Supporting information

Carbon nanotube/PTFE as a hybrid platform for lipase B from *Candida antarctica* in transformation of α-angelica lactone to alkyl levulinates

Anna Szelwicka,[1] Anna Kolanowska,[2] Piotr Latos,[1] Sebastian Jurczyk,[3] Slawomir Boncel[2] and Anna Chrobok[1*]*

[1)] Department of Organic Chemical Technology and Petrochemistry, Silesian University of Technology, Krzywoustego 4, 44-100 Gliwice, Poland

[2)] Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, Krzywoustego 4, 44-100 Gliwice, Poland

[3)] Institute for Engineering of Polymer Materials and Dyes, Skłodowskiej-Curie 55, 87-100 Toruń, Poland

Table of contents in Supporting Information

S1. GC-FID analysis

Table S1 Temperature program of GC-FID analysis.

Initial temperature (°C)	Rate of increasing	Holding time (min)

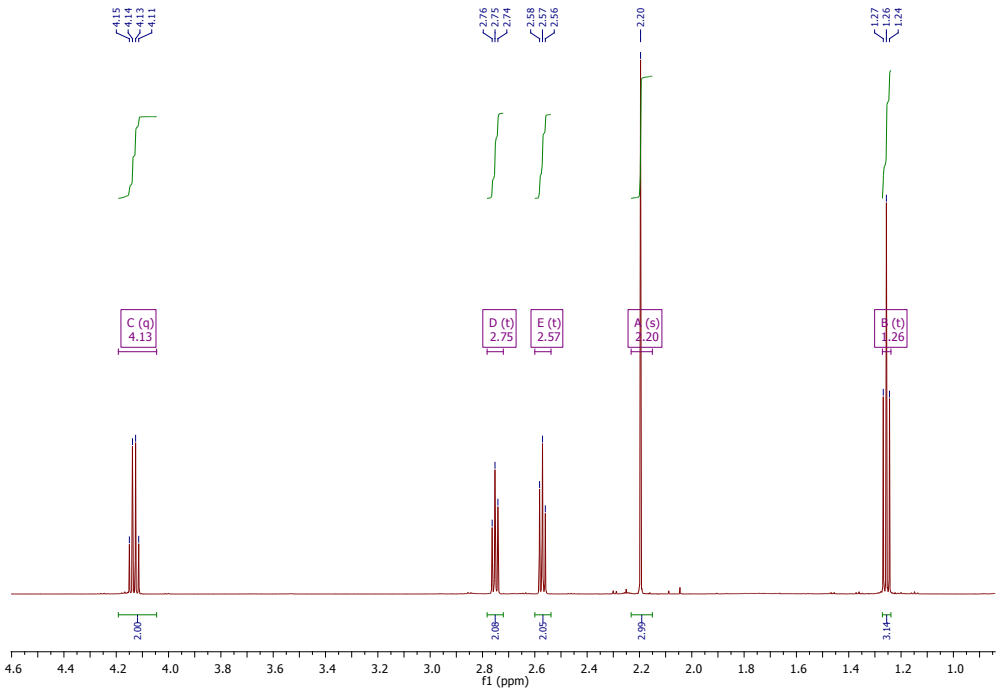
	temperature (°C/min)	
80	-	2
80	40	0
200	20	0
280	-	5

run time: 14 min

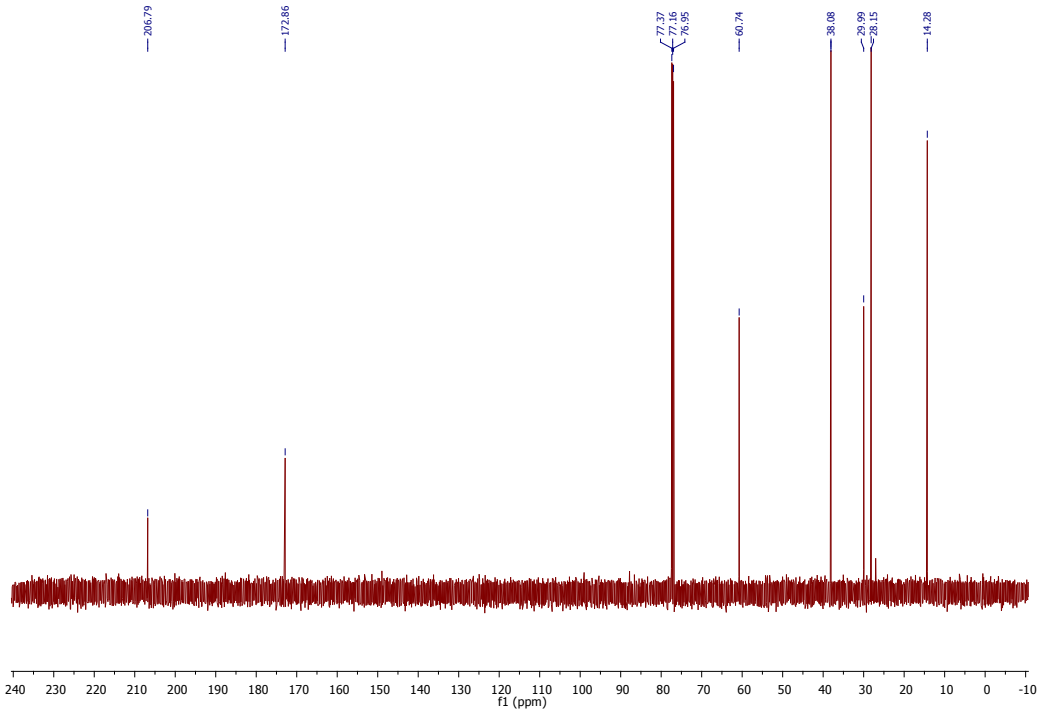
Table S2 Retention time and parameters of linear regression.

Compound	Retention time (min)	Linear regression equation	Correlation coefficient (r)
α-angelica lactone	2.053	y=0.4842x-0.0455	0.9999
ethyl levulinate	4.165	y=0.4950x-0.1556	0.9997
n-propyl levulinate	4.705	y=0.4554x-0.1254	0.9989
iso-propyl levulinate	4.375	y=0.5414x-0.0638	0.9987
n-butyl levulinate	5.193	y=0.8451x-0.2032	0.9997
iso-octyl levulinate	7.125	y=0.7205x-0.1854	0.9988
n-dodecyl levulinate	8.610	y=0.9964x-0.1061	0.9997

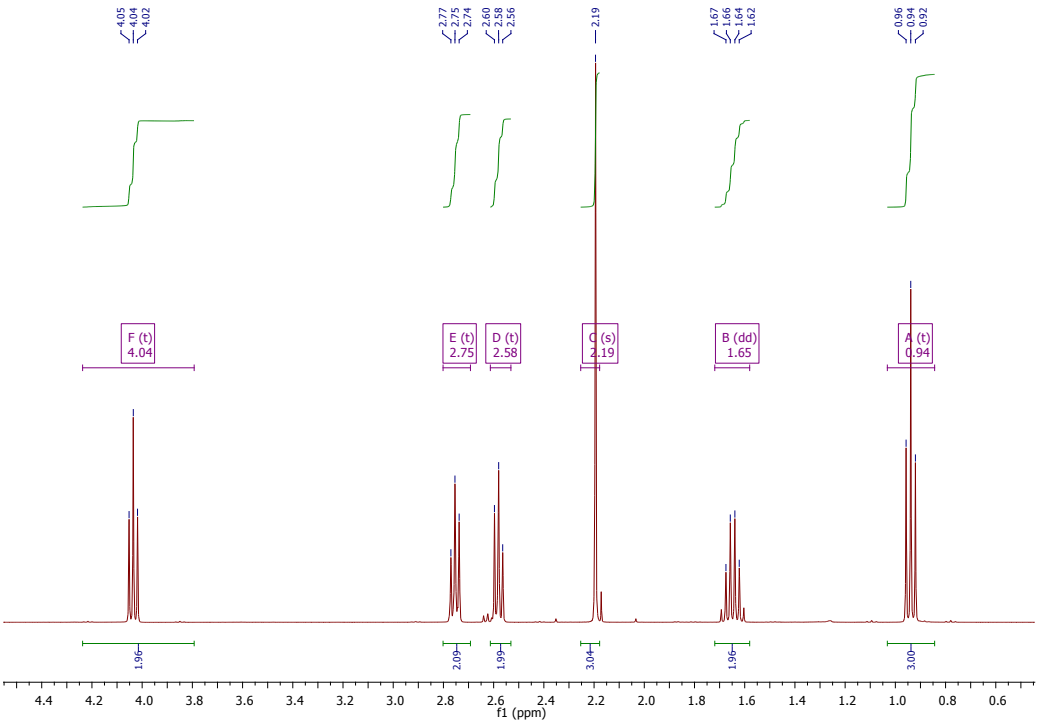
S2. ^{1}H and ^{13}C NMR spectra of products



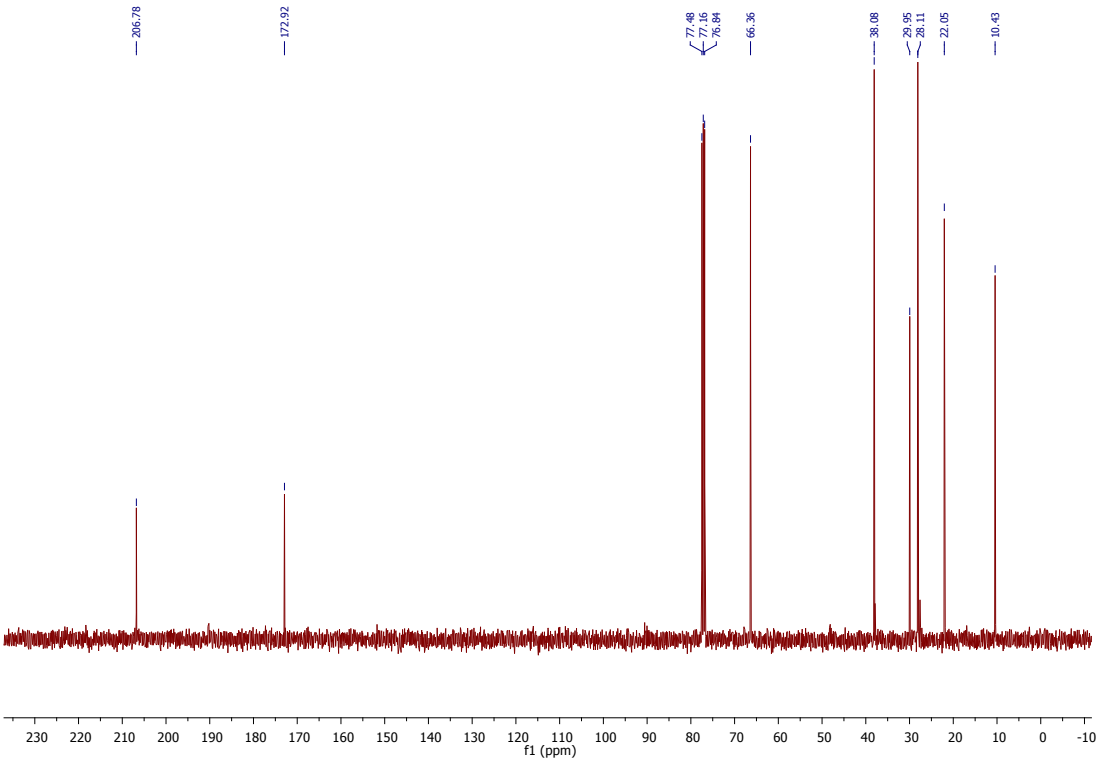
Scheme S1 ^{1}H NMR spectrum of ethyl levulinate.



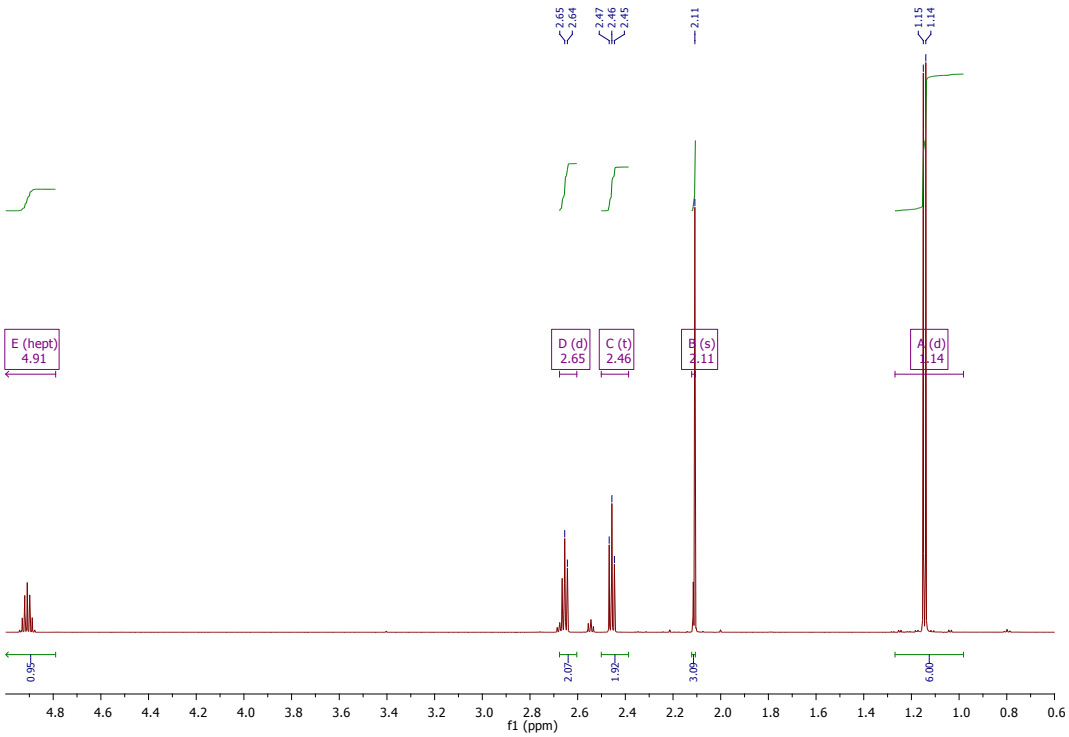
Scheme S2 ^{13}C NMR spectrum of ethyl levulinate.



