Electronic supporting information

# Upgrading grape pomace contained ethanol into hexanoic acid, fuel additives and a sticky polyhydroxyalkanoate: *an effective alternative to ethanol distillation*

Gonzalo A. Martinez,[a,*] Salvatore Puccio,[a] Joana M. B. Domingos,[a] Elena Morselli,[a] Claudio Gioia,[a] Paola Marchese,[a] Anna Maria Raspolli Galletti,[b] Annamaria Celli,[a] Fabio Fava[a] and Lorenzo Bertin[a]

[a] Department of Civil, Chemical, Environmental and Material Engineering (DICAM), University of Bologna, via Terracini, 28, I-40131 Bologna, Italy

[b] Department of Chemistry and Industrial Chemistry, University of Pisa, via G. Moruzzi 13, 56124, Pisa, Italy

* Corresponding author email: gonzalo.martinez3@unibo.it; Tel:+39-051 20 90314 | Fax: +39-051 20 90322

## 1. Production of MCCs from $GP_{Fresh}$

### 1.1. Operating conditions screening.

GP anaerobic fermentation was initially tested at different temperatures (37 and 55 °C) and pH levels (5, 6 and 7), **Figure S *1*** and **Figure S 2** show the obtained results. Later, the experiment was repeated just for those conditions at 37 °C in order to confirm the positive results obtained at the lower temperature. To this end, a lower solid content was used (less $GP_{Fresh}$) to improve miscibility. The resulting ethanol and organic acids concentration trends are shown on the main text, while **Figure S 3** shows the biogas production trends.

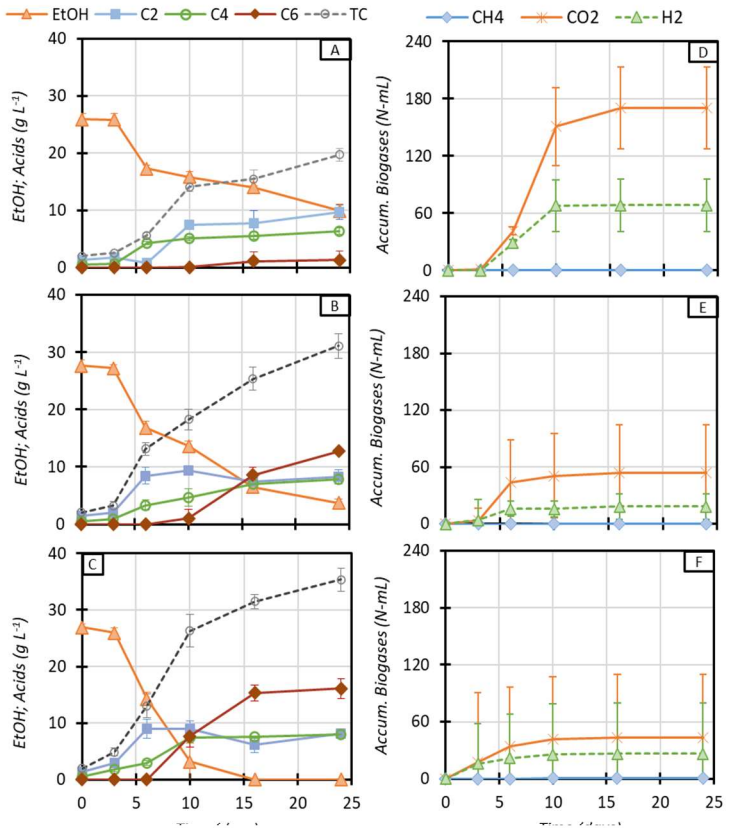



**Figure S 1:** Microbial consortium responses at 37°C. First column of graphs shows the concentration trends for ethanol (EtOH), acetic (C2), butyric (C4), hexanoic (C6) and total carboxylic acids (TC) for the experiments carried out at pH levels of 5 (A), 6 (B) and 7 (C). Second column of graphs show the corresponding accumulated biogas produced at pH 5 (D), 6 (E) and 7 (F).

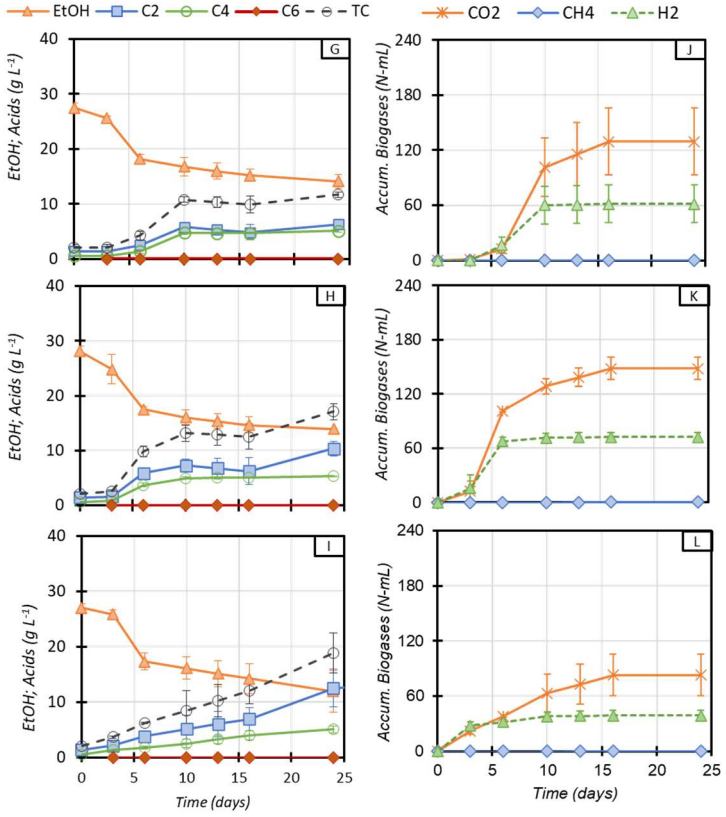



**Figure S 2:** Microbial consortium responses at 55°C. First column of graphs shows the concentration trends for ethanol (EtOH), acetic (C2), butyric (C4), hexanoic (C6) and total carboxylic acids (TC) for the experiments carried out at pH levels of 5 (A), 6 (B) and 7 (C). Second column of graphs show the corresponding accumulated biogas produced at pH 5 (D), 6 (E) and 7 (F).

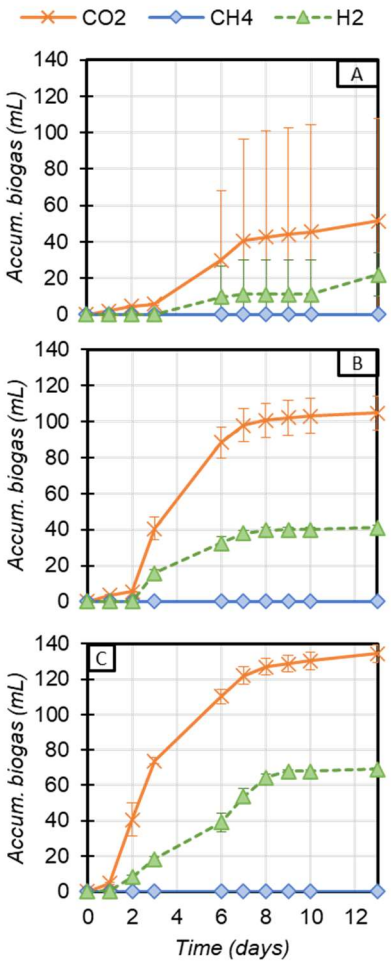



**Figure S 3:** Microbial consortium responses at 37°C for the experiments carried out at lower total solids, i.e. lower $GP_{Fresh}$ content. Accumulated biogas produced at pH 5 (A), 6 (B) and 7 (C).

A later anaerobic fermentation test was performed at the optimal conditions (pH 7 and 37°C) but using a Pyrex bottle with a dynamic headspace. This allowed to verify if the operating pressure might affect the chain elongation performance. Specifically, the experimental set-up bottles (**Figure S 4**) allowed to maintain the pressure close to the atmospheric. The obtained results at atmospheric pressure are shown in **Figure S 5** where they are compared with the reported results on the main text (**Figure 2C**).

