Supplementary information

Towards sustainable kinetic resolution, a combination of bio-catalysis, flow chemistry and ~~greener~~ more sustainable bio-based solvents

Andree Iemhoff,[a,b] James Sherwood,[a] Con R. McElroy[a] and Andrew J. Hunt[a,c*]

[a] Department of Chemistry, University of York, Heslington, York, UK. YO10 5DD

[b] Institut für Technische und Makromolekulare Chemie, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

[c] Materials Chemistry Research Center, Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand. (*Corresponding Author, E-mail: andrew@kku.ac.th)

Experimental procedures

Chemicals. Racemic 2-phenylpropionic acid was purchased from Sigma Aldrich (97%). Ethanol, hexane, ethyl acetate, toluene, chloroform and tetrahydrofuran were purchased from VWR. NMP, *d*-limonene, and γ-valerolactone were purchased from Sigma Aldrich. Propylene carbonate, diethyl carbonate, *p*-cymene and 2-methyltetrahydrofuran were purchased from Acros. DMF and methyl ethyl ketone were purchased from Fisher Scientific. Anisole was purchased from Alfa Aesar. All solvents were used as received without further purification. Lipase B (*Candida Antarctica*, ≥5,000 U/g, recombinant, expressed in Aspergillus niger*)*, supported on a macroporous acrylic resin (Novozyme 435) was purchased from Sigma Aldrich.

Batch esterification reactions. 2-Phenylpropionic acid (120 mg, 0.8 mmol, 1 Eq), supported enzyme (20 mg), biphenyl as internal standard (44 mg) and 4 mL of solvent were weighed into a 8 mL glass vial equipped with a magnetic stirring bar, which was put in an aluminium multipoint block to reach the desired reaction temperature while stirring at 300 rpm. The reaction was initiated by adding ethanol (60.7 µL, 1.04 mmol, 1.3 eq.). Aliquots (50 µL) were taken in regular intervals for GC analysis, which were filtered through glass wool and diluted with 1.1 mL $CHCl_3$ prior to analysis.

GC analysis was carried out with an Agilent Technologies 6890N Network system equipped with FID detector. A Restex stabilwax (30 m x 0.25 mm x 0.25 µm) column was used with Helium as a carrier gas at 65.6 kPA constant pressure (flow: 26.3 mL min^{-1}). The temperature programme was as follows: From 40 °C the temperature was ramped up to 250 °C at 30 °C min^{-1}. A split ratio of 30:1 was used and the detector was heated to 300 °C. The overall run time of the method was 20 minutes. The retention times of the compounds of interest were confirmed by measuring samples of the pure compound in chloroform: ethanol (3.1 min), 2-phenylpropionic acid ethyl ester (10.4 min), biphenyl (internal standard, 11.4 min) and 2-phenylpropionic acid (12.9 min). The concentration of the 2-phenylpropionic acid and the ethyl ester were determined with calibration curves.

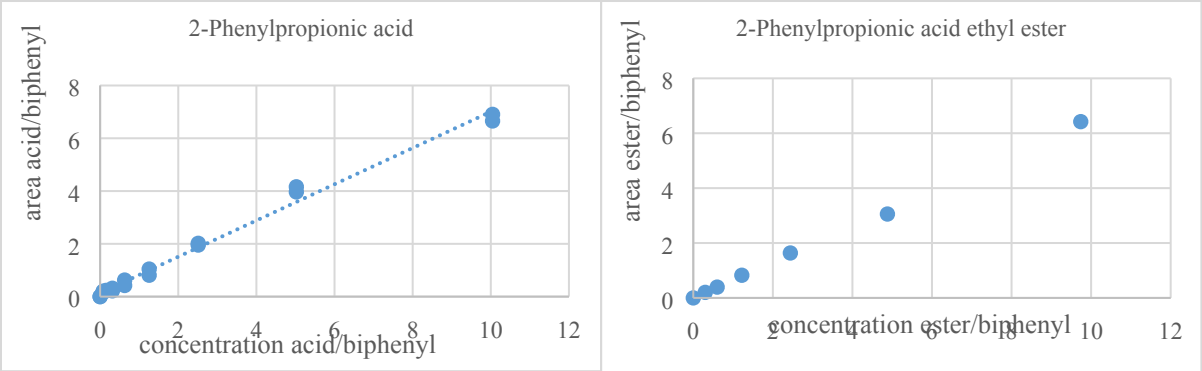

Figure S1. Calibration curves for 2-phenylpropionic acid and the respective ethyl ester with biphenyl as the internal stand.