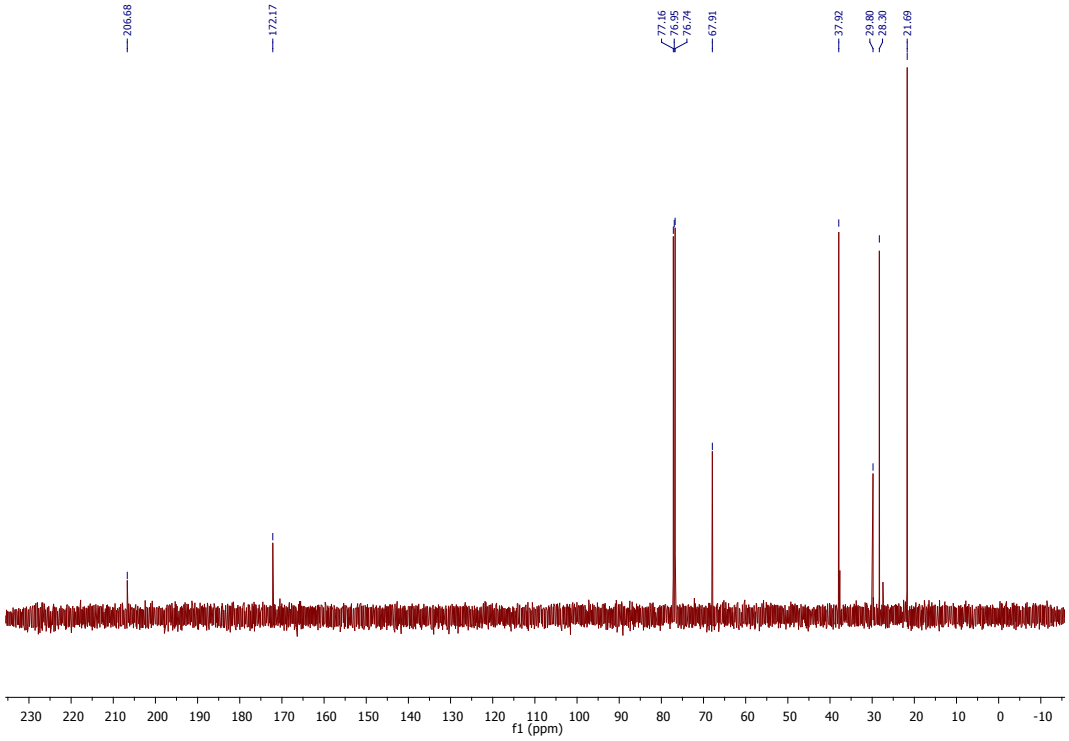
Scheme S3 ^{1}H NMR spectrum of *n*-propyl levulinate.



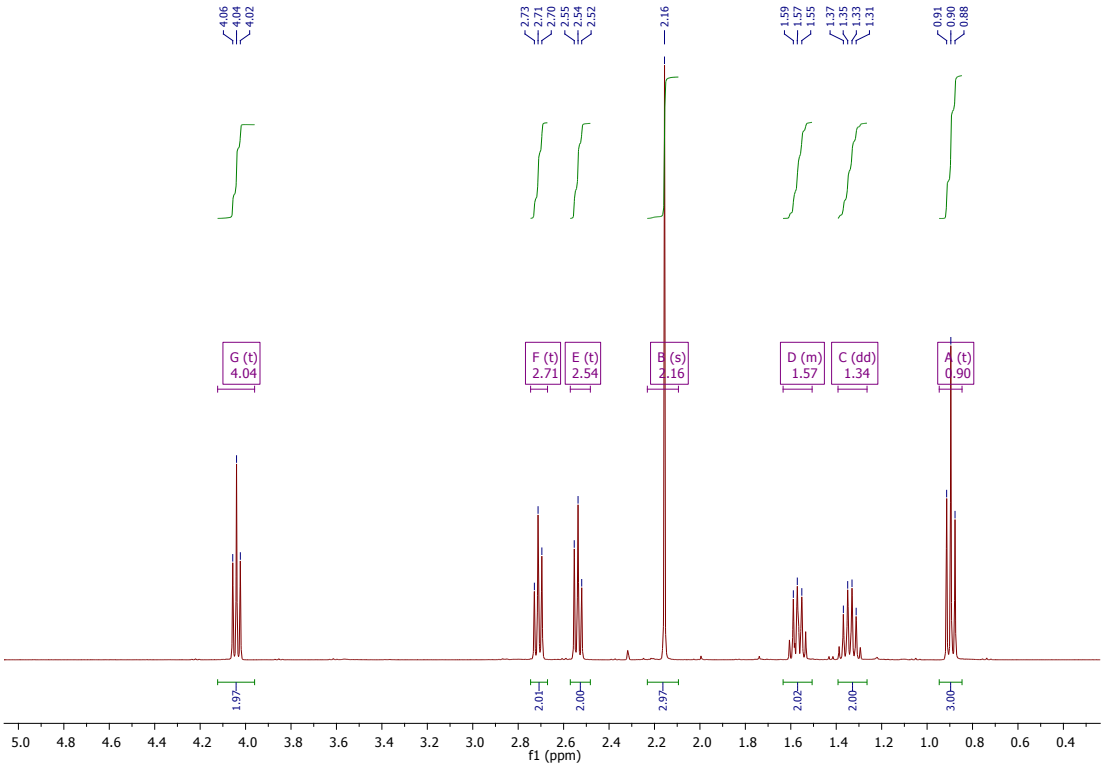
Scheme S4 ^{13}C NMR spectrum of *n*-propyl levulinate.



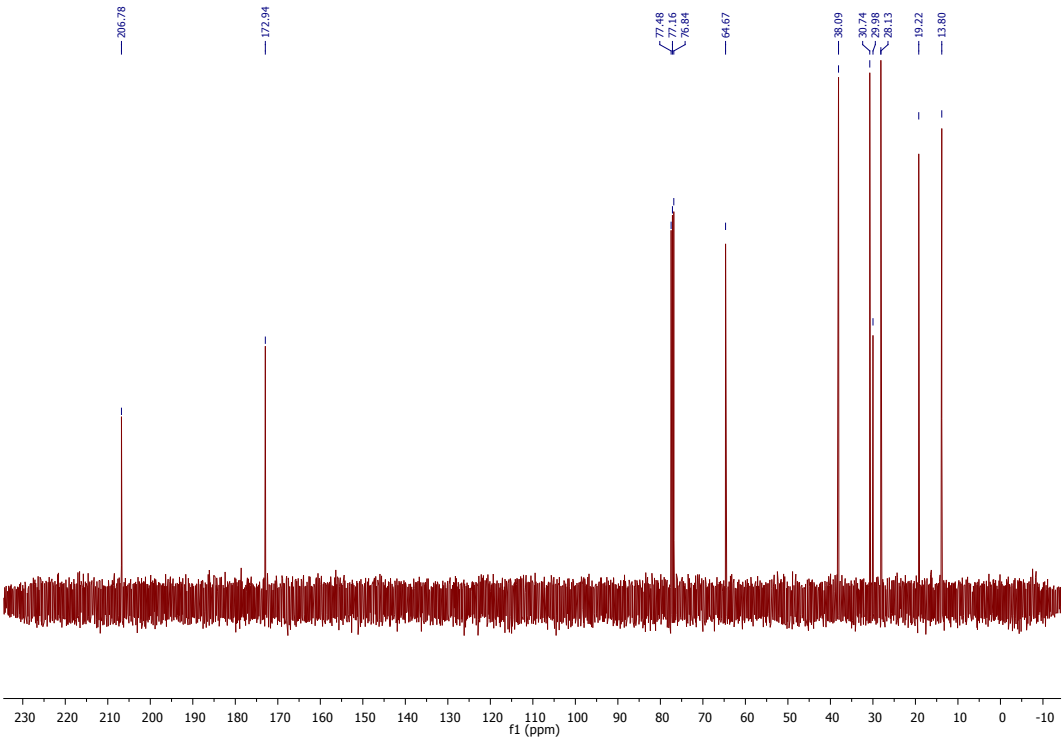
Scheme S5 ^{1}H NMR spectrum of *iso*-propyl levulinate.



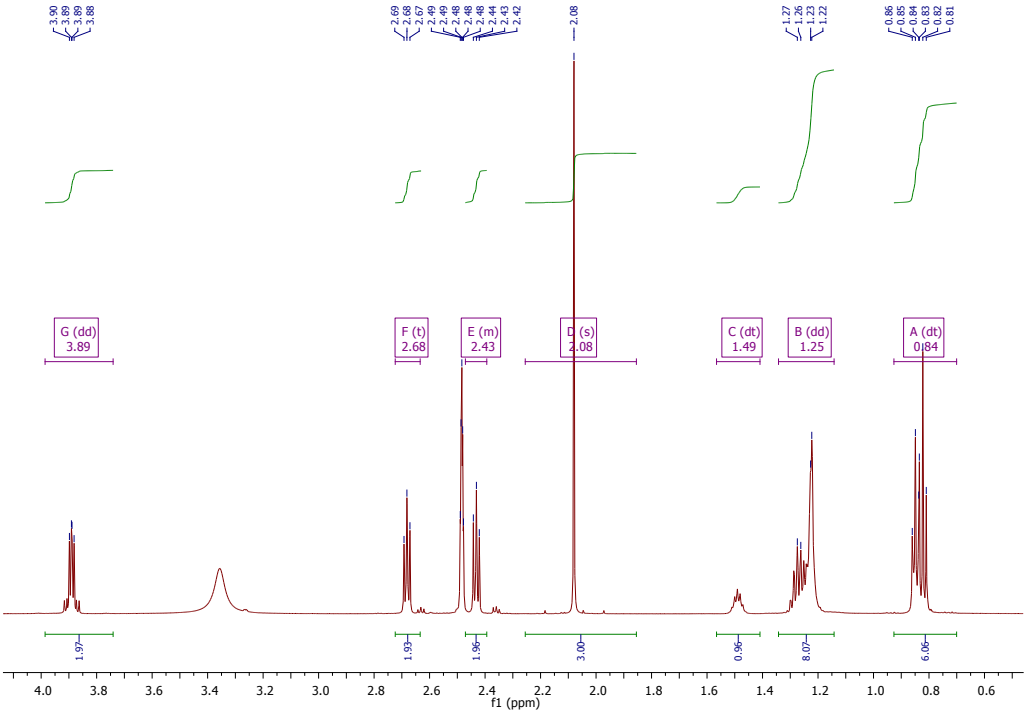
Scheme S6 ^{13}C NMR spectrum of *iso*-propyl levulinate.



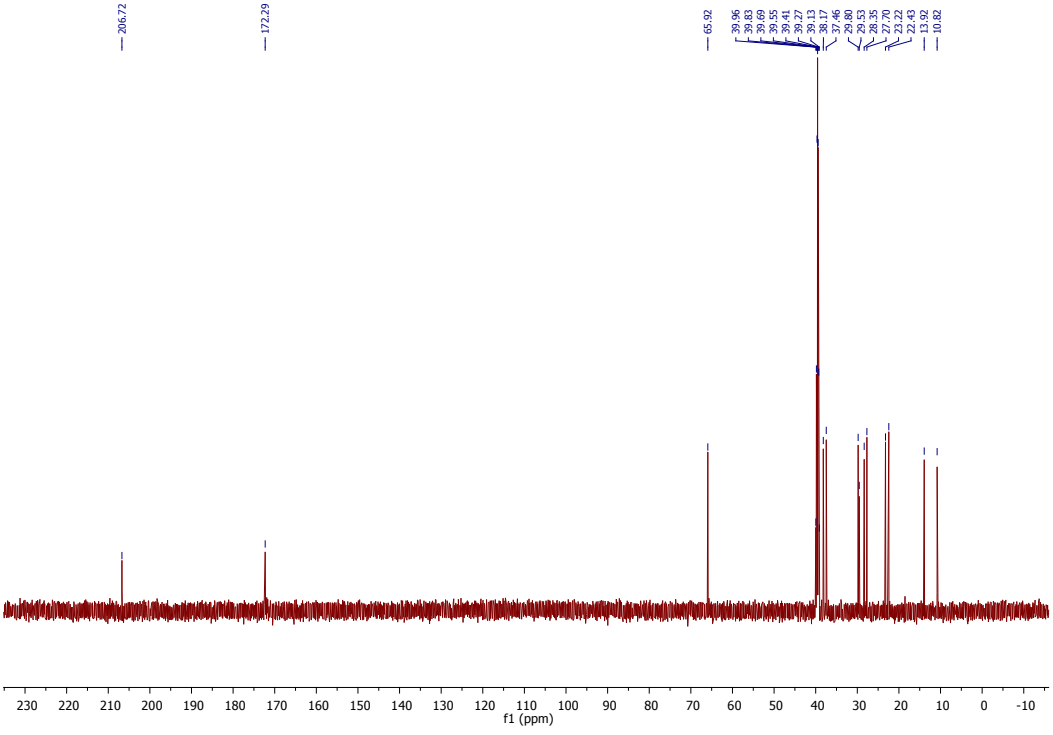
Scheme S7 ^{1}H NMR spectrum of *n*-butyl levulinate.