**Figure S 4:** experimental setup used for working at low pressure conditions

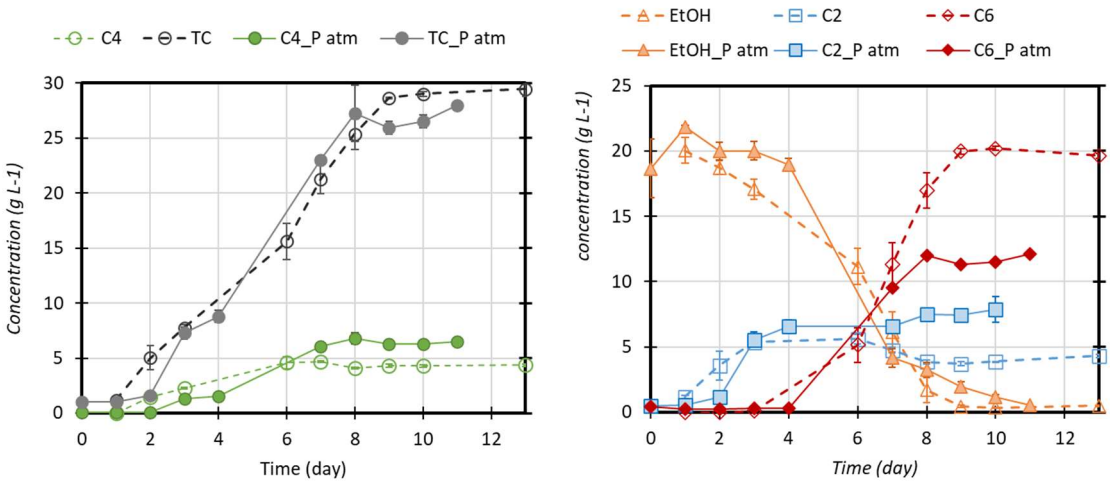



**Figure S 5:** Microbiome responses to different pressure conditions, namely P>$P_{atm}$ and at almost atmospheric pressure (P atm).

Ethanol and overall carboxylate concentration trends resulted highly similar to that obtained under pressure condition (30 g/L). However, acetic and butyric acid started to be produced slightly later and the final hexanoic acid concentration resulted significantly lower.

### 1.2. Production of highly concentrated hexanoic acid

Anaerobic fermentation of $GP_{Fresh}$ at 20L scale was carried out in order to assess the production of C6 at high concentration and to verify the feasibility of applying a simple separation step such as C6 insolubilization by broth acidification. **Figure S 6** shows the obtained concentration trends along the fermentation time.

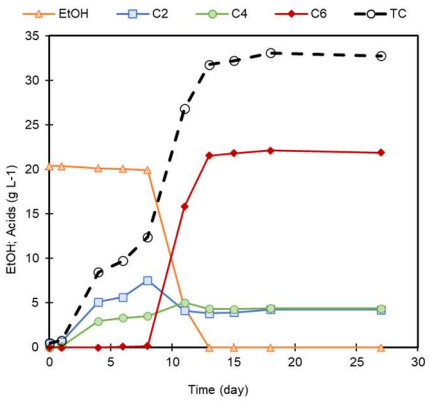



**Figure S 6**: Metabolites concentrations along the fermentation time.

After filtrating and centrifugating the fermentation broth (main text, Experimental section), C6 was separated from the aqueous solution by using the experimental set-up shown in **Figure S 7**.

To determine its purity, three samples were prepared by diluting the recovered C6 at different ratios and analysed by HPLC. These samples were compared with the results obtained by preparing the same sample dilutions with a commercial C6 (**Figure S 8A**). Besides, the same six samples were used for determining the chemical oxygen demand (COD) and to compare results again between $GP_{Fres}$ derived C6 and commercial standard (**Figure S 8B**). Furthermore, the concentrated C6 purity was analysed by means of ion chromatography, **Figure S 9** and **Figure S 10** to **Figure S 15** show total ion chromatogram and the mass spectra of the identified compounds.