Isolation of 2-phenylpropionic ethyl ester. 2-Phenylpropionic acid (1.0 g, 6.7 mmol), ethanol (7.5 mL, 128.8 mmol, 19 eq.) were dissolved in 50 mL Toluene. 250 µL hydrochloric acid (37 %) were added and the mixture was refluxed until the water formation in a Dean Stark trapped stopped. The organic phase was washed with ice water (80 mL) and unreacted acid and ethanol were extracted with saturated aqueous Na_2CO_3 solution. After drying with $MgSO_4$ the organic solution was filtered, and the toluene was removed *in vacuo*. The crude product was purified by column chromatography with cyclohexane:ethyl acetate (95:5, v:v) as eluent and silica gel as stationary phase. The product was obtained as a colourless oil (810.8 mg, 4.45 mmol, 66 %). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 1.14 (t, J = 7 Hz, 3H), 1.43 (d, J = 7 Hz, 3 H), 3.64 (q, J = 7 Hz , 1H), 4.01 (m, 2H) 7.23 (m, 5 H) /ppm.

Control reaction. To demonstrate the necessity of a (bio)catalyst, the reaction was performed in *p*-cymene without enzyme or any other catalyst. No conversion was detected after 24 hours. For a sample of supported enzyme that was denaturised at 150 °C for 36 hours, a conversion of 0.4% was measured in *p*-cymene after 96 hours. Ethanol was chosen as reagent since it was identified as the most efficient candidate for kinetic resolution of Ketoprofen in other publications.[1,2] Moreover, an increasing amount of ethanol (up to 50 eq.) was shown to have an adverse effect on the conversion, thus the experiments were performed with a slight excess of 1.3 equivalents. The mass of catalyst was chosen to be 20 mg as more added catalyst did not increase the conversion after 24 hours in *p*-cymene.

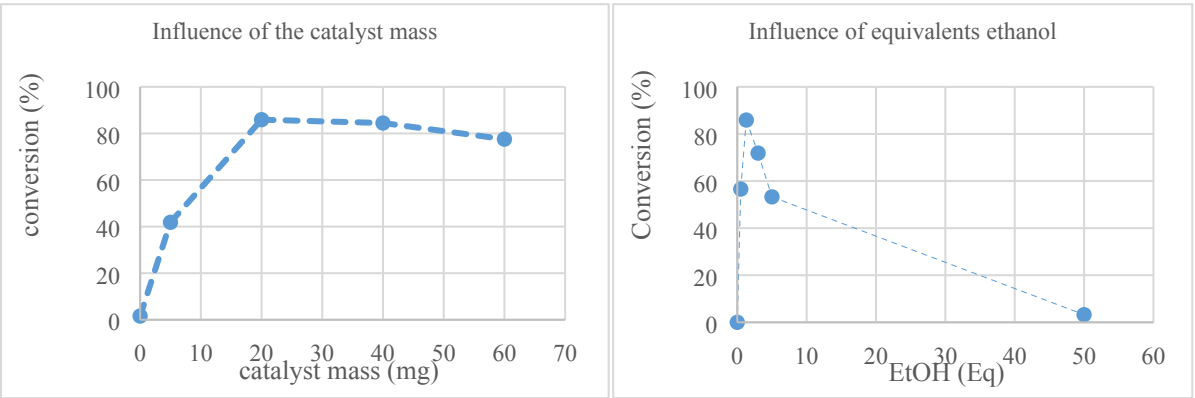

Figure S2. Influence of the catalyst mass and equivalents of ethanol on the esterification of 2-phenylpropionic acid.

Calculation of initial rate

The initial rate of the reaction was calculated from the amount of 2-phenylpropionic acid ethyl ester that was formed in the first 8 hours. It corresponds to the slope of the linear fit of concentration over time.

