resolution of *rac*-1-phenylethyl acetate catalyzed by Beads 1. The retention times were 3.6, 3.9, 14.7 and 18.5 min for (*R*)-1-phenylethyl acetate, (*S*)-1-phenylethylacetate, (*R*)-1-phenylethanol and (*S*)-1-phenylethanol, respectively.

References

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