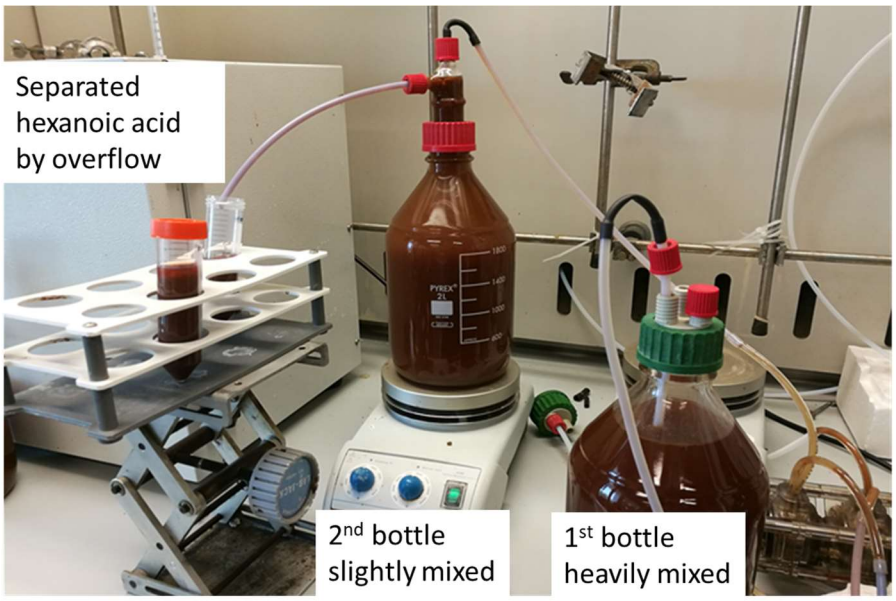



**Figure S 7**: Experimental set-up for separating the insolubilized hexanoic acid.

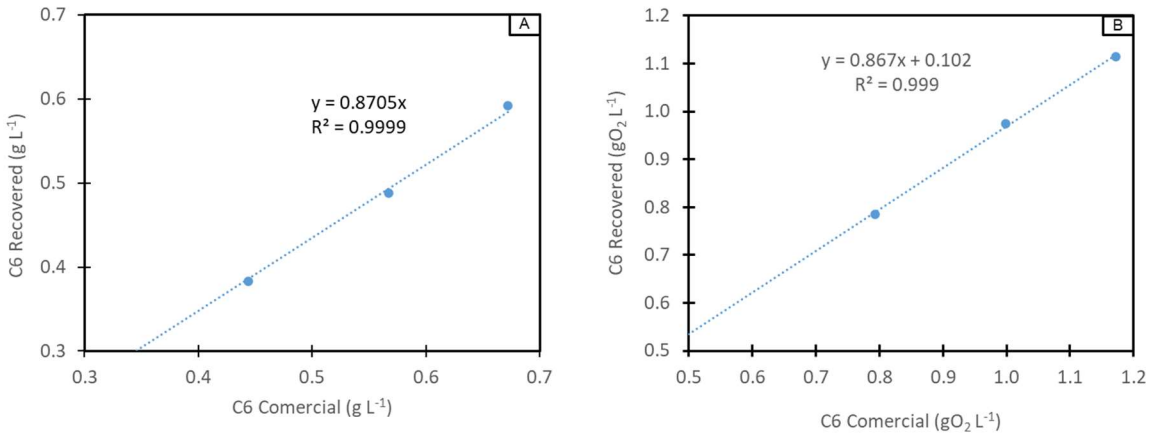



**Figure S 8**: Purity assessment of the $GP_{Fresh}$ derived C6 from HPLC (A) and COD (B) analyses.

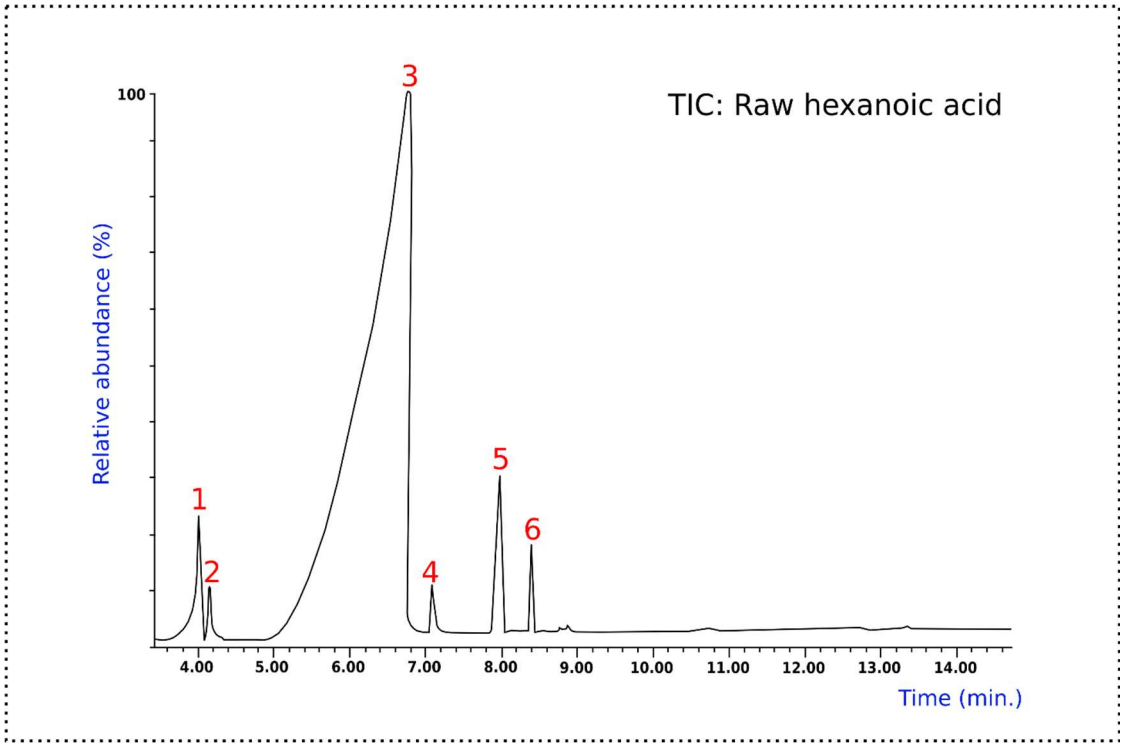



**Figure S 9:** Total ion chromatogram (TIC) of raw hexanoic acid allowed to detect butyric (1), valeric (2), hexanoic (3), heptanoic (4), octanoic (5) and decanoic (6) acids.