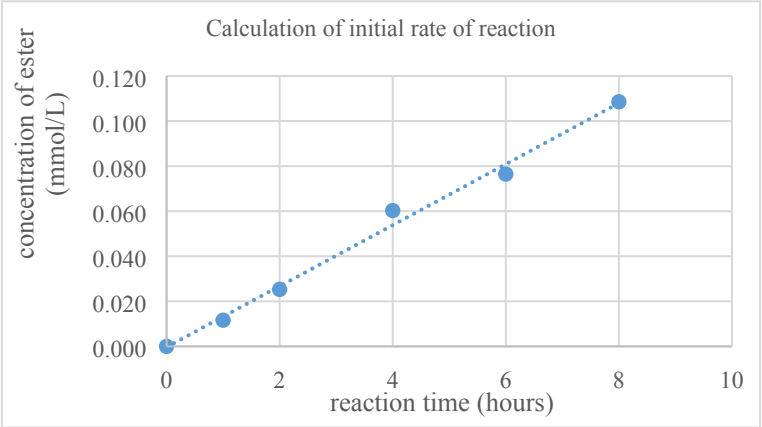

Figure S3. Calculation of the initial rate of reaction by a linear fit of the increasing concentration of 2-phenylproionic acid ethyl ester.

Linear solvation energy relationship

Polarisability/dipolarity (π*), and the square of Hildebrand´s solubility parameter (δ_H^2) were found not to be statistically relevant for the correlation. All the solvents used for the reaction were aprotic, meaning α was also not relevant. Table S1 presents the coefficients for the relationships discussed in the main article, along with P-values and overall coefficient of determination, for equations in the form $XYZ = XYZ_0 + b\cdot\beta + c\cdot V_m + d\cdot\log(P)$. The number of solvents included in the regression analysis is given as 'n'. The full set of solvents in which the reaction proceeds is 12. Ten solvent correlations ignore ethyl acetate and THF, which could not be modelled correctly. The solvent set for the water stripping calculation is different.

Table S1. Correlation data.

XYZ	XYZ_0	b		c		d		R^2	n
ln(r)	-0.18	-2.44	0.00237	0.0183	0.00377	-	-	0.8753	10
ln(r)	0.20	-	-	-	-	0.48	0.00571	0.6361	10
ln(r)	-1.27	-3.33	0.0223	0.0271	0.0214	-	-	0.7064	12
ln(r)	-0.69	-	-	-	-	0.72	0.00599	0.5467	12
$\ln(W_d/W_a)$	-3.37	4.98	0.000294	-0.00877	0.2671	-	-	0.8451	11
$\ln(W_d/W_a)$	-4.38	5.19	0.000133	-	-	-	-	0.8175	11
$\ln(W_d/W_a)$	-0.49	-	-	-	-	-0.78	0.0286	0.4296	11

Catalyst recycling

For the recycling experiments the catalyst was filtered, washed with *p*-cymene and acetone and dried in a petri dish at ambient temperature. The following cycle was carried out in the same procedure as described above for the batch reactions. For cycle two, the amount of the reagents and solvents were adjusted to the amount of catalyst that was recovered.

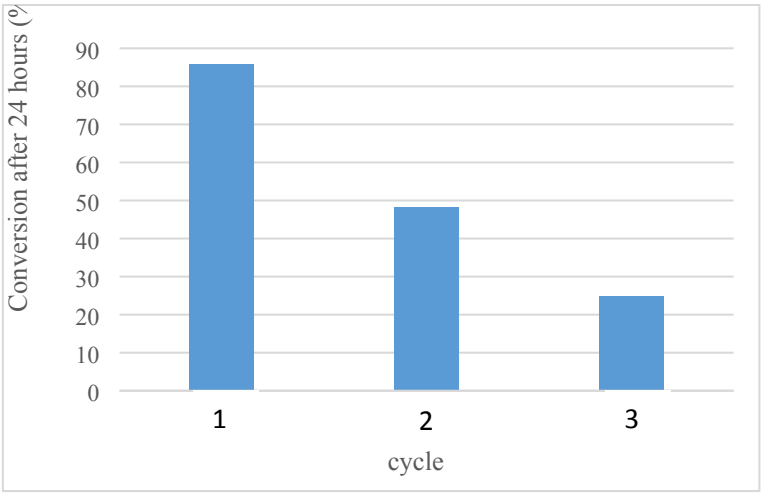

Figure S4. Effect of recycling of CAL-B supported on acrylic resin on conversion in batch experiments.