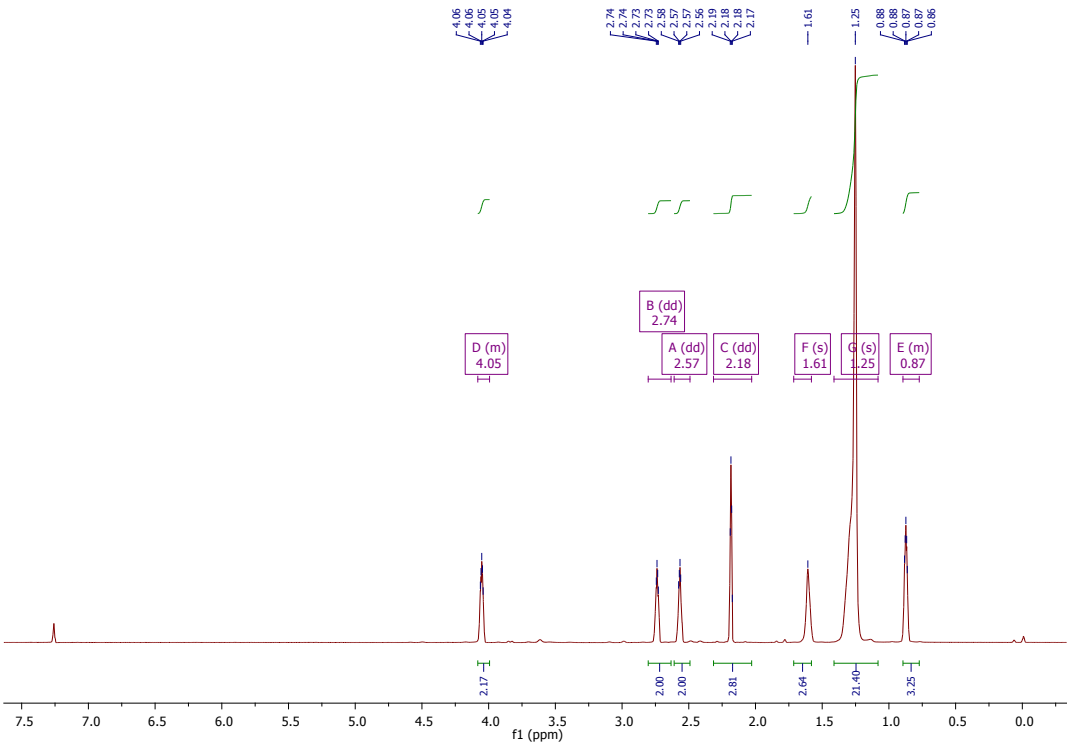
Scheme S8 ^{13}C NMR spectrum of *n*-butyl levulinate.



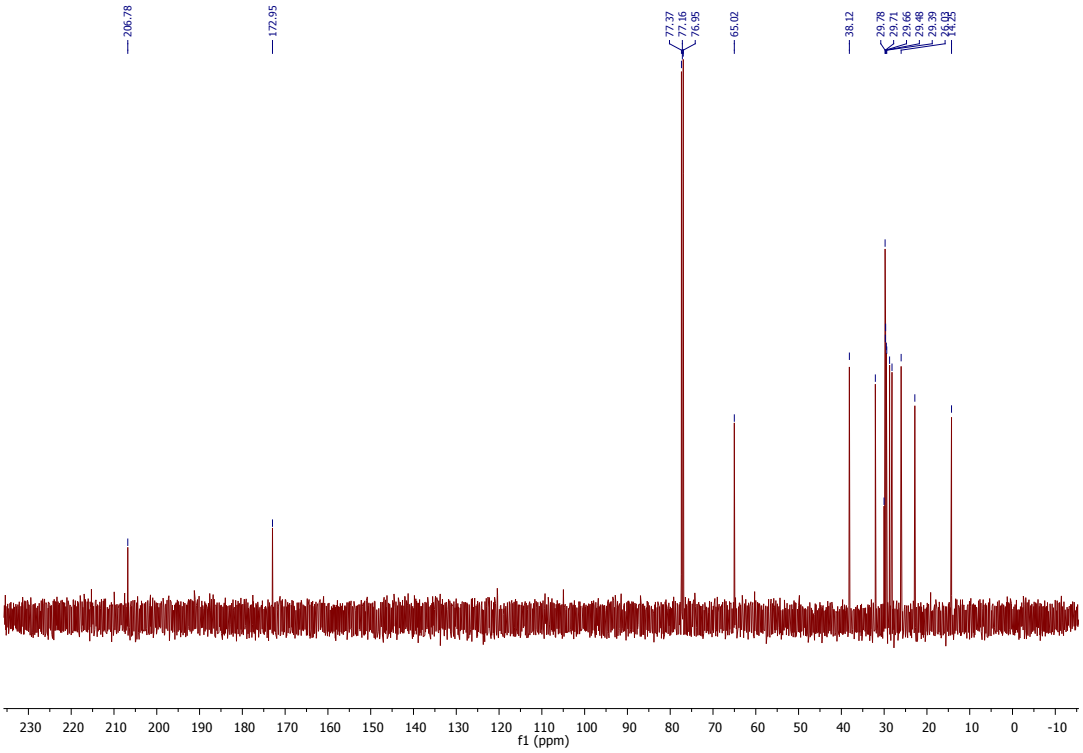
Scheme S9 ^{1}H NMR spectrum of i*so*-octyl levulinate.



Scheme S10 ^{13}C NMR spectrum of *iso*-octyl levulinate.



Scheme S11 ^{1}H NMR spectrum of *n*-dodecyl levulinate.



Scheme S12 ^{13}C NMR spectrum of *n*-dodecyl levulinate.

S3. Deactivation of CALB

Lowry's protein detection method

Amount of lipase in the filtrate was calculated *via* Lowry's method of protein detection using a UV-VIS technique. UV-VIS spectra were performed using Jasco V-650 spectrophotometer at room temperature in aqueous solution and the absorbance at wavelength λ=670 nm was measured. This technique confirmed the protein was below the detection limit in the filtrate after all reaction cycles.

Preparation of the calibration curve for Lowry's protein detection method

To the 25 mL flask an aqueous solution of 3-30 μL/mL of *Candida antarctica* lipase B was introduced. The calibration curve was made *via* mixing of 1 mL of protein solution with 5 mL of 2% solution of Na_2CO_3 in 0.1 M aqueous solution of NaOH. After 10 min, a 0.5 mL of Folin-Ciocalteu reagent was added and the absorbance at wavelength λ=670 nm was measured after 30 min. Each measurement was repeated twice and the calibration curve with R^2 equal 0.979 was achieved in consequence.

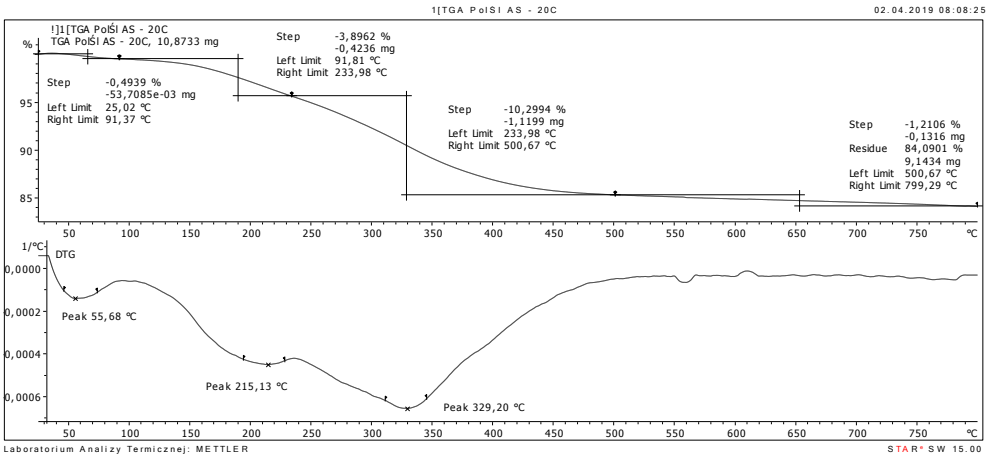


Fig. S1 The TG curves of CALB/MWCNT-PTFE(0.10 wt.%) biocatalyst after 7th reaction cycle.

S4. TGA curves of standards, supports and biocatalysts with various amount of PTFE

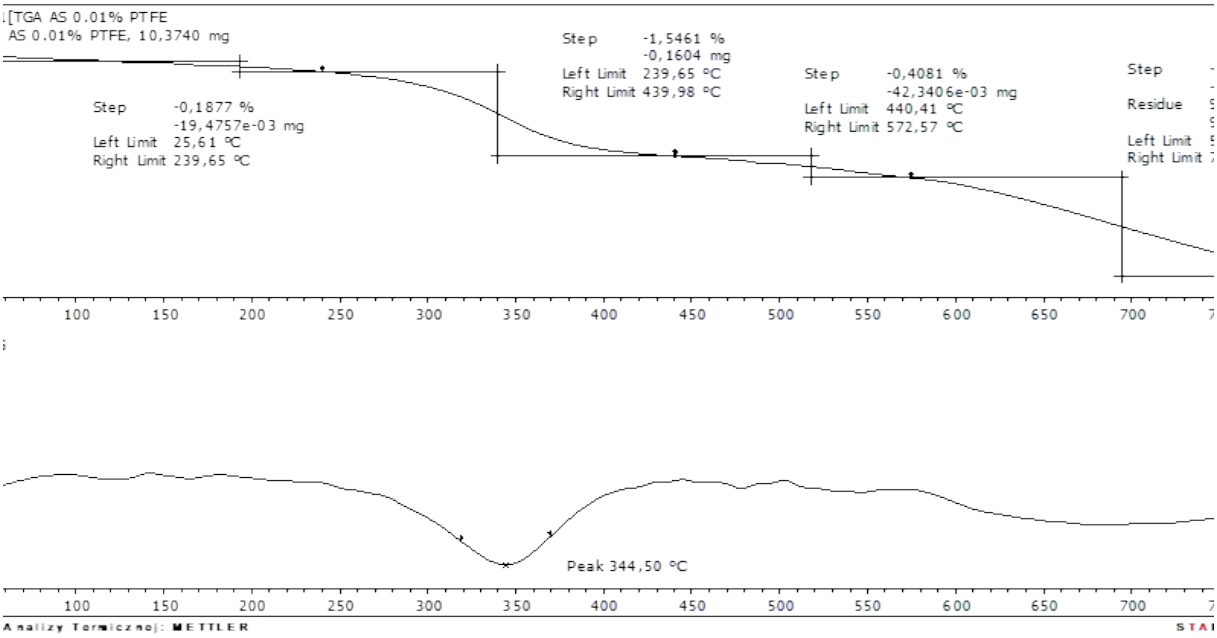


Fig. S2 The TG curves of CALB/MWCNT-PTFE(0.01 wt.%) support.

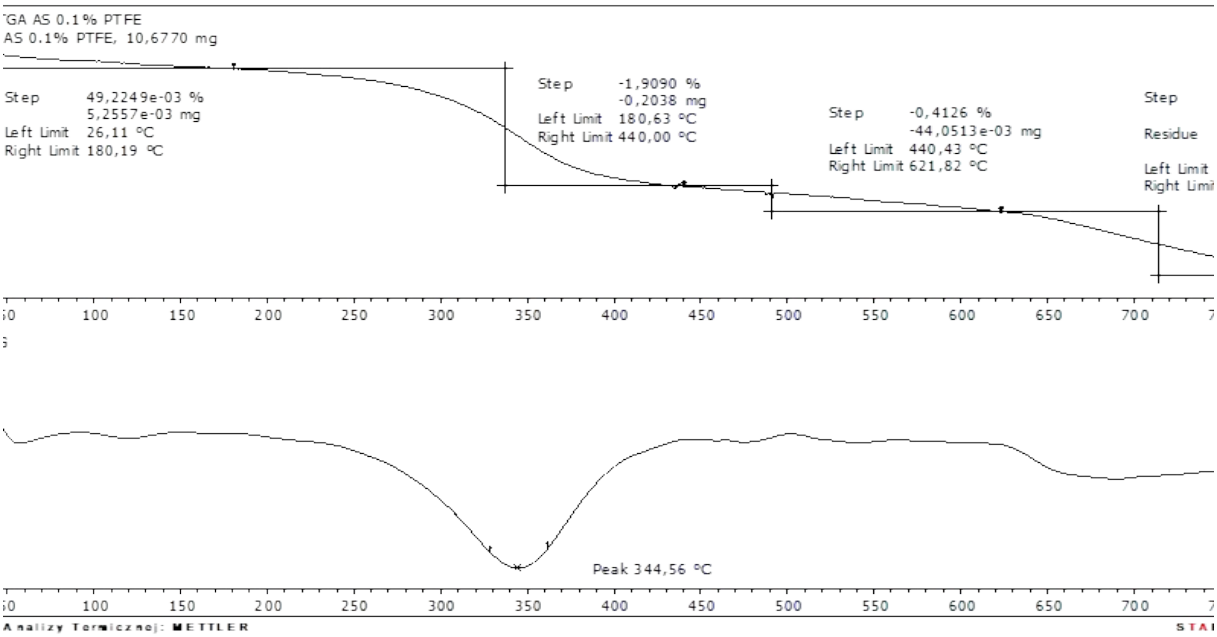


Fig. S3 The TG curves of CALB/MWCNT-PTFE(0.10 wt.%) support.

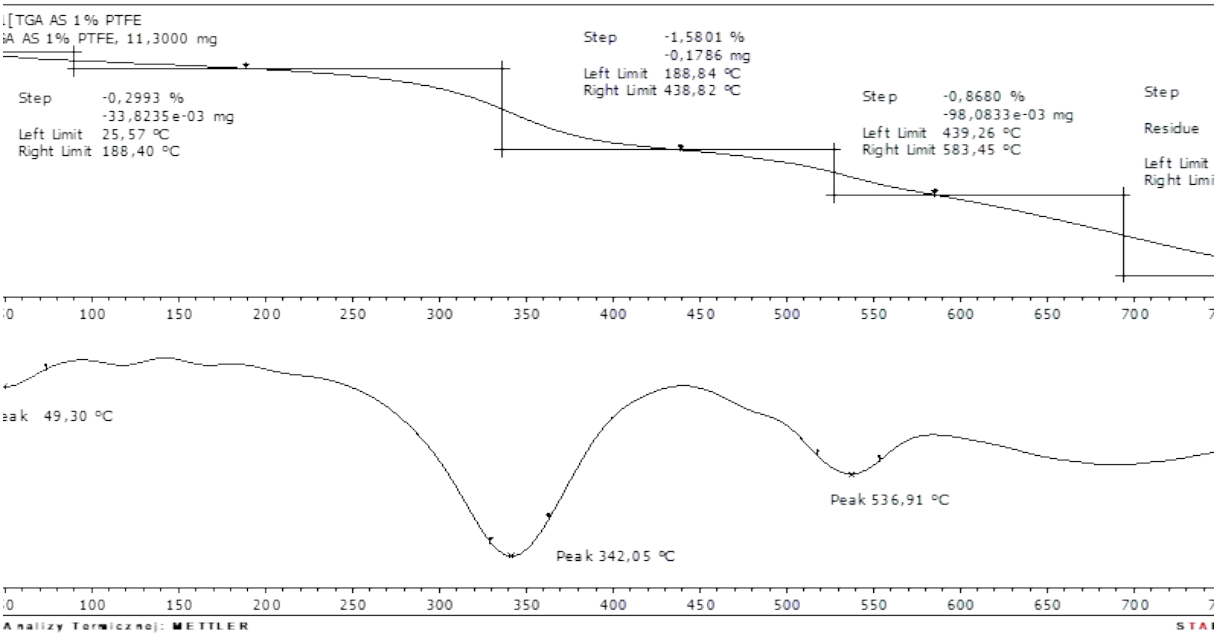


Fig.S4 The TG curves of CALB/MWCNT-PTFE(1.00 wt.%) support.