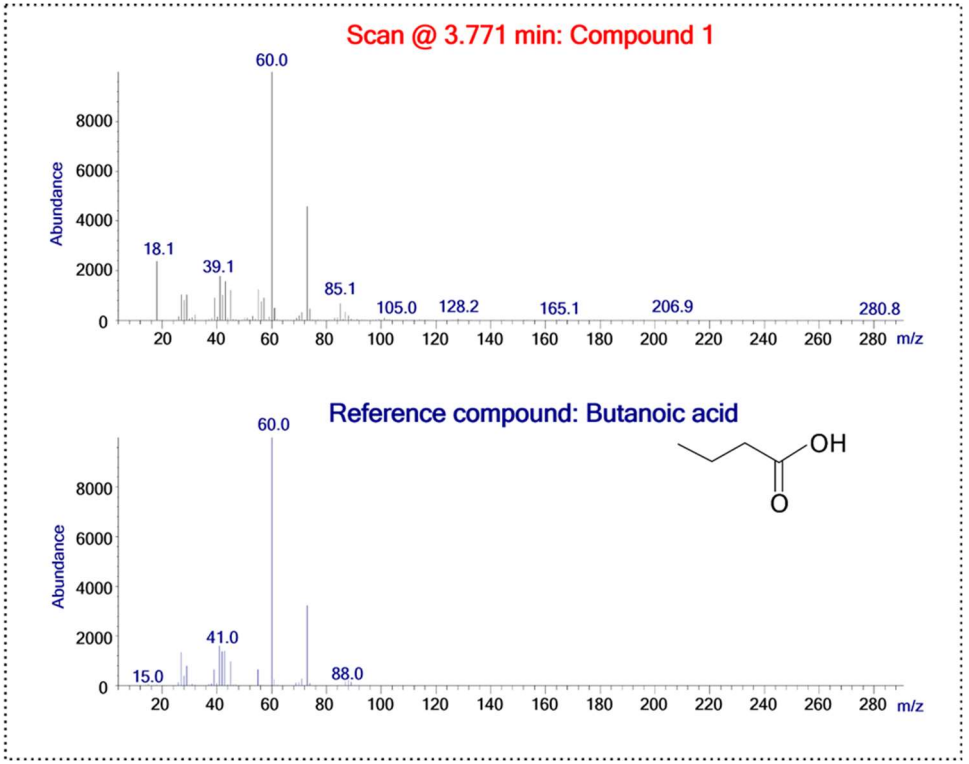



**Figure S 10:** Mass spectra of the identified butyric acid (compound/peak 1) and that of the standard reference.

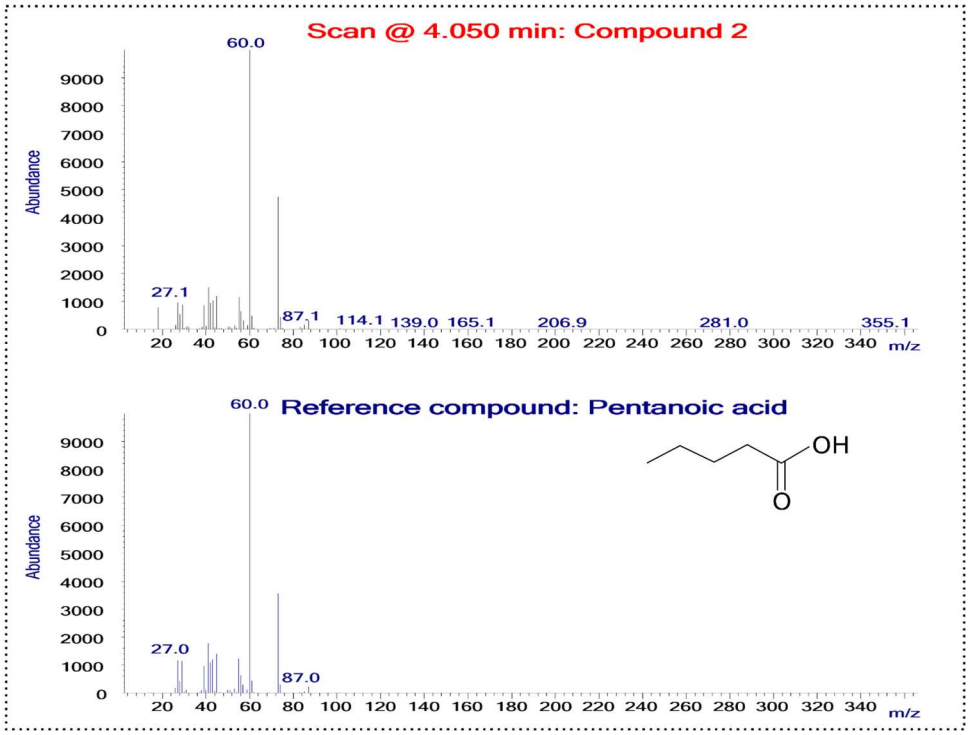



**Figure S 11:** Mass spectra of the identified valeric acid (compound/peak 2) and that of the standard reference.

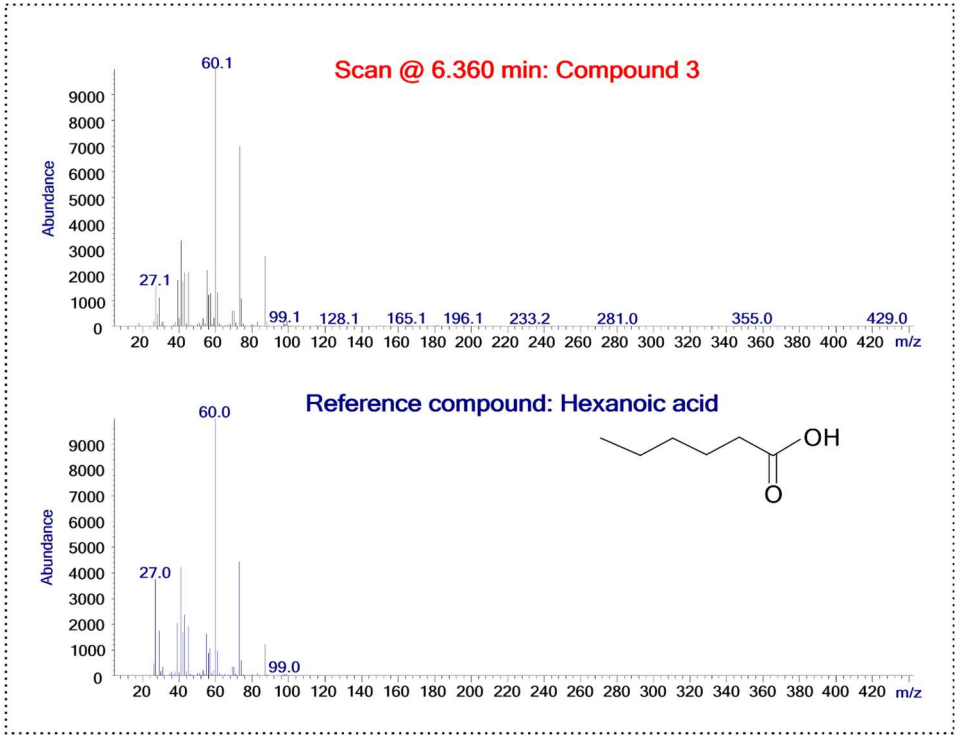



**Figure S 12:** Mass spectra of the identified hexanoic acid (compound/peak 3) and that of the standard reference.

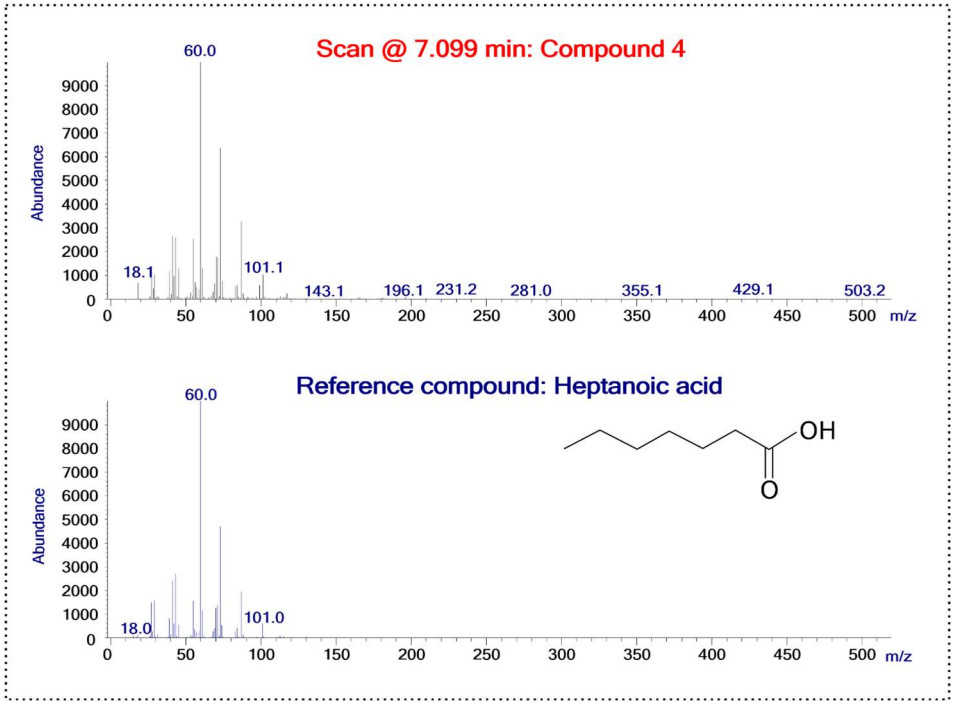



**Figure S 13**: Mass spectra of the identified heptanoic acid (compound/peak 4) and that of the standard reference.

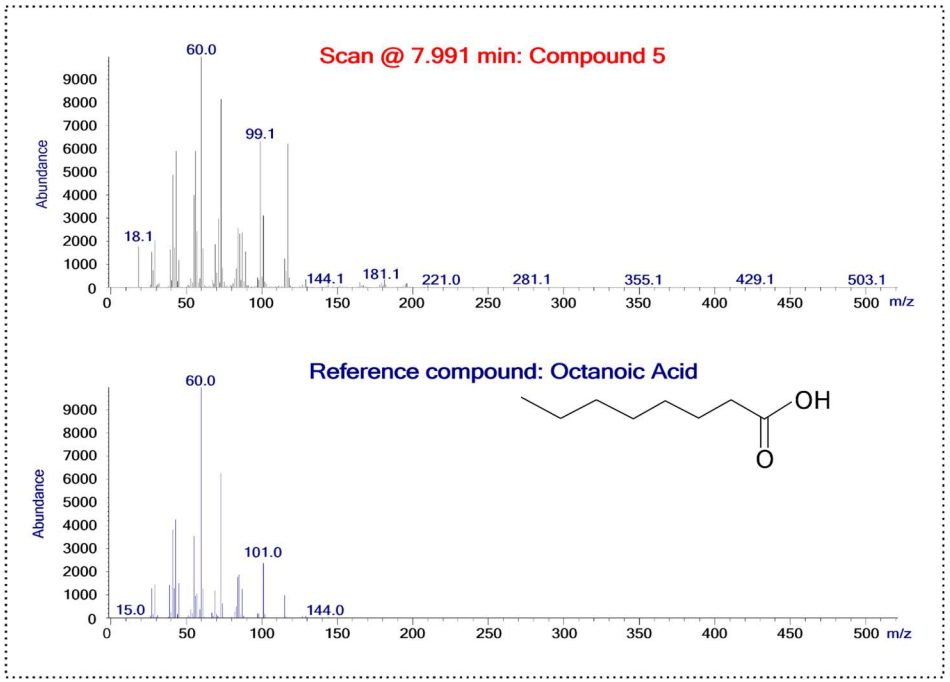



**Figure S 14:** Mass spectra of the identified octanoic acid (compound/peak 5) and that of the standard reference.

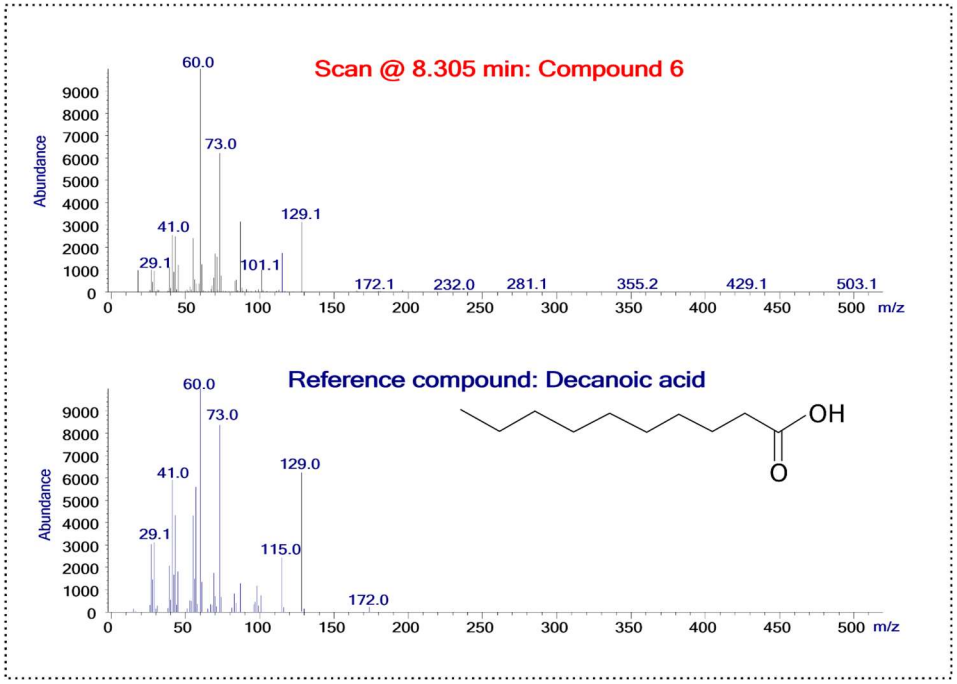



**Figure S 15:** Mass spectra of the identified decanoic acid (compound/peak 6) and that of the standard reference.

Initially, n-caprylate (C8) production was ignored due to the fact that the VFAs standard mixture contained only VFAs including a number of C atoms between 2 (acetic acid) and 7 (heptanoic acid). Later on, a C8 standard was injected, confirming that this acid was also produced. A final concentration of 0.4 g $L^{-1}$ was estimated for the octanoic acid.