Flow setup

A HPLC column (diameter = 4.6 mm, length = 150 mm) was packed with 700 mg of Novozym 435. The solid bed was tightened with cotton wool at both ends, yielding a length of 135 mm of supported enzyme in the column. The void volume was calculated to be 70.5%, based on the density of acrylic resin (as stated by the manufacturer and the bulk density of the porous support, as determined with a measuring cylinder). With these parameters the void volume within the catalyst bed was calculated to be 1.58 mL.

Volume of the packed bed:

$$\pi r^2 \times 135\ mm = \ \pi \times (2.3\ mm)^2 \times 135\ mm = 2.24\ mL$$

Percentage of void volume in packed bed reactor:

$$1 - \frac{bulk\ density}{carrier\ density} = 1 - \frac{0.3122 \frac{g}{cm^3}}{1.06 \frac{g}{cm^3}} = \ 70.5\ \%$$

Stainless steel tubing was used to connect the packed column to a HPLC pump (Jasco PU - 1585 Intelligent HPLC Pump) to control the flow rate. To maintain the temperature at 40 °C the column was placed inside a column oven (Merck LaChrom L7300). The catalyst bed was pretreated with pure *p*-cymene for 10 min at 0.5 mL min^{-1} before switching to a solution of 2PPA (50 mg mL^{-1}) and ethanol (19.94 mg mL^{-1}, 1.3 eq.) in *p*-cymene at 9 µL min^{-1}. The setup was left running overnight to obtain steady conversion. Between experiments, the column was detached from the flow setup, sealed and stored in a refrigerator (4 °C). The conversion of 2PPA was determined by gas chromatography (see above for method) and quantified by an external standard.

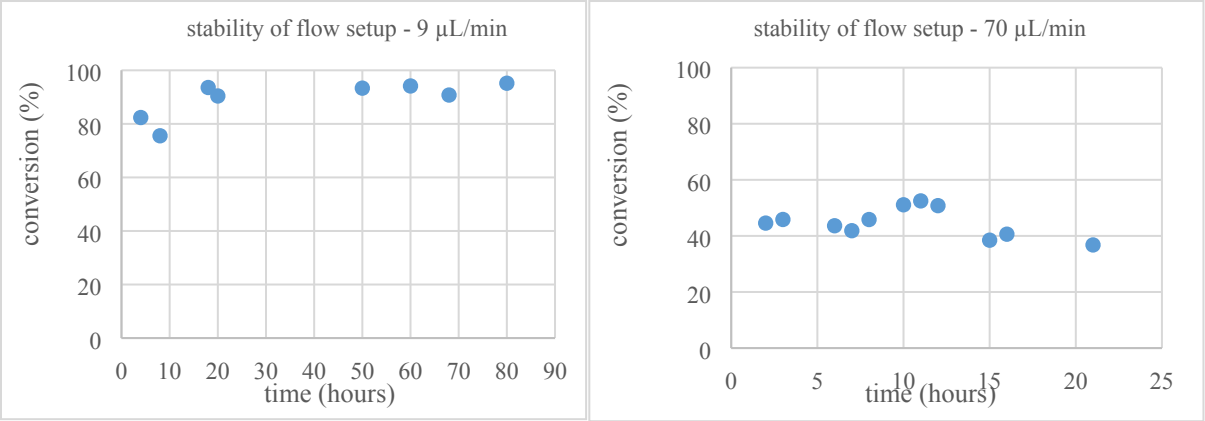

Figure S5. Stability of the conversion in flow setup at different flow rates with a concentration of 50 mg/mL. For 70 µL/min a decrease in conversion can be observed after 20 hours on stream.

Furthermore, the concentration was increased to 250 mg mL^{-1} and tested at two different flow rates. At 9 µL min^{-1} conversion drops to 75% and at 20 µL min^{-1} 45% conversion are detected. In the light of this, increasing the concentration seems a valuable alternative regarding the slow flow rates that are necessary to achieve sufficient conversions.