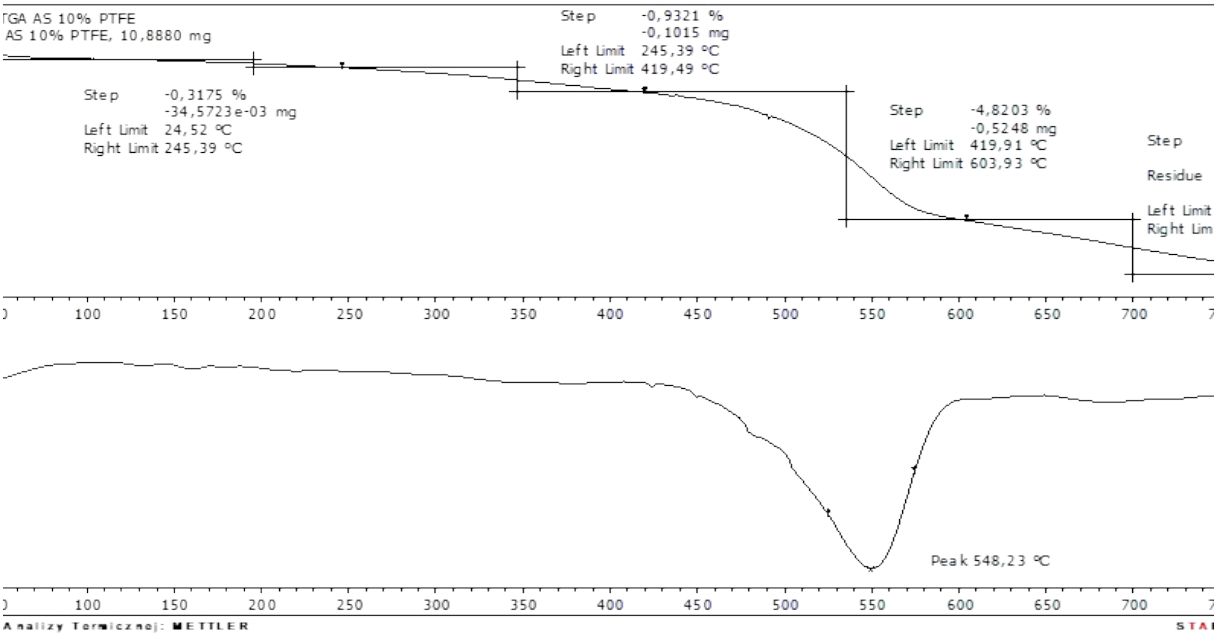


Fig. S5 The TG curves of CALB/MWCNT-PTFE(10.00 wt.%) support.

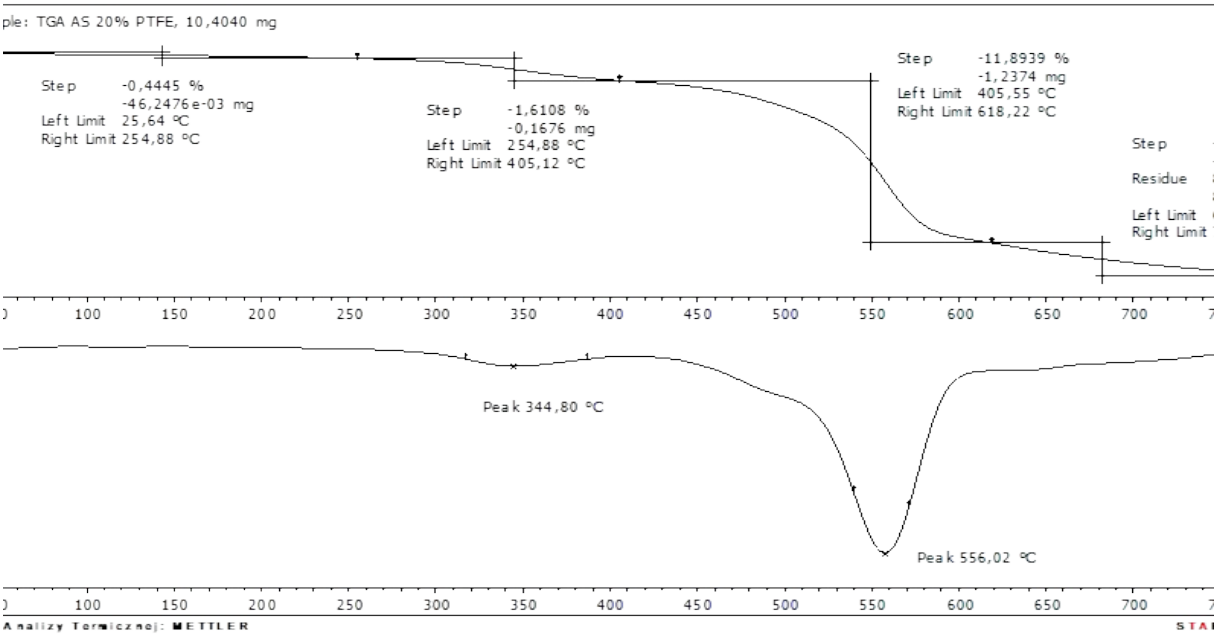


Fig. S6 The TG curves of CALB/MWCNT-PTFE(20.00 wt.%) support.

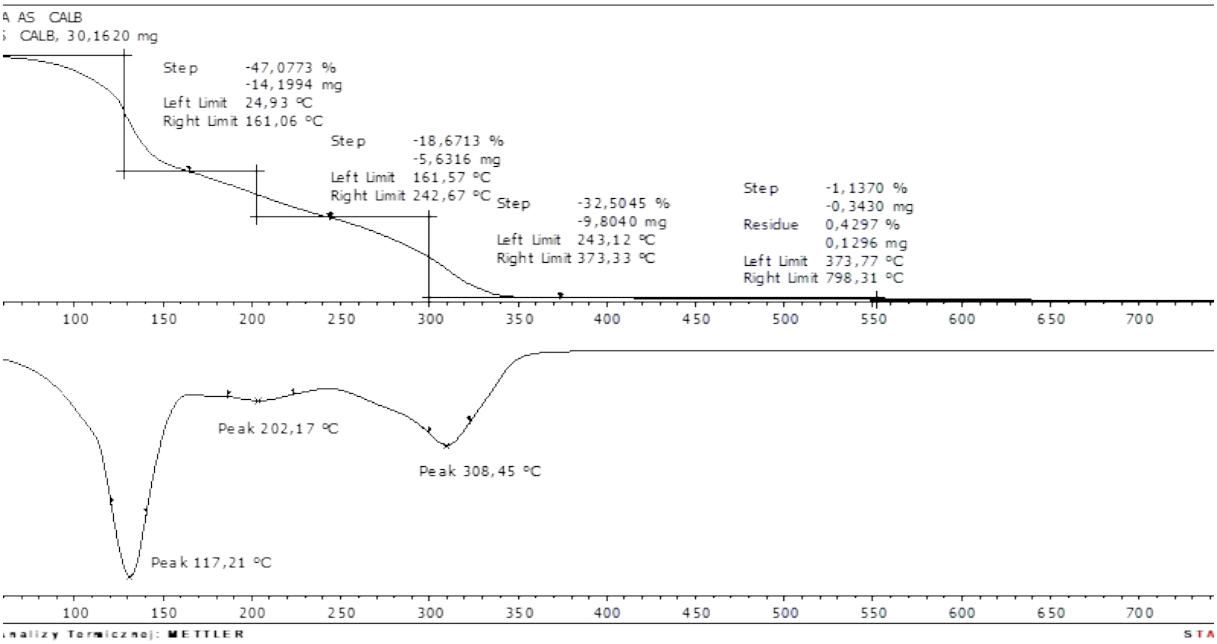


Fig. S7 The TG curves of solution of CALB.

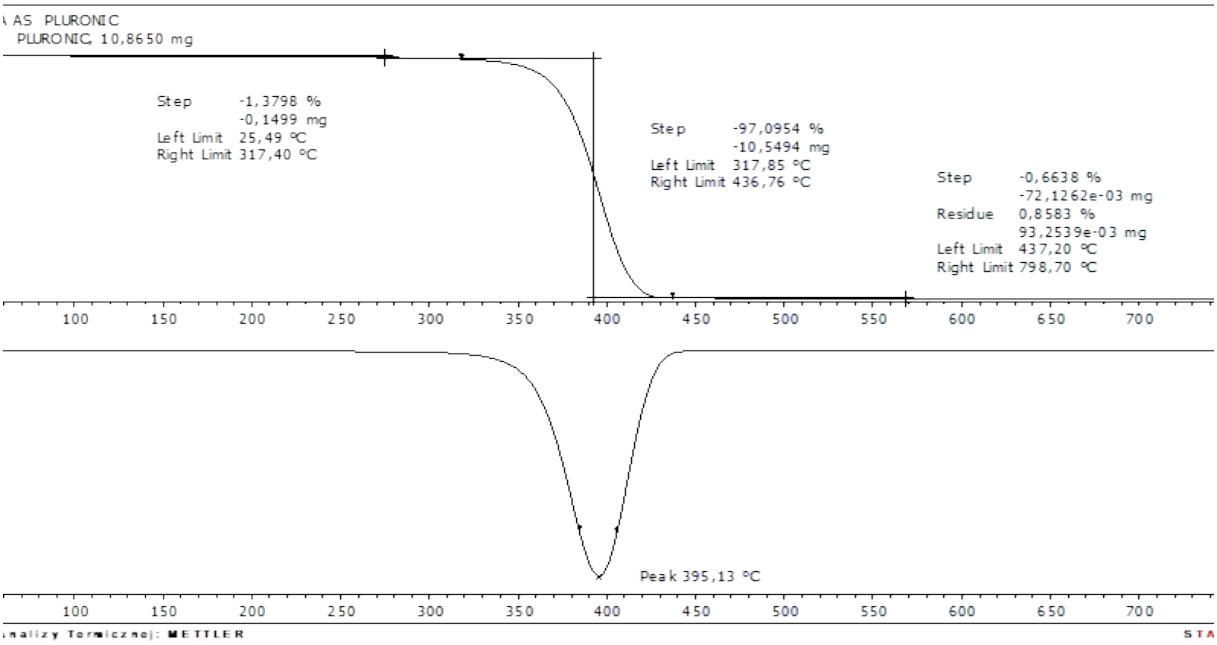


Fig. S8 The TG curves of surfactant (Pluronic F127).

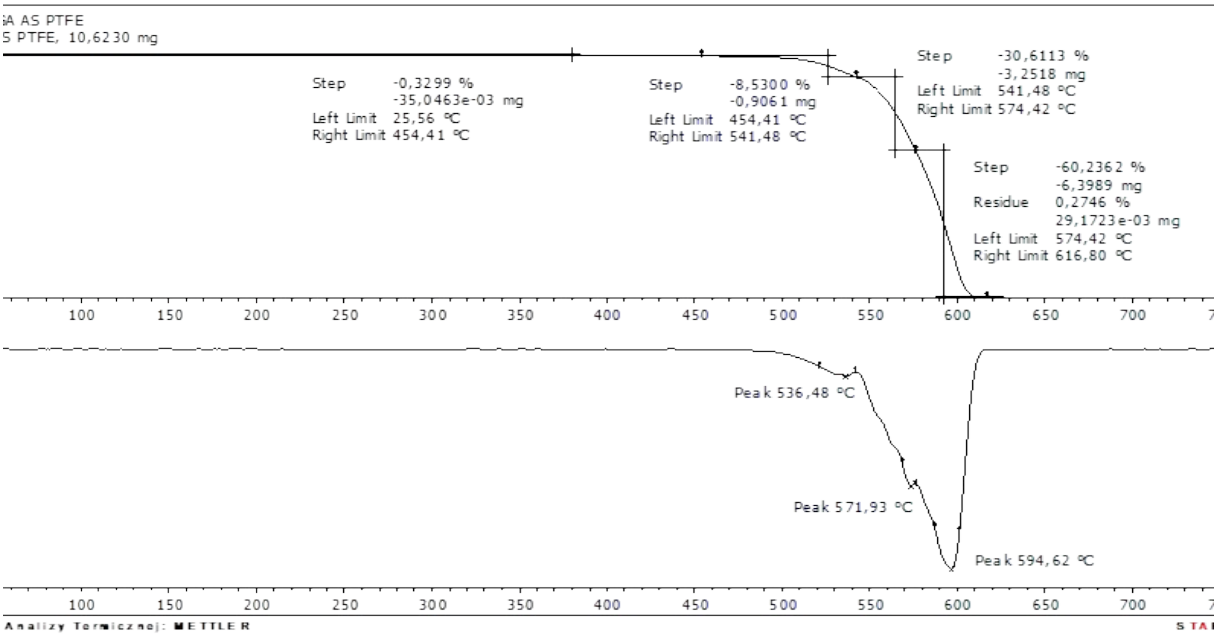


Fig. S9 The TG curves of PTFE.