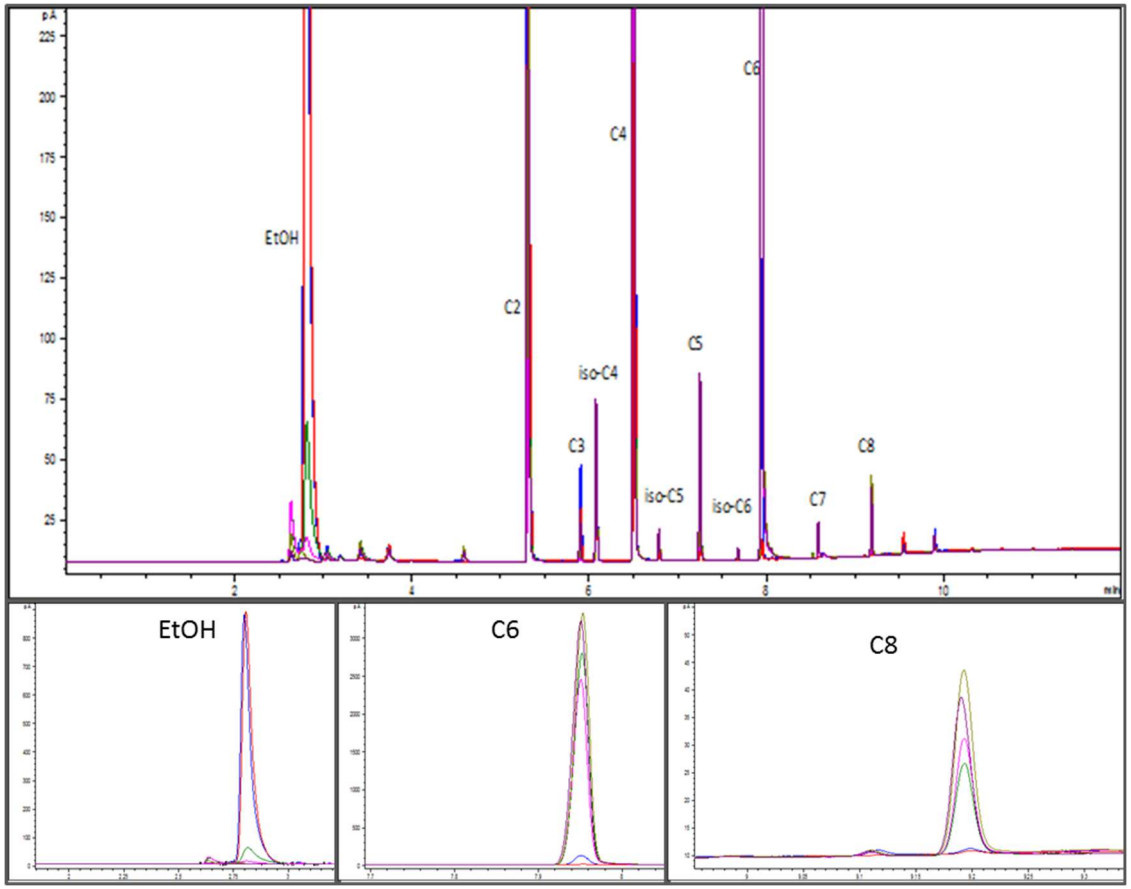



**Figure S 16:** GC chromatograms for ethanol (EtOH) and carboxylic acids (Cn) determination during the anaerobic fermentation at 37ºC and pH 7. Concentrations during the first days are represented by chromatogram profiles in blue and red, while final days concentrations are represented by the violet and the yellow-greenish lines.

## 2. Production of ester and alcohol from the separated hexanoic acid

The mixture containing the derived ester and alcohol was analysed by means of ion chromatography, **Figure S 17** shows the total ion chromatogram whereas **Figure S 18** to **Figure S 22** present the mass spectra of the identified compounds.