Microscopic images of used catalyst

Microscopic images were recorded on a Leica S6 D stereomicroscope.

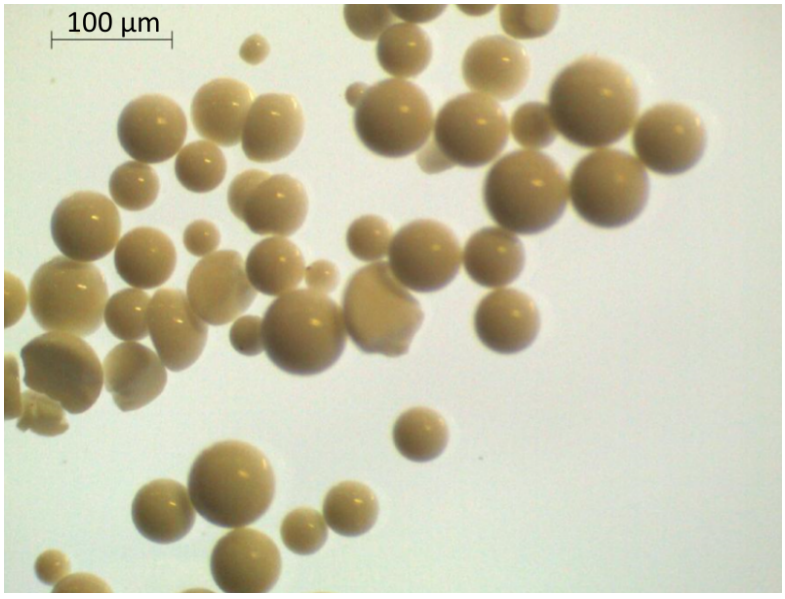

Figure S6. Microscopic image of virgin CAL B supported on acrylic resin.

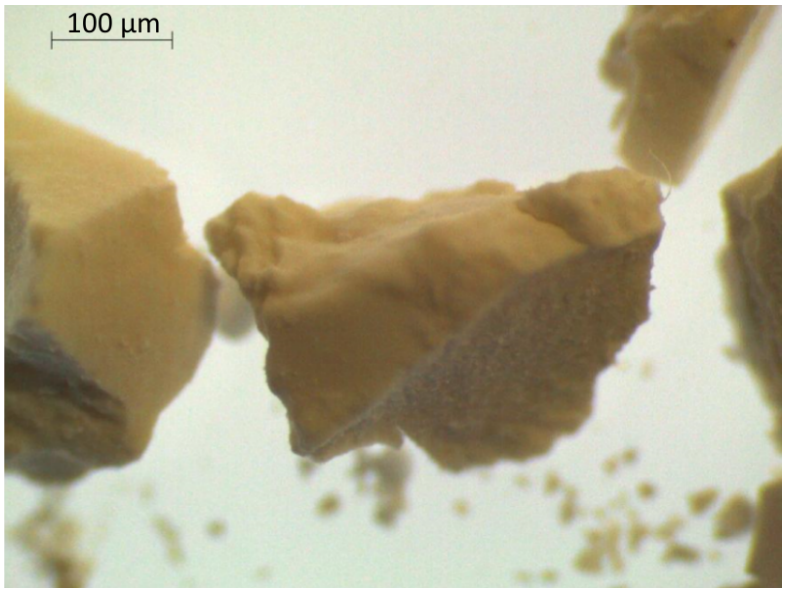

Figure S7. Microscopic image of supported catalyst after recycling and drying from batch experiments. The bead like structure of the porous support is lost and the polymer has aggregated to larger, unregular particles.