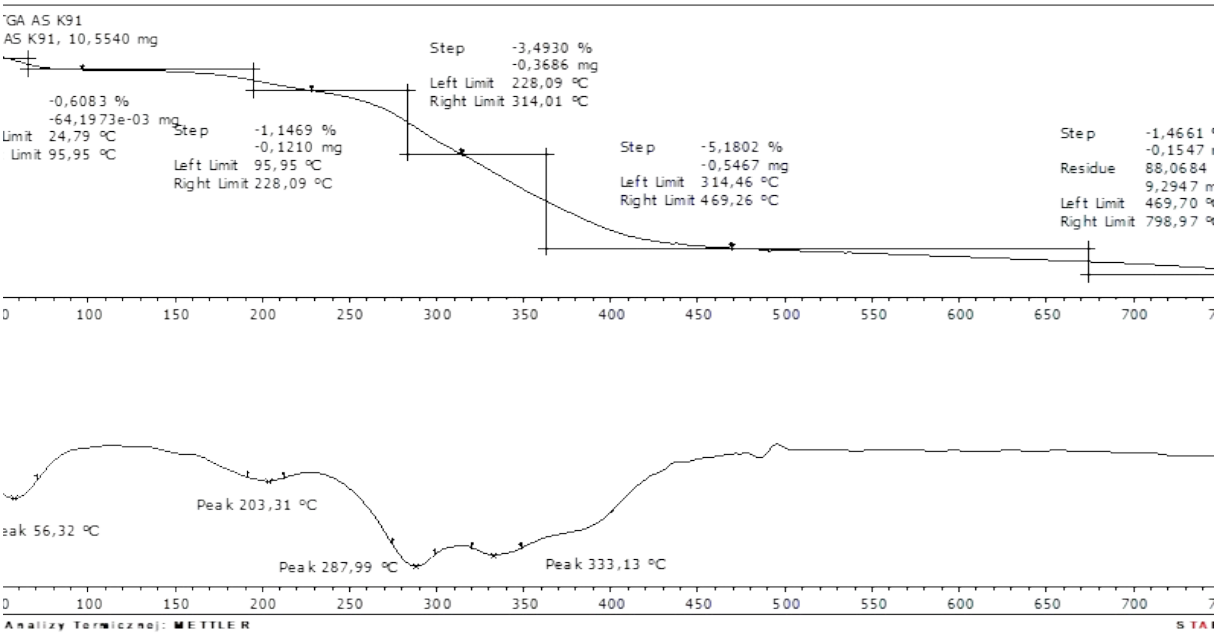


Fig. S10 The TG curves of CALB/MWCNT-PTFE(0.01 wt.%) biocatalyst.

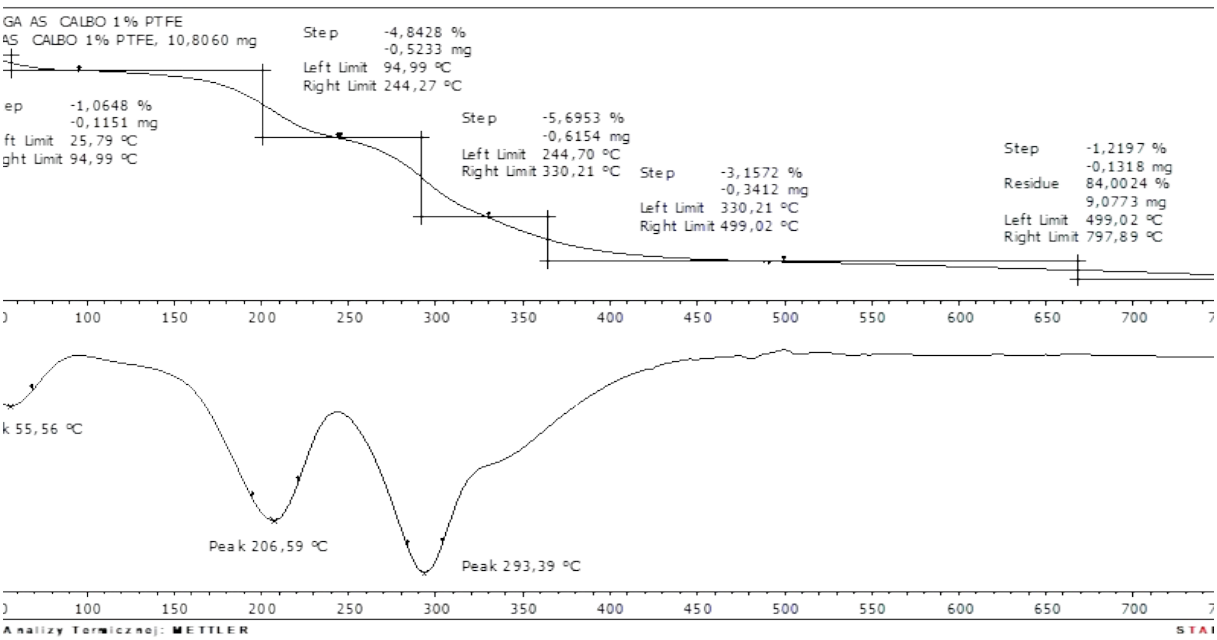


Fig. S11 The TG curves of CALB/MWCNT-PTFE(0.10 wt.%) biocatalyst.

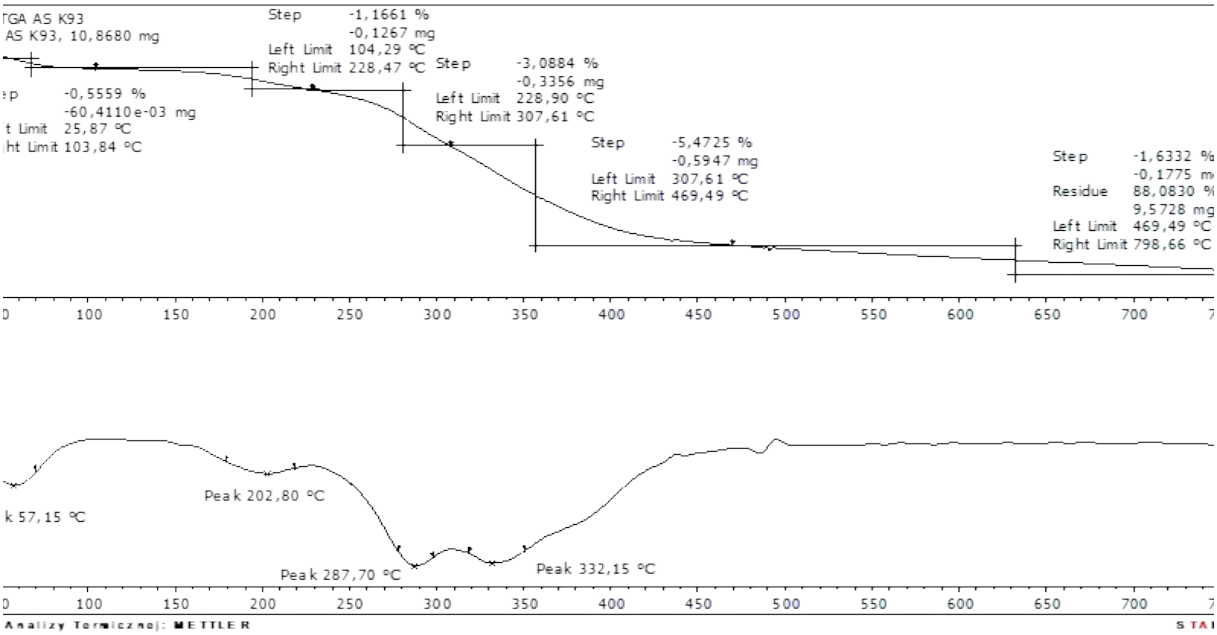


Fig. S12 The TG curves of CALB/MWCNT-PTFE(0.50 wt.%) biocatalyst.

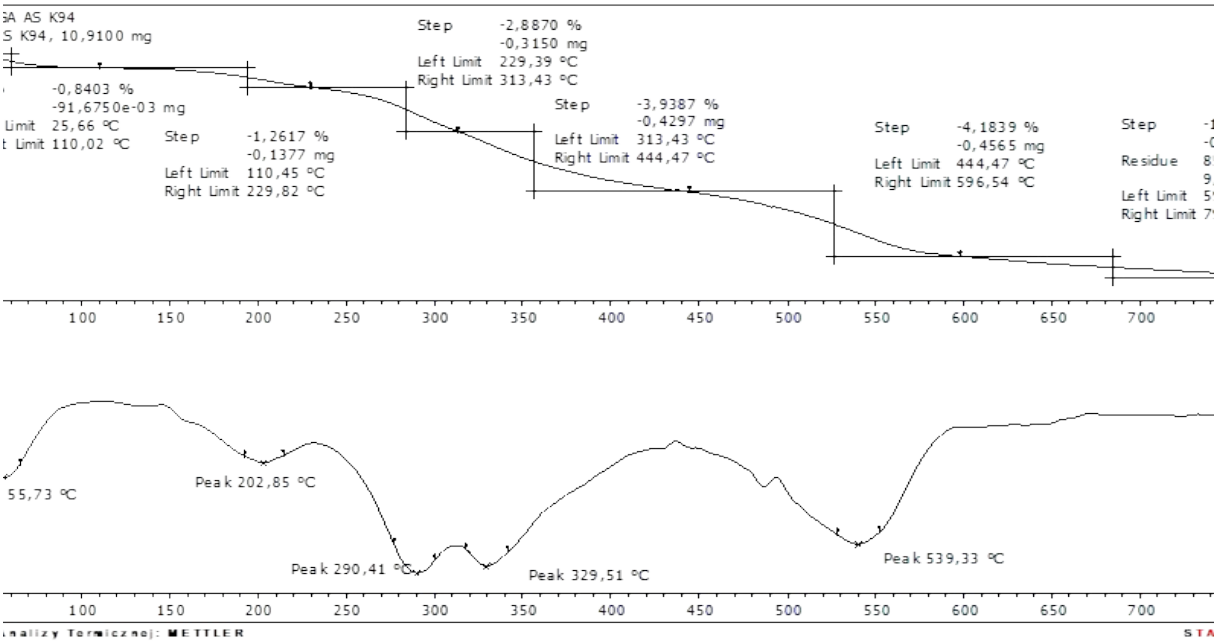


Fig. S13 The TG curves of CALB/MWCNT-PTFE(1.00 wt.%) biocatalyst.

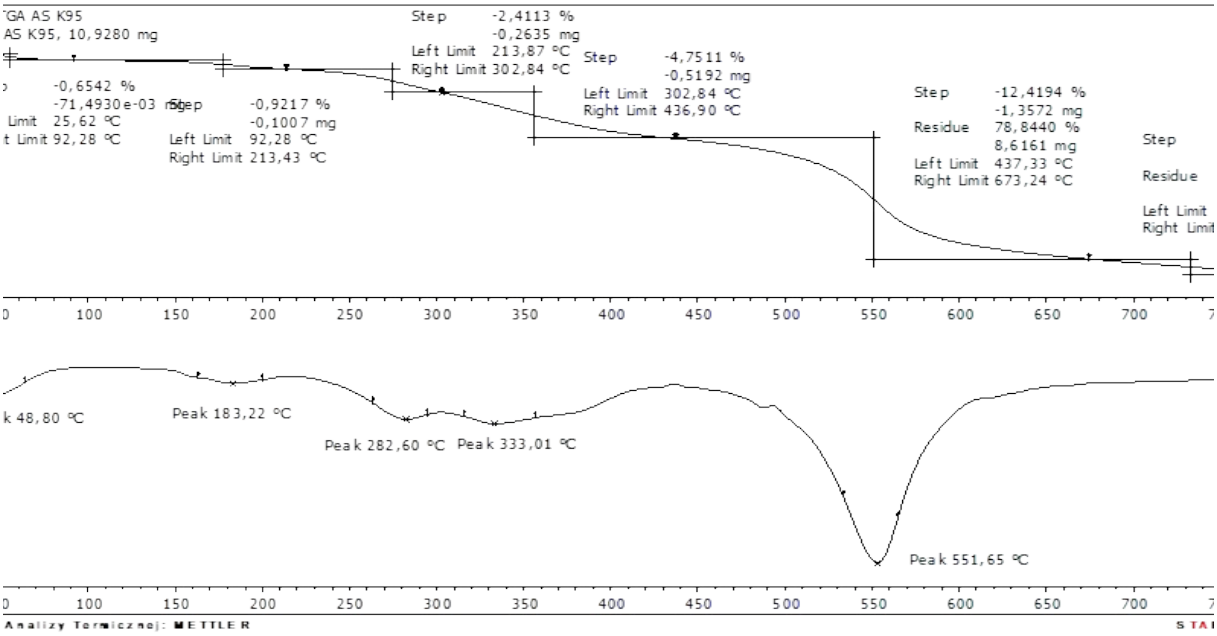


Fig. S14 The TG curves of CALB/MWCNT-PTFE(10.00 wt.%) biocatalyst.

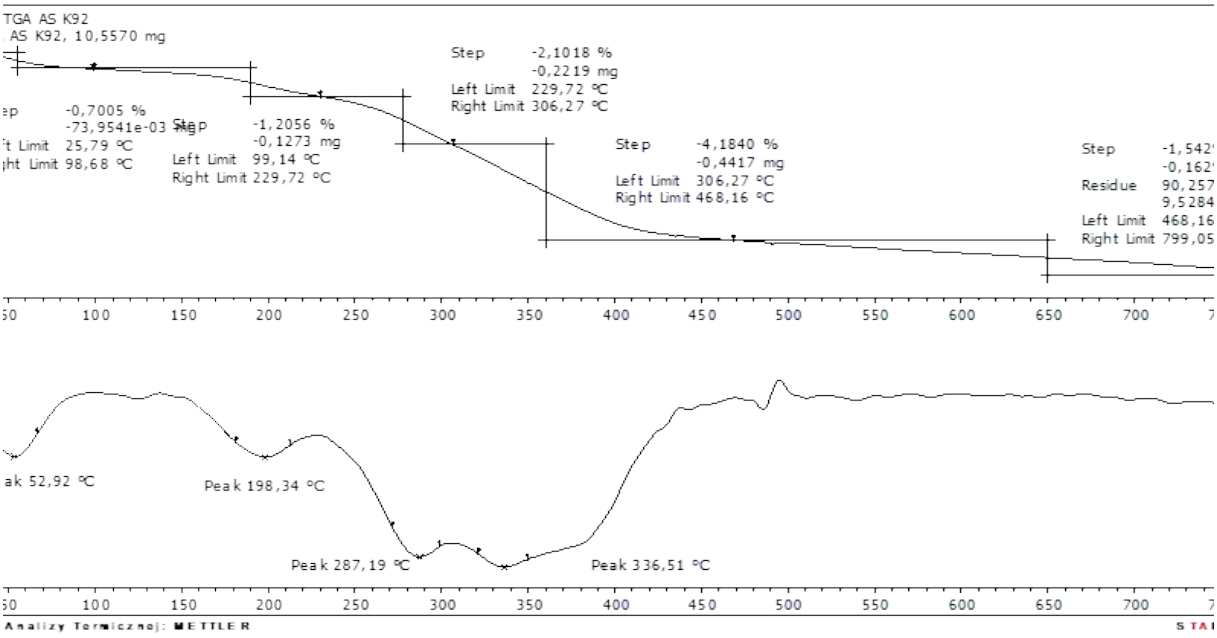


Fig. S15 The TG curves of CALB/MWCNT-PTFE(20.00 wt.%) biocatalyst.