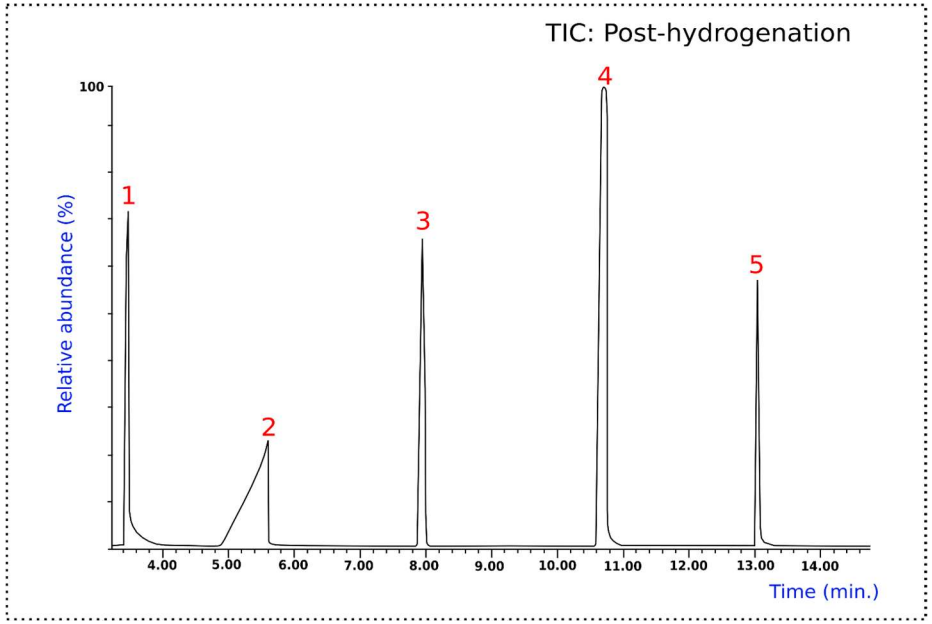



**Figure S 17:** Total ion chromatogram (TIC) of the hydrogenation product.

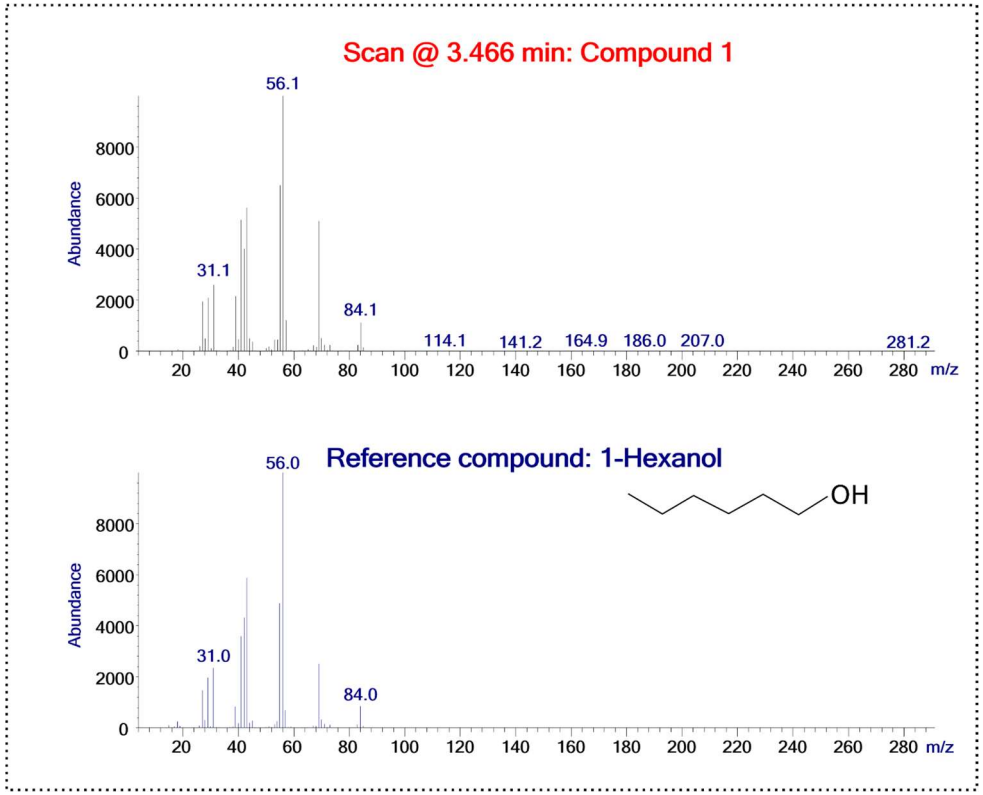



**Figure S 18:** Mass spectra of the identified 1-hexanol (compound/peak 1) and that of the standard reference.

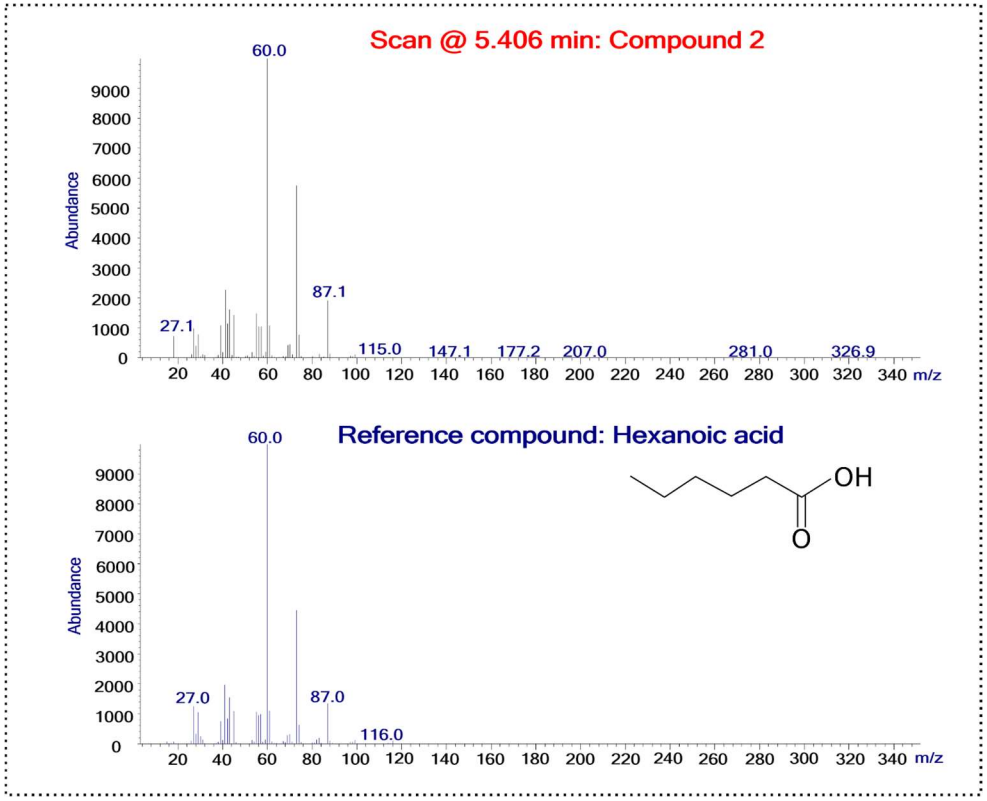



**Figure S 19:** Mass spectra of the identified hexanoic acid (compound/peak 2) and that of the standard reference.

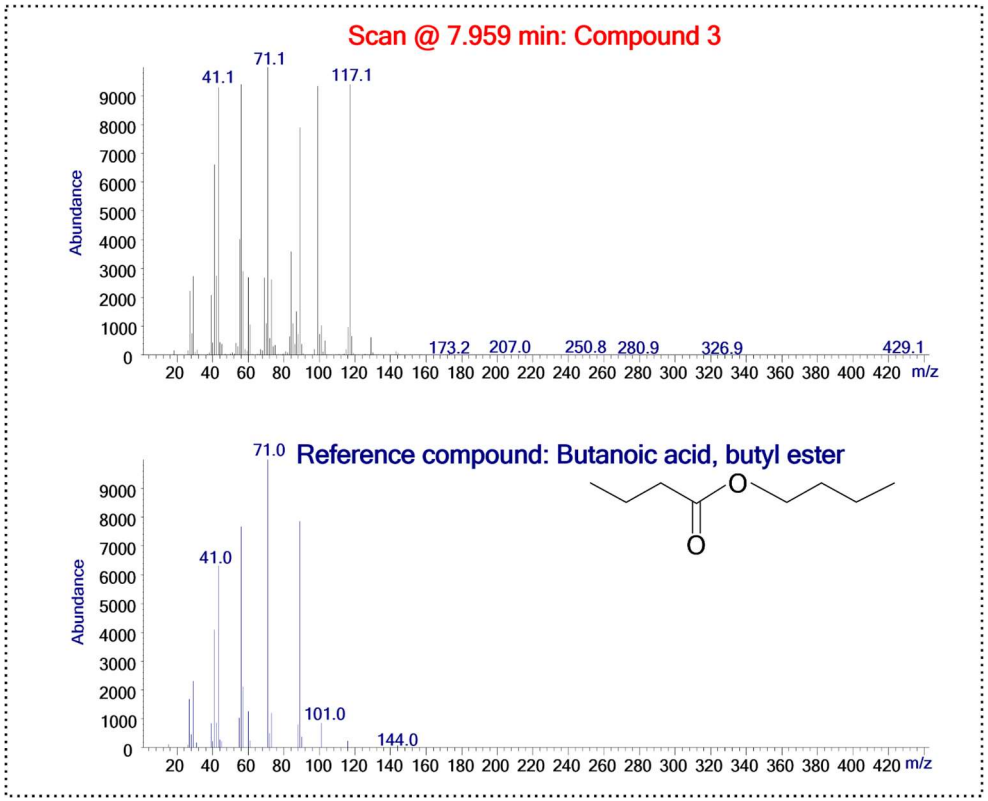