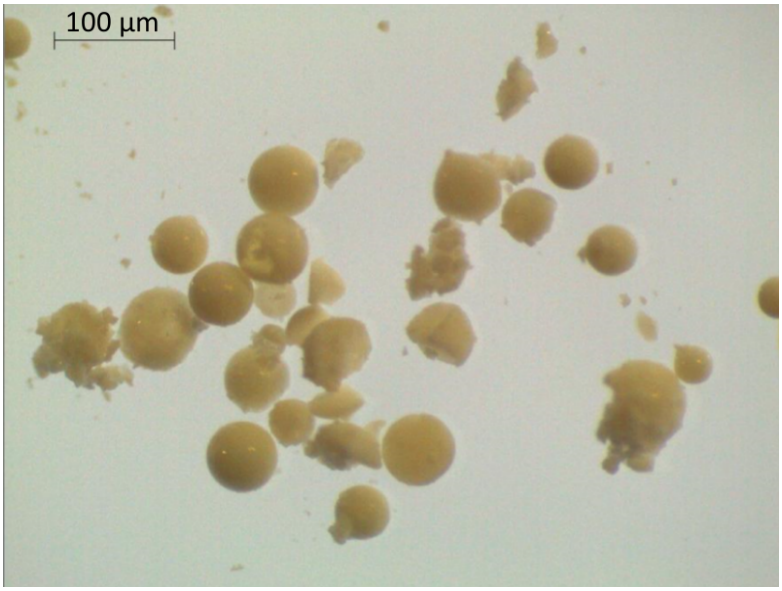

Figure S8. Microcopic image of catalyst after use in flow system. The original structure of the polymer support appears to be mostly intact. Some beads exhibit a coarser structure compared to the unused catalyst, which can be attributed to swelling of the polymer in *p*-cymene.

Chiral HPLC

Chiral stationary phase HPLC was performed on an Agilent 1200 series chromatograph equipped with a Chiralpak IB column and UV-detection at 210 nm. A mixture of n-hexane and isopropanol (98:2, v:v) was used as the eluent. The samples were prepared by extraction of unreacted 2-phenylpropionic acid and ethanol with saturated aqueous $NaHCO_3$ solution and drying of the *p*-cymene solution over $MgSO_4$. The concentration of the ester was determined by GC-FID and the samples were diluted with *n*-hexane to obtain samples with a concentration of 2-phenylpropionic acid ethyl ester between 1 and 2 mg mL^{-1}. The flow rate was 0.5 mg mL^{-1}.

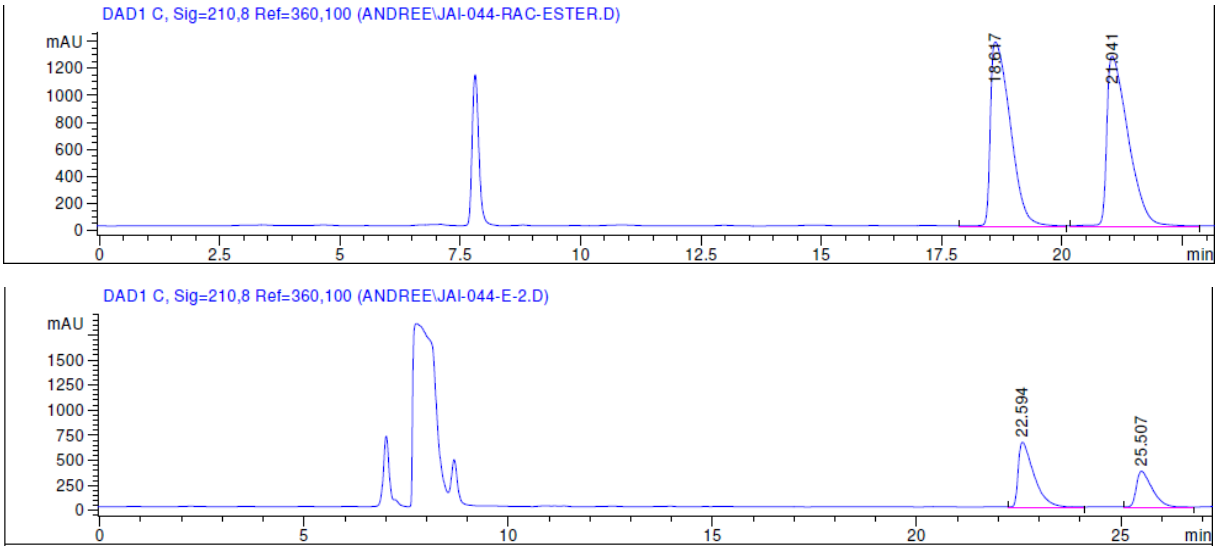

Figure S9. Chiral HPLC of racemic 2-Phenylpropionic acid ethyl ester in p-cymene (above) and ester synthesised in flow at 70 µL/min. The two additional peaks near the p-cymene signal at 7.5 min can be attributed to impurities in the solvent.