S5. TGA curves of biocatalysts with various amount of CALB

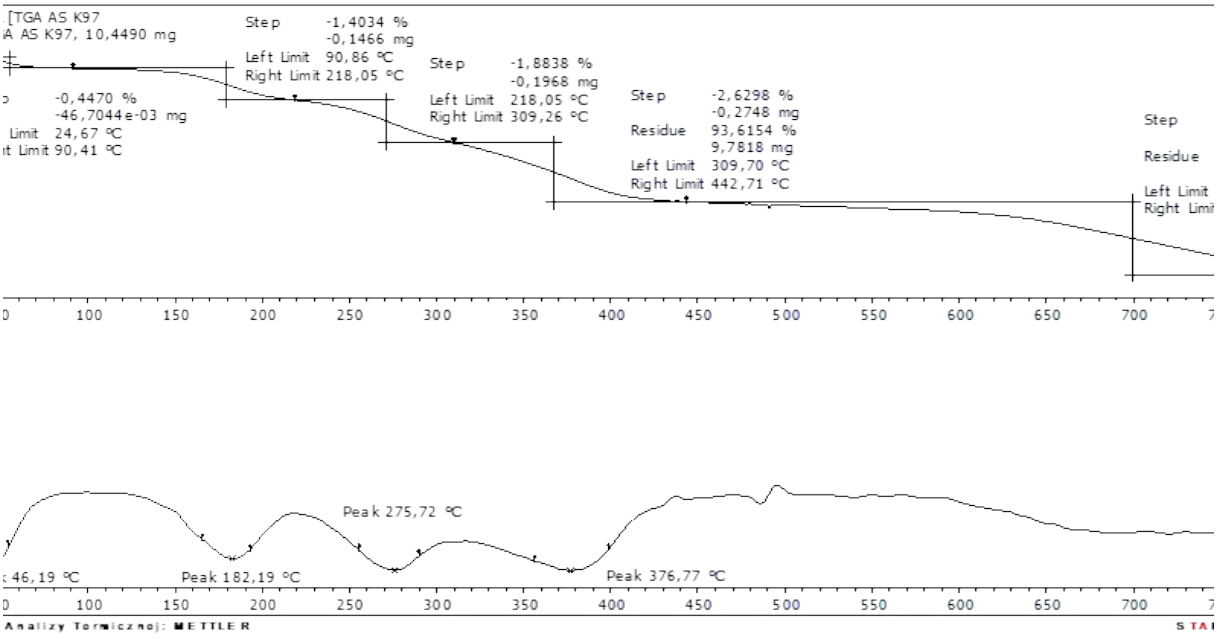


Fig. S16 The TG curves of CALB/MWCNT-PTFE(0.10 wt.%) biocatalyst with CALB: support MR=1:1.

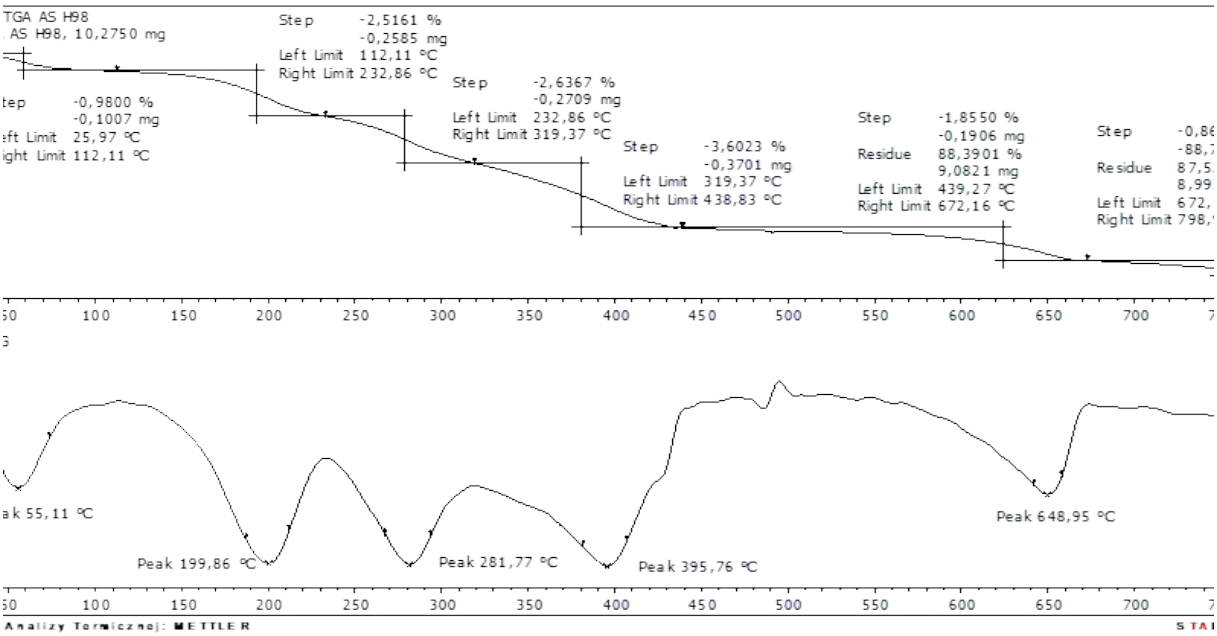


Fig. S17 The TG curves of CALB/MWCNT-PTFE(0.10 wt.%) biocatalyst with CALB: support MR=1:2,5.

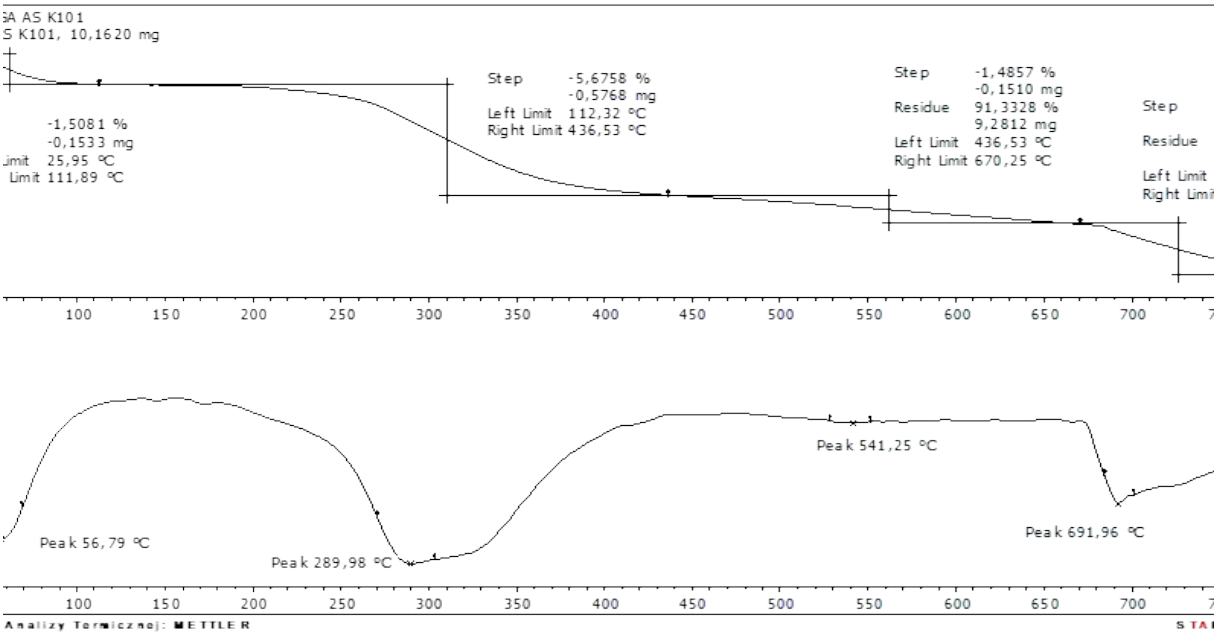


Fig. S18 The TG curves of CALB/MWCNT-PTFE(0.10 wt.%) biocatalyst with CALB: support MR=1:5.

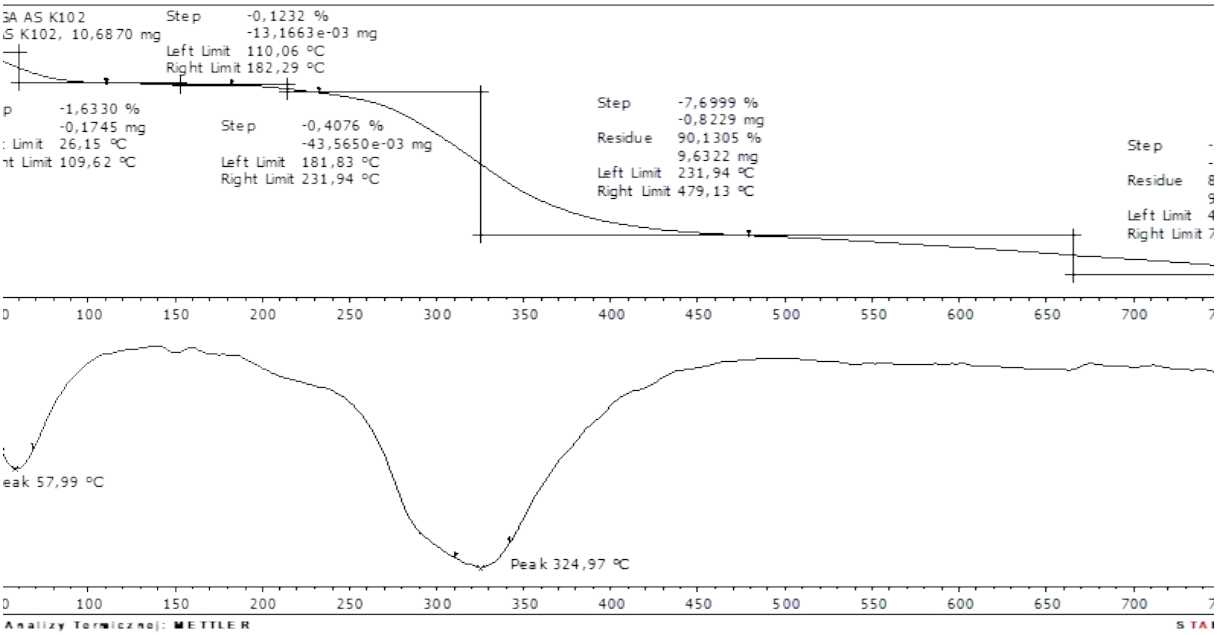


Fig. S19 The TG curves of CALB/MWCNT-PTFE(0.10 wt.%) biocatalyst with CALB: support MR=1:10.

S6. SEM/EDS images of supports with various amount of PTFE

Fig. S20 SEM image of CALB/MWCNT-PTFE(0.01 wt.%) support with results of EDS analysis.

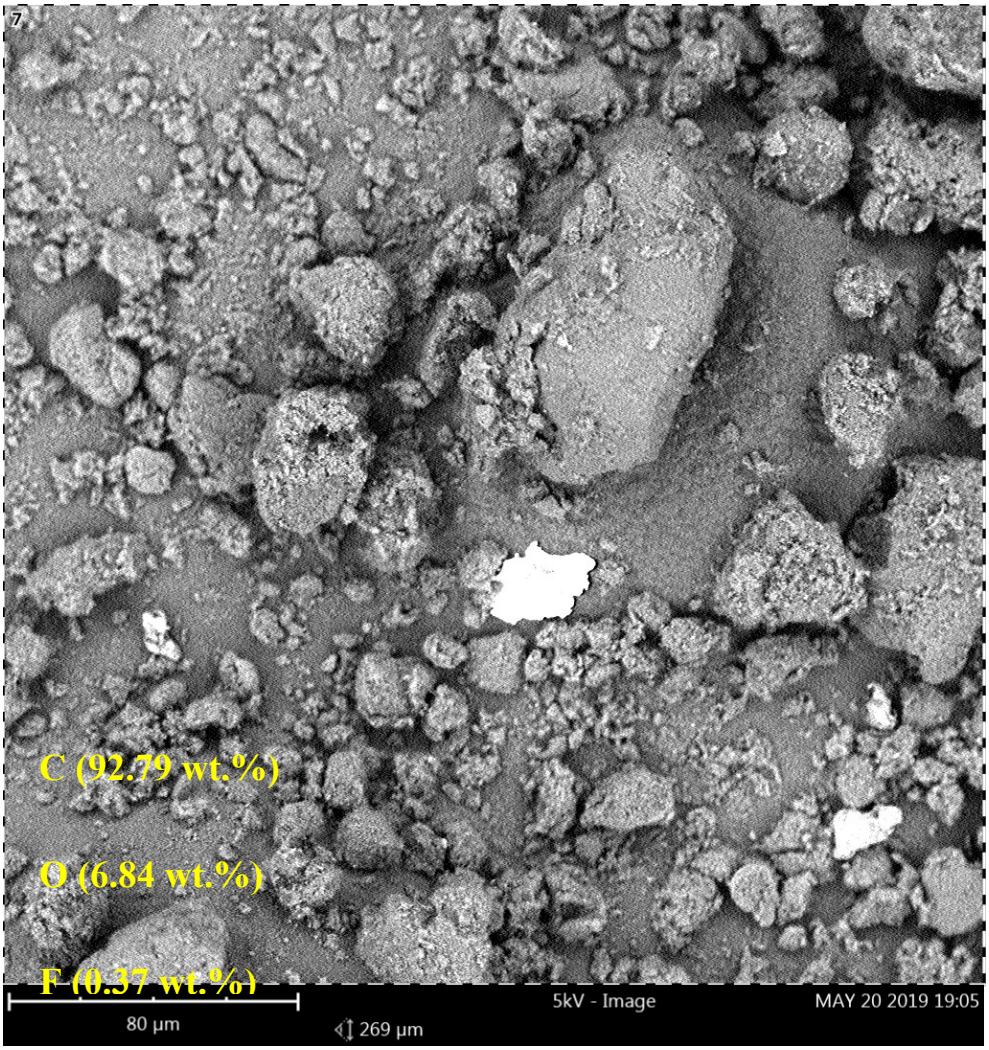


Fig. S21 SEM image of CALB/MWCNT-PTFE(0.50 wt.%) support with results of EDS analysis.