**Figure S 20:** Mass spectra of the identified butyl butyrate (compound/peak 3) and that of the standard reference.

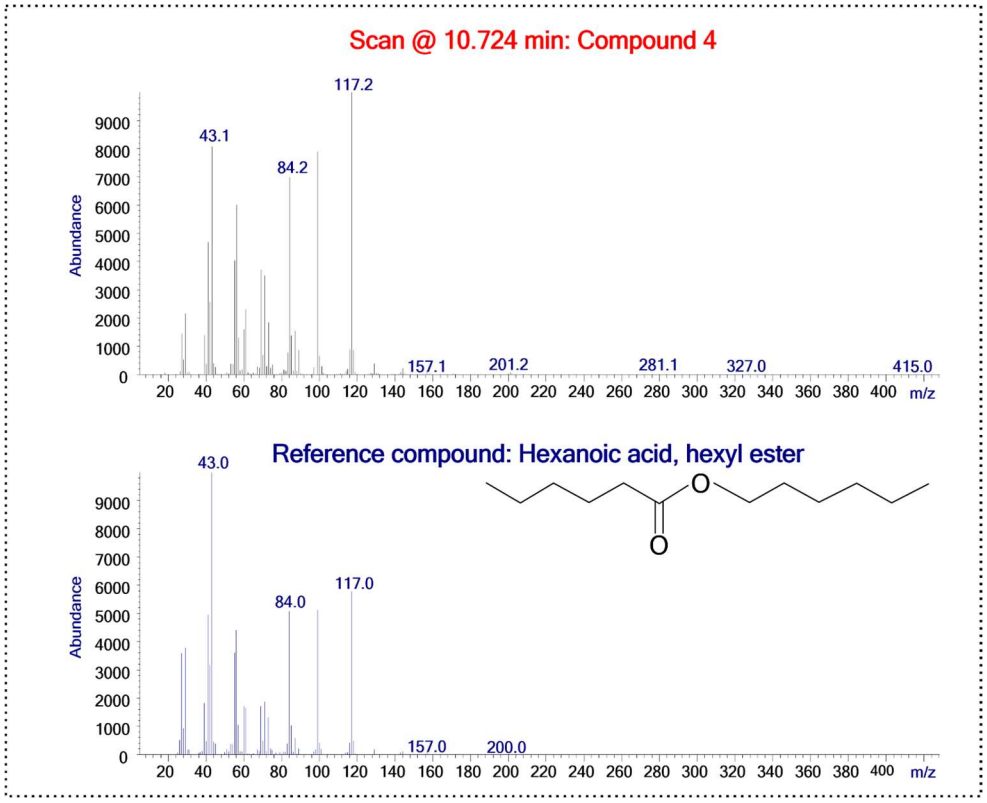



**Figure S 21:** Mass spectra of the identified hexyl hexanoate (compound/peak 4) and that of the standard reference.

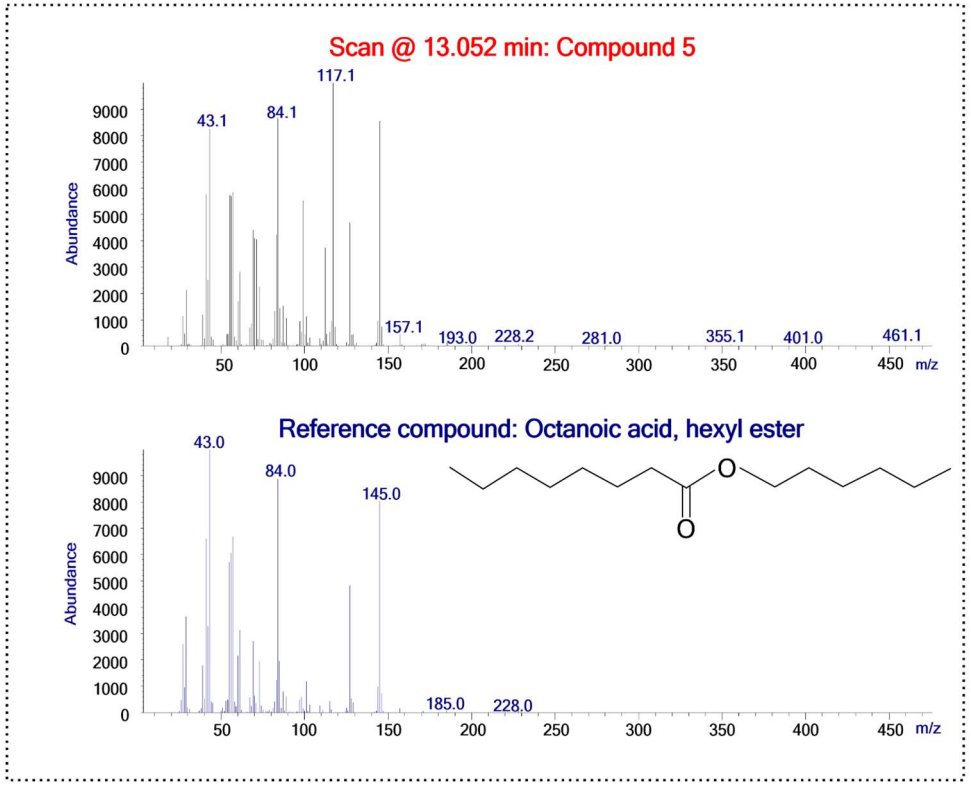
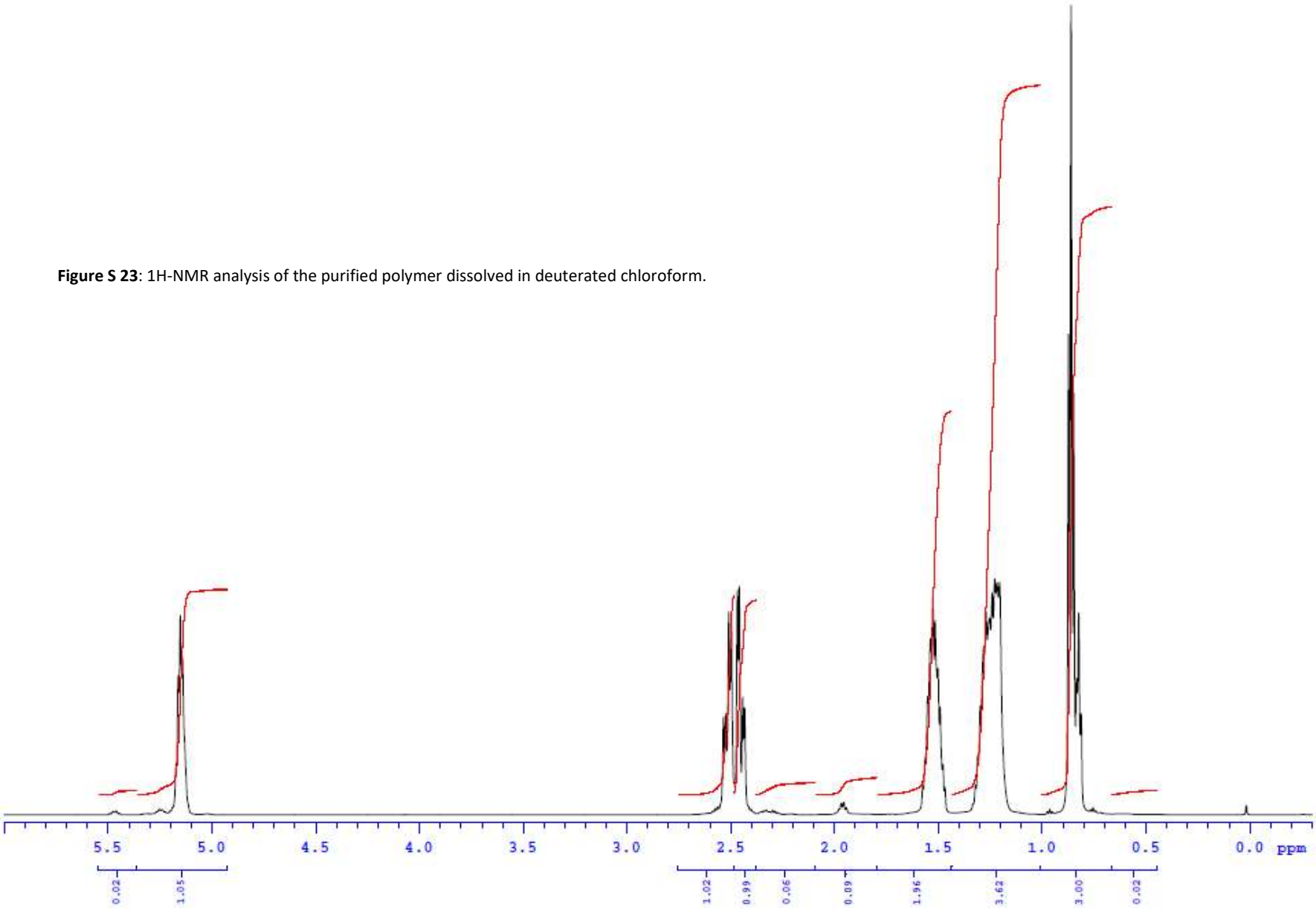