Table S2. Performance of the flow system at different flow rates determined by GC-FID and chiral HPLC. Enantiomeric values (E) were calculated according to Sih *et al.* For 9 µL min^{-1} the enantiomeric value could not be calculated.

Flow rate (µL min^{-1})	**Residence time (hours)**	**Conversion (%)**	**ee_{ester} (%)**	**E**
9	2.93	92.08	-	-
20	1.32	81.90	4.35	1.25
30	0.88	68.82	14.13	1.72
50	0.53	50.02	29.94	2.08
70	0.38	44.35	28.72	2.23
90	0.29	34.72	34.14	2.41

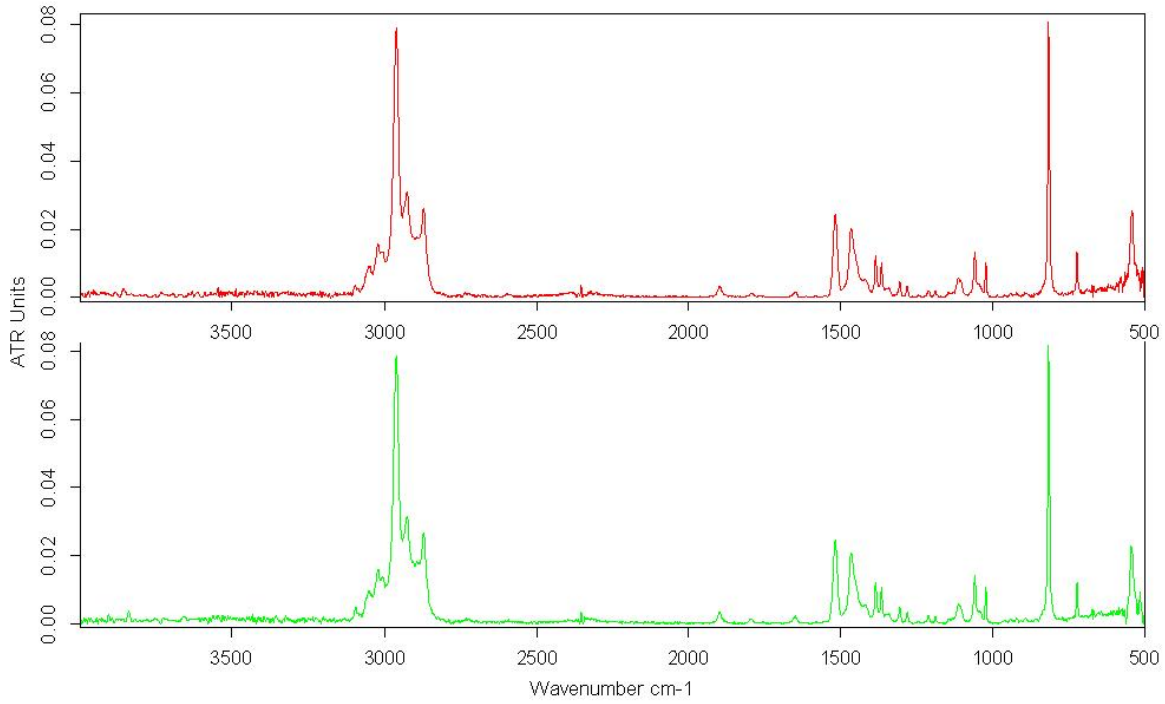

Figure S10. IR-spectra of the p-cymene solution (red) that was used for pretreating the flow setup shows no *evidence of* dissolved polymer support (in comparison to a blank samples of *p*-cymene (green)).

References

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Manufacturer information on acrylic resin used for Novozyme 435 can be found at *http://www.lenntech.com/Data-sheets/Lewatit-VP-OC-1600-L.pdf*, accessed 10/10/2017.