Fig. S22 SEM image of CALB/MWCNT-PTFE(1.00 wt.%) support with results of EDS analysis.

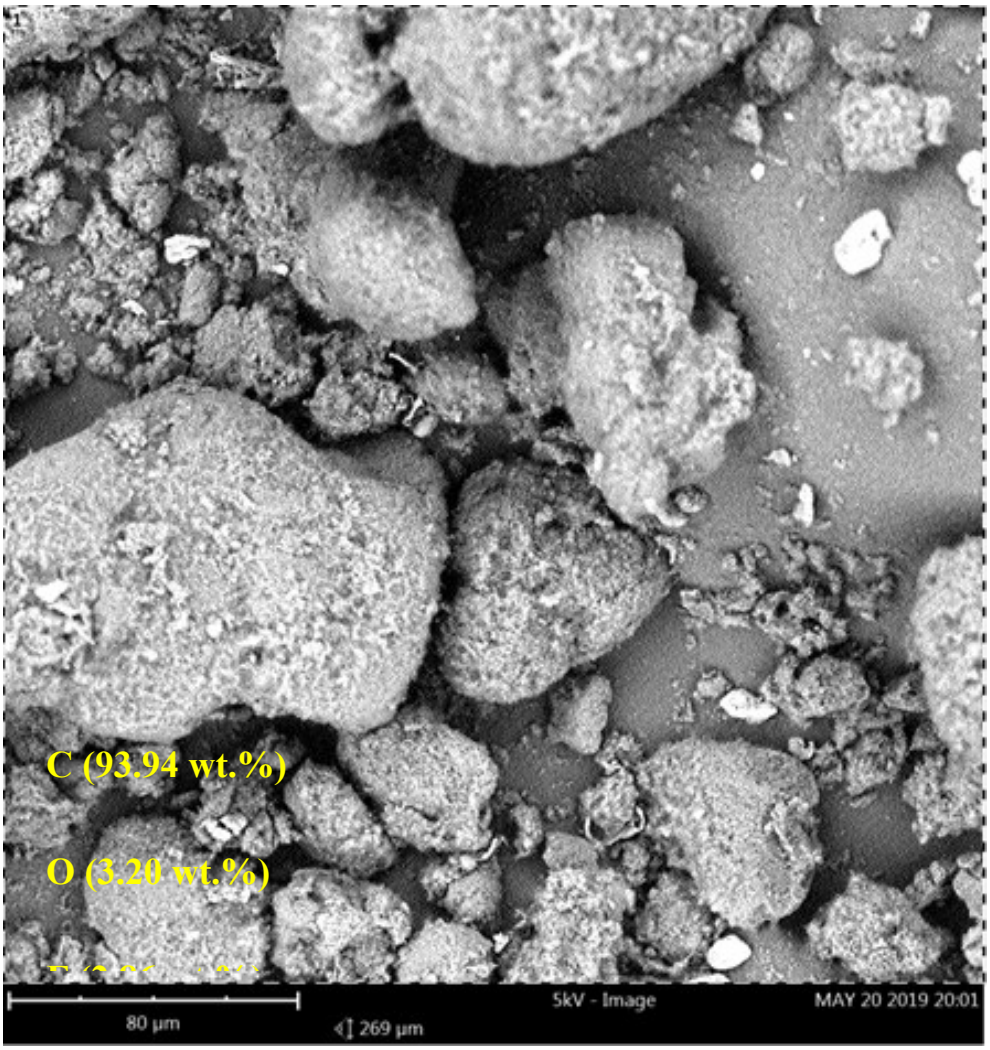


Fig. S23 SEM image of CALB/MWCNT-PTFE(10.00 wt.%) support with results of EDS analysis.

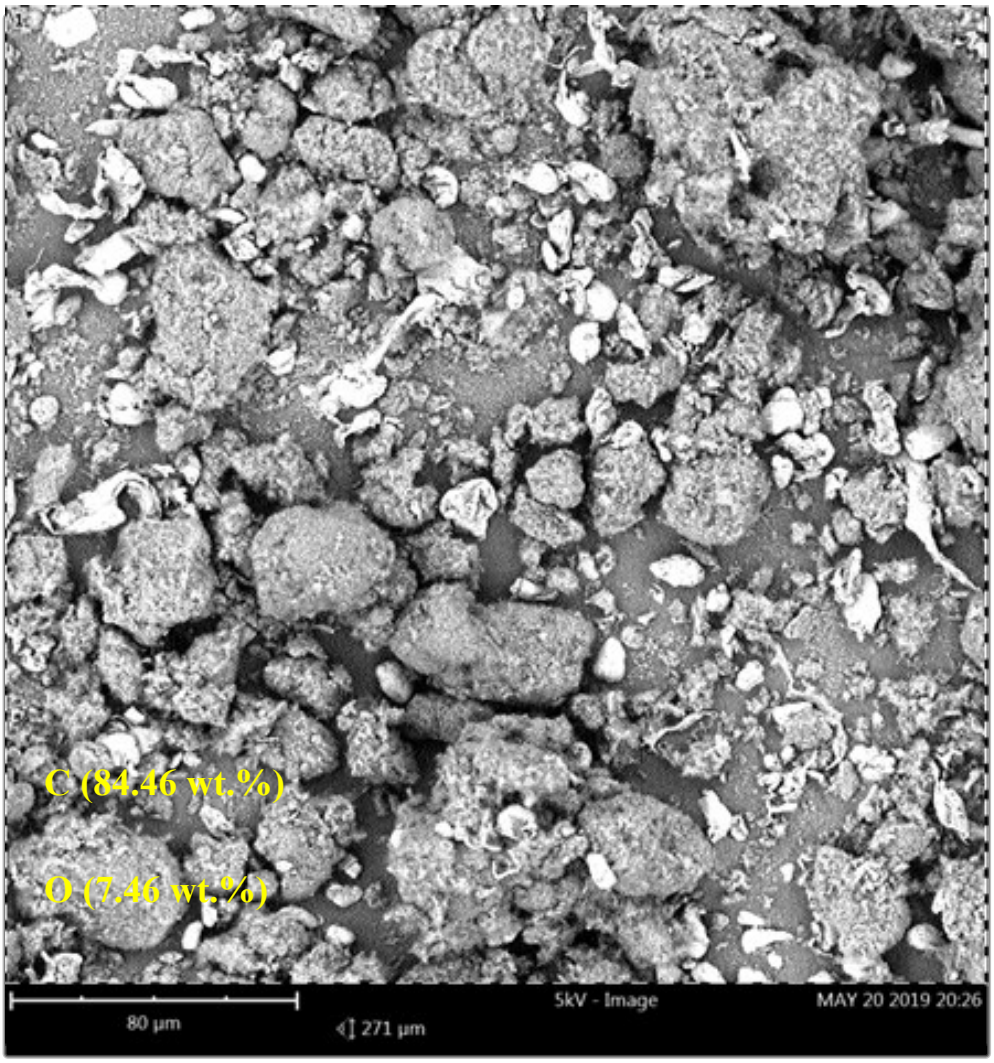


Fig. S24 SEM image of CALB/MWCNT-PTFE(20.00 wt.%) support with results of EDS analysis.

S7. TEM/EDS images of selected supports with various amount of PTFE

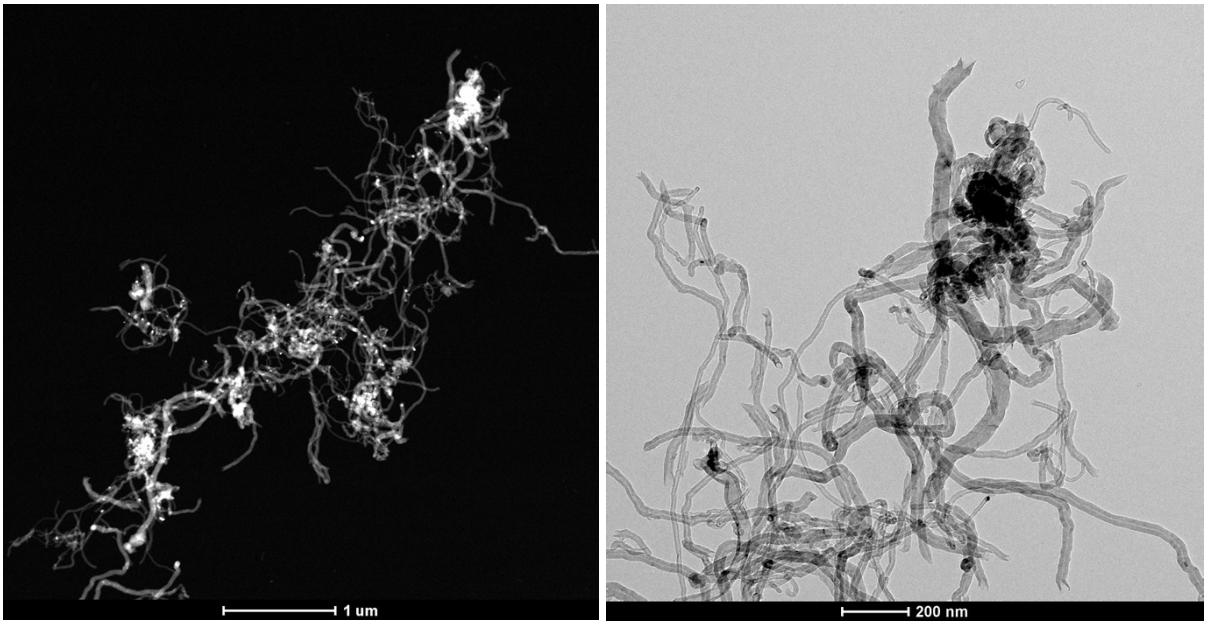


Fig. S25 TEM images of CALB/MWCNT-PTFE(0.10 wt.%) support.

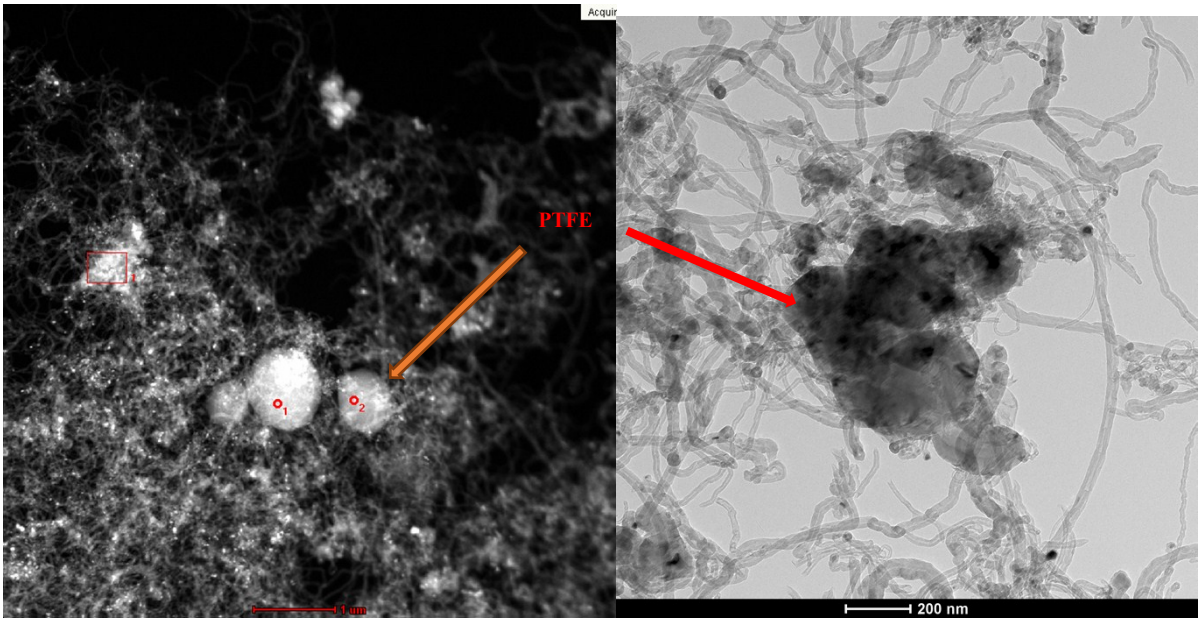


Fig. S26 TEM images of CALB/MWCNT-PTFE(0.10 wt.%) biocatalyst.

S8. The influence of the stirring speed on the reaction rate

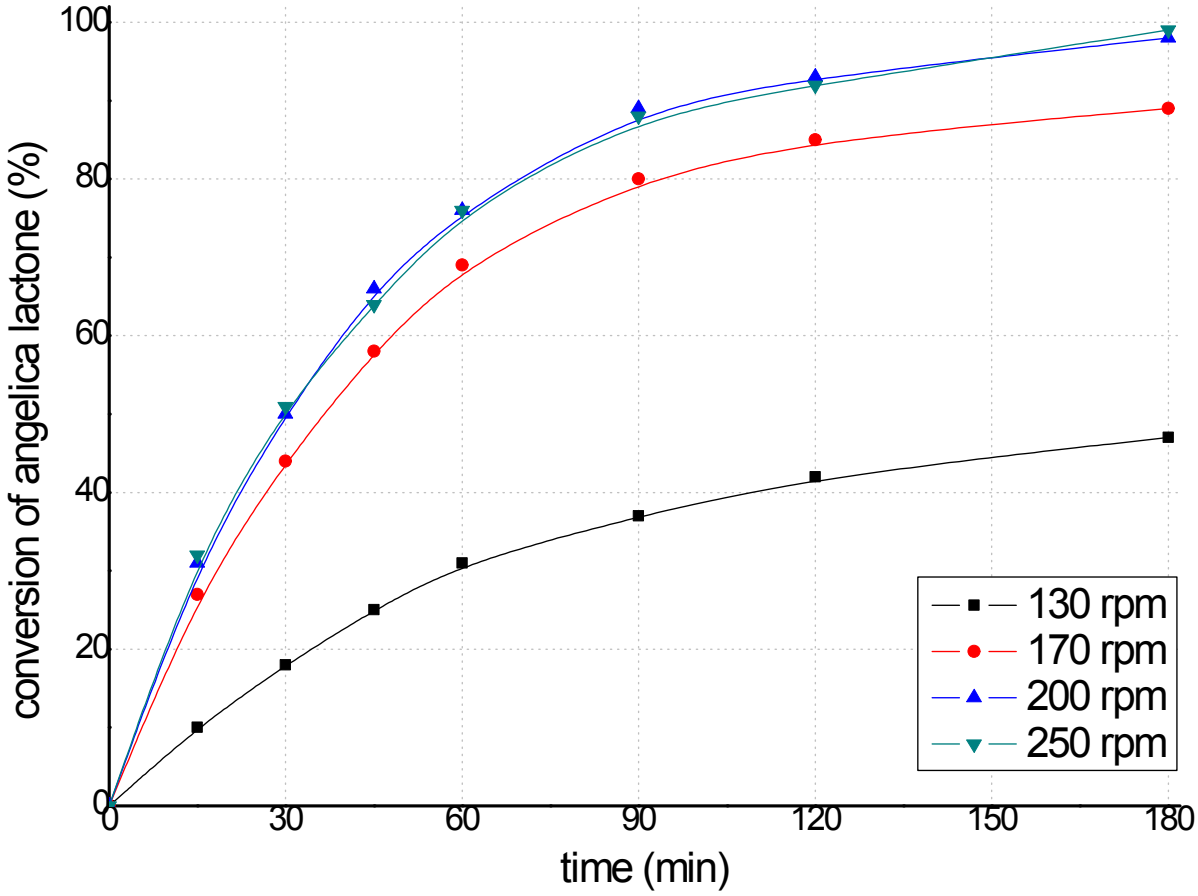


Fig. S27 The influence of the stirring speed on the reaction rate.