**Figure S 22:** Mass spectra of the identified hexyl octanoate (compound/peak 5) and that of the standard reference.

## 3. Production of mcl-PHAs

### 1.1. Polymer characterization.

**Figure S 23**: 1H-NMR analysis of the purified polymer dissolved in deuterated chloroform.



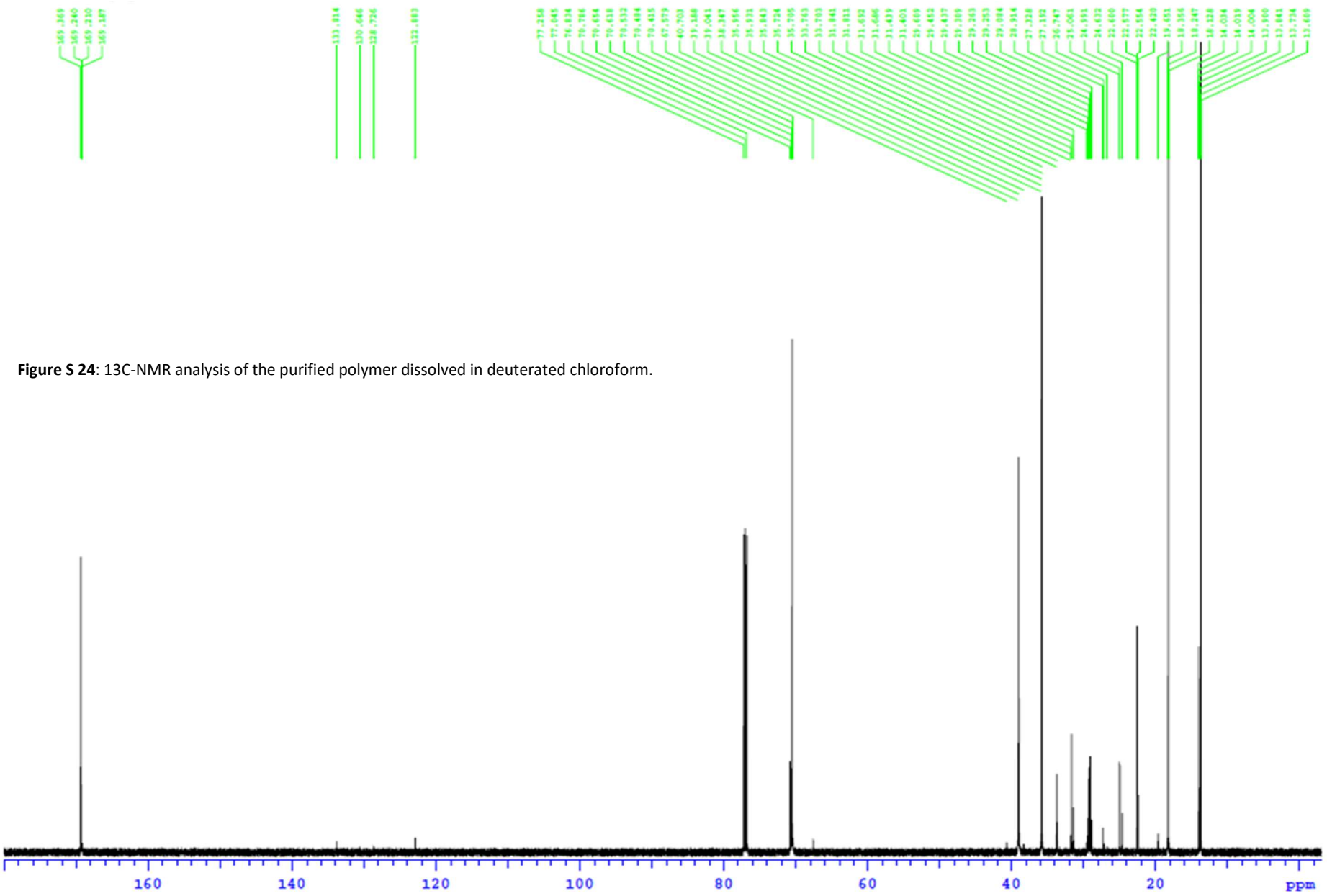



**Figure S 24**: 13C-NMR analysis of the purified polymer dissolved in deuterated chloroform.

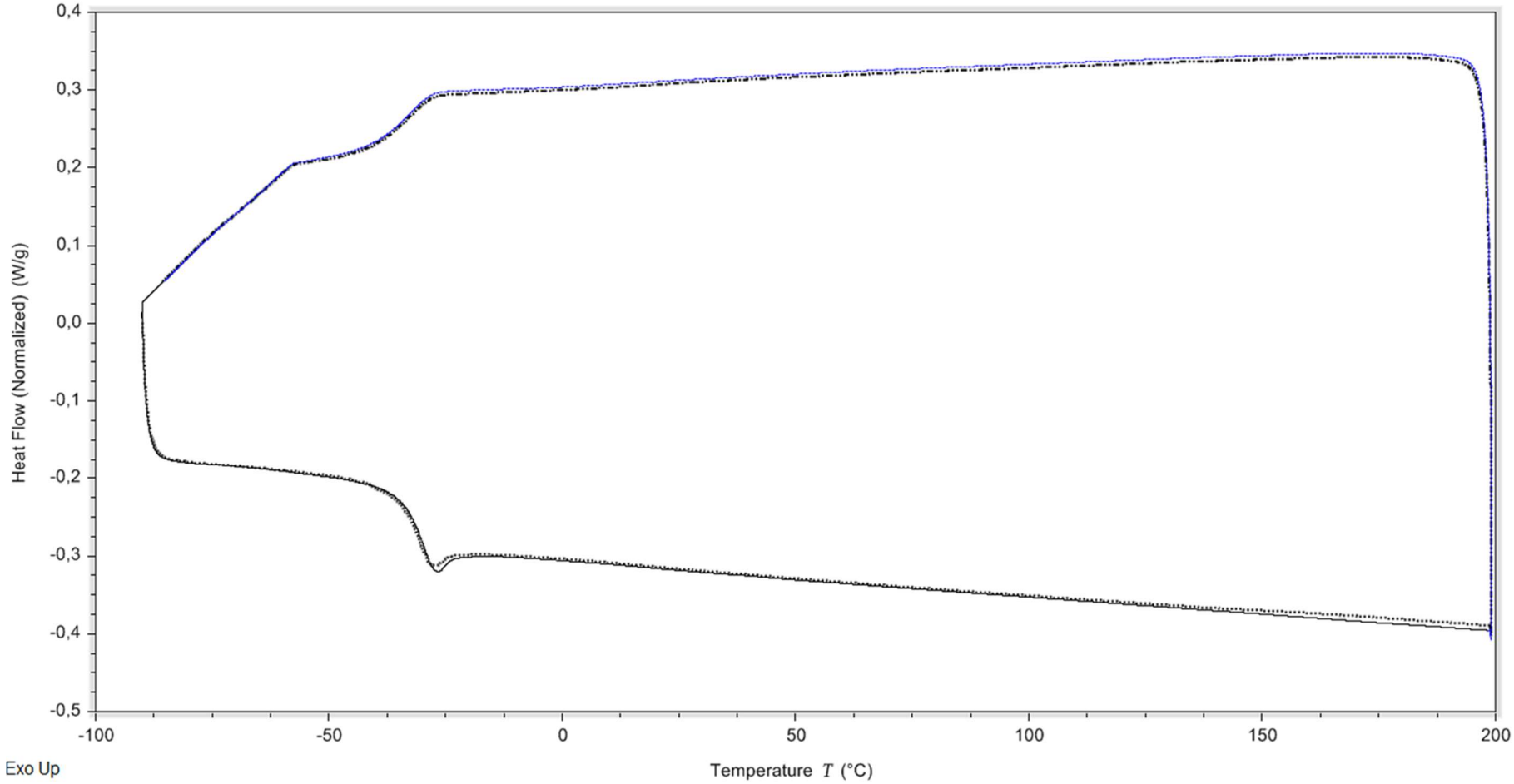



**Figure S 25**: Differential scanning calorimetry analysis of the purified polymer. Two cycles at 10K $min^{-1}$.

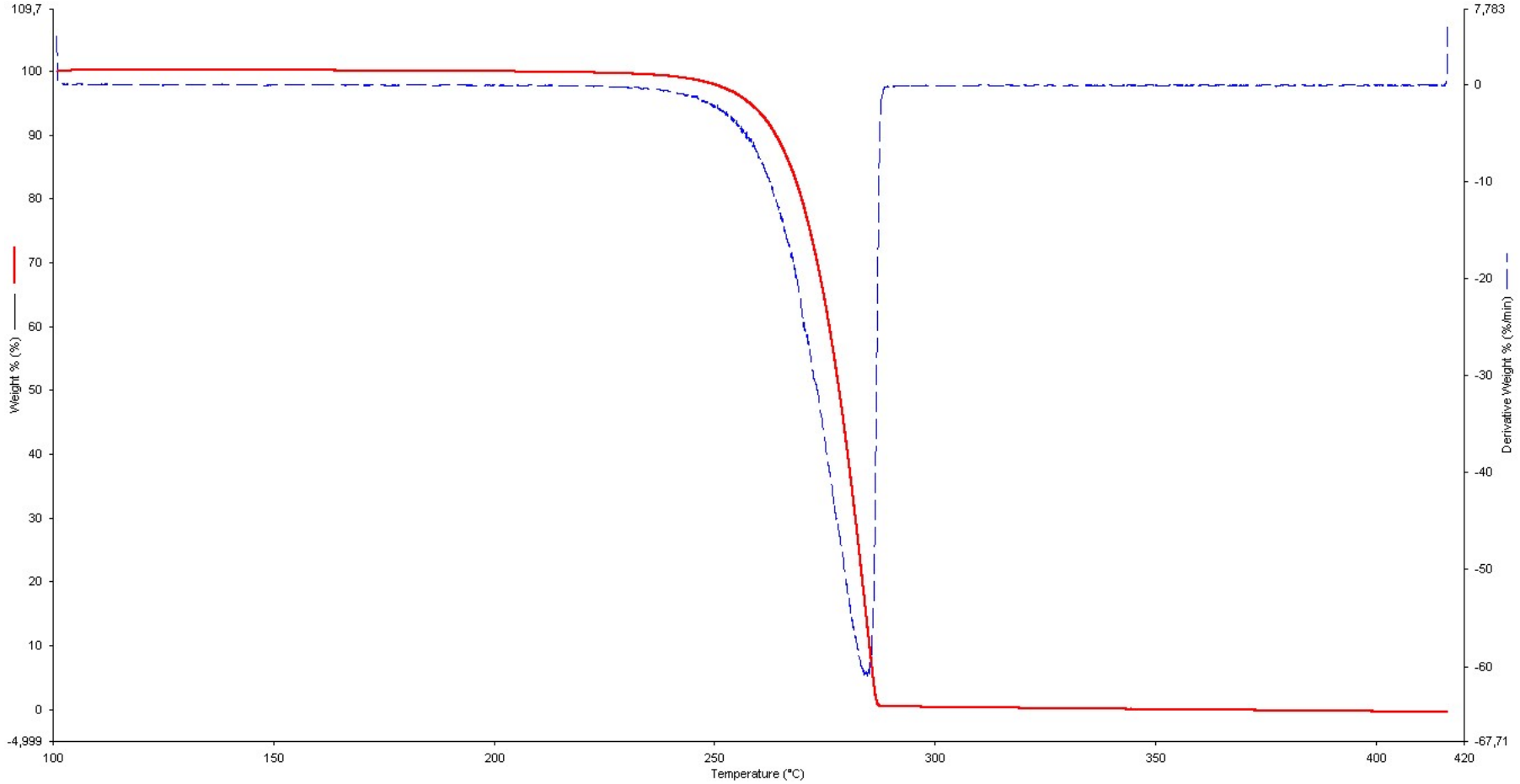



**Figure S 26**: thermogravimetric analysis of the purified polymer, with a degradation temperature of 287 °C.