Reaction conditions: α-AL 0.098 g (1 mmol), *n*-BuOH 0.149 g (2 mmol), toluene 1 mL/1 mmol of α-AL, biocatalyst 0.150 g/1 mmol of α-AL, 20 °C.. All experiments were triplicated (standard deviation 1%).

S9. The influence of the molar ratio of reactants

In Table S3 the composition of the post reaction mixture obtained using various molar ratio of α-Al to *n*-BuOH from 1:1 to 1:8 and in *n*-butanol as solvent was presented. Due to the lack of a calibration curve for pseudo-*n*-butyl levulinate (pseudo-BLV) the raw estimation was performed based on the ratio of peak area on the GC chromatogram.

Table S3 Yield of *n*-butyl levulinate in processes carried out with various molar ratio of α-AL to n-BuOH

Molar ratio of α-AL:*n*-Bu	Time (min)	Conversion of α-AL (%)	Yield of BLV (%)	Composition of the post-reaction mixture (%)			
				α-AL	LA	pseudo-BLV	BLV
1:1	90	99 ± 1	81 ± 2	1	1	17	81
1:2	**120**	**>99 ± 1**	**99 ± 2**	**-**	**-**	**1**	**99**
1:4	180	>99 ± 1	99 ± 2	-	-	1	99
1:8	240	99 ± 1	78 ± 2	1	1	20	78
1:11 without toluene	120	99 ± 1	51 ± 2	1	1	48	50

Reaction conditions: α-AL 0.098 g (1 mmol), toluene 1 mL/1 mmol of α-AL, CALB/MWCNT-PTFE(0.10 wt.%) biocatalyst 0.150 g/1 mmol of α-AL, 20 °C, 250 rpm. All experiments were triplicated (standard deviation 2%). Shortcuts: LA-levulinic acid, pseudo-BLV- pseudo-*n*-butyl levulinate.

These experiments clearly showed that two-fold molar excess of *n*-BuOH in respect to the α-AL is required in order to achieve nearly stoichiometric yield of corresponding ester. Higher concentration of *n*-butanol in the reaction environment negatively affected activity of biocatalyst. On the other hand, equimolar amounts of both reagents led to the presence of traces the α-AL in the post-reaction mixture, as well as poorer selectivity and formation of significant amounts of pseudo-BVL.

S10. The influence of the amount of CALB immobilized on the MWCNT-PTFE(0.10 wt.%) hybrid support on the biocatalyst activity

In the next step, series of experiments with biocatalysts containing various lipase loadings were performed (Figure S28) with a simultaneous determination of CALB loading *via* TGA analysis (TableS4). For this purpose, MWCNT-PTFE(0.10 wt.%) was chosen as a hybrid support. The biocatalysts were prepared using different mass of CALB in the relation to the hybrid support (CALB:MWCNT-PTFE(0.10 wt.%); mass ratio MR from 1:1 to 10:1) in the immobilization step.

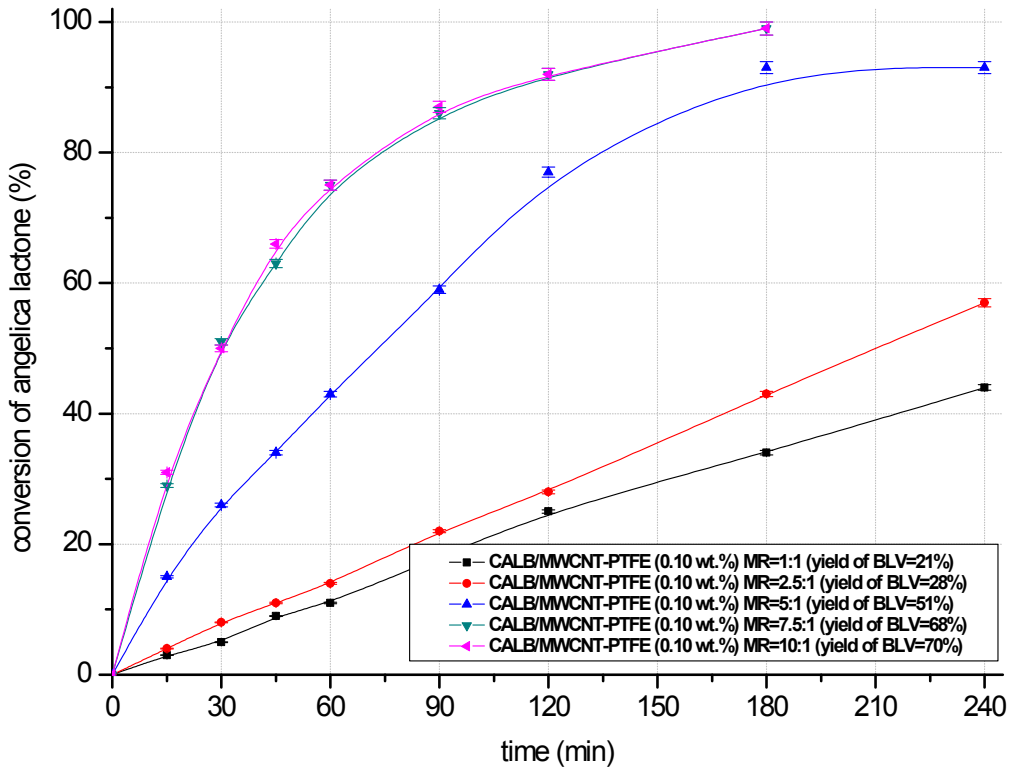


Fig. S28 The influence of the mass ratio of CALB and MWCNT-PTFE(0.10 wt.%) support in the immobilization step on the activity of biocatalyst in the synthesis of *n*-butyl levulinate.

Reaction conditions: α-AL 0.098 g (1 mmol), *n*-BuOH 0.149 g (2 mmol), toluene 1 mL/1 mmol of α-AL, biocatalyst 0.050 g/1 mmol of α-AL, 20 °C, 250 rpm. All experiments were triplicated (standard deviation 1%). The yield of n-butyl levulinate at the end points is given in brackets.

Table S4 The influence of MR (mass of CALB in the relation to the mass of hybrid support CALB:MWCNT-PTFE(0.10 wt.%) used in the immobilization step on CALB loading in biocatalysts (MR determined via TGA/DTG analysis).

MR	CALB loading (wt.%)
1:1	8.8
2.5:1	13.2
5:1	15.6
7.5:1	**22.5**
10:1	20.3

The experiments were carried out in the presence of a lower amount of biocatalyst than the optimal one, in order to slow down the reaction and to present, in the more pronounced manner, the differences between activities of the corresponding catalytic systems. The most promising results were achieved when CALB/MWCNT-PTFE(0.10 wt.%) MR = 7.5:1 was used as biocatalyst. In fact, higher amounts of CALB led to the use of higher amounts of the enzyme without any significant influence on the reaction rate clearly showing the saturation point as well as *plateau* in the activity. On the other hand, smaller amounts of CALB in biocatalyst were insufficient. Furthermore, TGA analysis confirmed the highest amount of lipase in CALB/MWCNT-PTFE(0.10 wt.%) MR = 7.5:1 catalyst and almost the same amount of CALB immobilized using MR 10:1 what is consistent with the results of its activity.

S11. The influence of the biocatalyst loading

The studies concerning the influence of the amount of biocatalyst were carried out with the use of CALB/MWCNT-PTFE(0.10 wt.%) catalyst (0.010-0.200 g per 1 mmol of α-AL) (Figure S29). The highest reaction rate was obtained when 0.150 g of the biocatalyst was applied. Here again, higher amount of the biocatalyst led to obtain approximately the same conversion of α-AL in the same time.

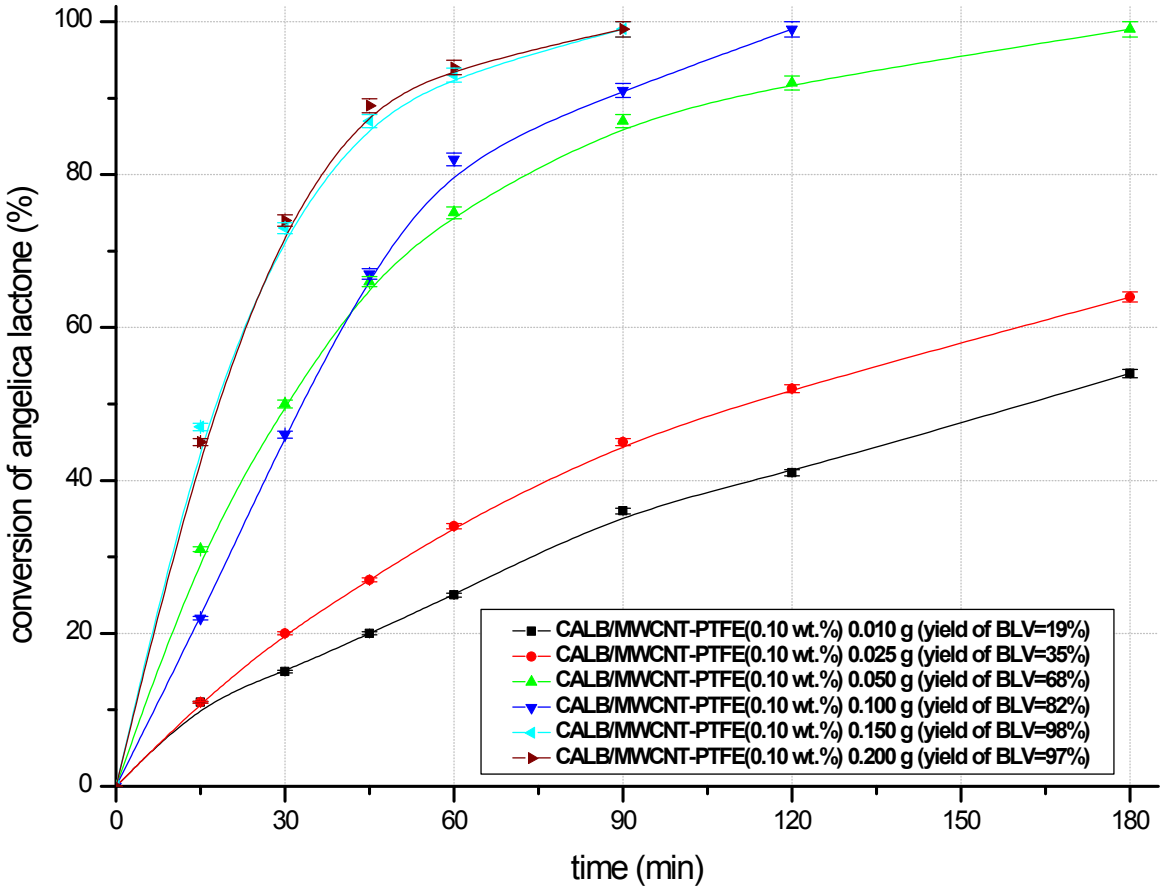


Fig. S29 The influence of the CALB/MWCNT-PTFE(0.10 wt.%) loading on the activity of biocatalyst in the synthesis of n-butyl levulinate.

Reaction conditions: α-AL 0.098 g (1 mmol), *n*-BuOH 0.149 g (2 mmol), toluene 1 mL/1 mmol of α-AL, 20 °C, 250 rpm. All experiments were triplicated (standard deviation 1%). The yield of *n*-butyl levulinate at the end points is given in brackets.

S12. Selectivity towards BLV in various solvents

Table S5 Composition of the post-reaction mixture in various solvents.

Solvent	Time, min	Yield of α-AL, (%)[a]	α-AL	LA	pseudo-BLV	BLV[b]	Reaction rate constant of synthesis of BLV at 20 °C (s^{-1})
toluene	**120**	**99 ±2**	**-**	**-**	**1**	**99**	**$42.0 \cdot 10^{-5}$**
cyclohexane	180	99 ±2	-	-	1	99	$34.0 \cdot 10^{-5}$
acetonitrile	240	72 ±2	1	-	30	69	$6.5 \cdot 10^{-5}$
THF	240	59 ±2	-	35	7	58	$5.8 \cdot 10^{-5}$
acetone	240	56 ±2	1	-	44	55	$5.5 \cdot 10^{-5}$
n-butanol	240	56 ±2	1	1	41	57	$8.4 \cdot 10^{-5}$
cyclohexanone	240	57 ±2	-	-	42	58	$10.2 \cdot 10^{-5}$
dichloromethane	240	71 ±2	-	22	4	74	$12.1 \cdot 10^{-5}$

Reaction conditions: α-AL 0.098 g (1 mmol), *n*-Bu 0.149 g (2 mmol), solvent 1 mL/1 mmol of α-AL, CALB/MWCNT-PTFE(0.10 wt.%) biocatalyst 0.150 g/1 mmol of α-AL, 20 °C, 250 rpm. All experiments were triplicated (standard deviation 2%).

[a] determined *via* GC analysis using calibration curve

[b] determined *via* GC analysis after calculating % of total area of peaks