

Supplementary material

A comparison of the total methyl group peak areas around 20 and 10 ppm, from the PHB and PVH respectively, gives the molar ratio of the PHB and PHV components. Comparing the CP and direct excitation experiments (Figure S1) it can be seen that there is some small discrepancy in the ratio of PHB/PVH methyls. Since the level of PHV is so low, the error using the CP experiment is probably sufficient for comparison purposes. However, when calculating the molar ratio of PHB and PHV in the other CP experiments, where no direct excitation experiment was measured, a correction factor was used based on the difference in Figure S1.

In order to try to characterise the nature of the side chain CH₂ peaks from the HV in the area 24-32 ppm, a variable contact CPMAS NMR experiment was performed on the VFA/urea sample. Figure S2 shows the peak areas of the HB methyls (3HB₄), HB methylenes (3HB₂) and HV side chain methylenes (HV₄) from the broad and narrow peaks in this area versus the cross polarisation contact time. Each component fits to a different model: the I-S or the I-I*-S model^{S1}. The I-S model usually gives a reasonable fit when the hydrogen nuclei surrounding the carbons can be treated as one thermal bath from which the magnetisation is transferred to the carbons in a cross polarisation experiment, and the peak area of the carbons versus contact time *t* can be described by the following equation^{S1}:

$$I(t) = I_0(1 - T_{IS}/T_{1\rho}^H)^{-1}[\exp(-t/T_{1\rho}^H) - \exp(-t/T_{IS})]$$

where:

T_{CH} is the cross polarisation time of the H to the C

$T_{1\rho}$ is the ¹H spin lattice relaxation time in the rotating frame

This model is often applicable in the case of amorphous regions of a typical hydrocarbon polymer or rigid carbons with no directly bonded H.

The I-I*-S model is observed when the system under study is more rigid and the initial transfer of magnetisation to the carbons is dominated by the strong ¹H-¹³C dipolar interaction of the closest H neighbours. After this initial fast transfer of magnetisation, a slower transfer is observed due to spin diffusion from Hs further away from the carbon of interest. The evolution of the magnetisation with the cross polarisation contact time *t* is then given by^{S1}:

$$\text{CP } I(t) = I_0 \exp(-t/T_{1\rho}^H) \left[1 - \lambda \exp(-t/T_{df}) - (1 - \lambda) \exp\left(-\frac{3}{2}t/T_{df}\right) \exp\left(-\frac{1}{2}t^2/T_2^2\right) \right]$$

where:

T_2 is a time which characterises the strength of the close dipolar interaction

T_{DF} is the time characterising the rate of spin diffusion

$T_{1\rho}$ is the ^1H spin lattice relaxation time in the rotating frame

It is clear from figure S2 that the cross polarisation dynamics of the more narrow peaks, normally associated with the crystalline HB matrix (3HB_4^{X} and 3HB_2^{X}), fit more closely to the I-I*-S model, as expected for a more rigid system. Table S1 shows the parameters derived from a fit of the appropriate model to each peak shown in figure S2.

Table S1. Fitted parameters^{S2} found from the variable contact cross polarisation experiment on the sample VFA/urea.

Peak	Best fit model	$T_2/\mu\text{sec}$	$T_{\text{DF}}/\mu\text{sec}$	$T_{1\rho}/\text{msec}$
3HB_4^{X}	I-I*-S	47	520	45
3HB_2^{X}	I-I*-S	18	600	45
		$T_{\text{CH}}/\mu\text{sec}$	$T_{1\rho}/\text{msec}$	
3HB_4^{A}	I-S	220	11	
3HB_2^{A}	I-S	27	8	
HV_4^{X}	I-S	57	21	
HV_4^{Y}	I-S	56	10	
HV_4^{Z}	I-S	390	14	

The two moieties 3HB_4^{X} and 3HB_2^{X} have very similar ^1H $T_{1\rho}$ values and spin diffusion times, as expected if both are within the same ordered crystalline lattice.

The broader 3HB_4^{A} and 3HB_2^{A} peaks give a better fit to the I-S model and their ^1H $T_{1\rho}$ values are shorter than for the 3HB_4^{X} and 3HB_2^{X} species. This suggests that the broader peaks are more likely associated with the more mobile amorphous regions of the polymer. The difference in the T_{CH} values for 3HB_4^{A} and 3HB_2^{A} is attributed to the fast rotation of the 3HB_4^{A} methyl group which partially averages the C-H dipolar interaction leading to a longer T_{CH} for the 3HB_4^{A} . This phenomenon is also seen in the T_2 values for the 3HB_4^{X} and 3HB_2^{X} species.

If the hydroxyvalerate (HV) part of the copolymer is within the crystalline region, the ^1H $T_{1\rho}$ value associated with the cross polarisation to the carbon should be similar to that of the crystalline part of the HB polymer, assuming a random copolymer, due to efficient spin diffusion between the HB and HV. All three simulated peaks from the HV_4 side chain CH_2 groups fit better to an I-S model of the intensities versus contact times. They also have a lower ^1H $T_{1\rho}$ value than those observed for the crystalline groups 3HB_4^{X} and 3HB_2^{X} , implying that these groups are within areas of slightly different mobility in the polymer. However, since the quality of the data is rather poor, it cannot be said for certain if these peaks are from truly separate regions or made up of several peaks from both the crystalline and amorphous regions. If the HV is indeed only present in the amorphous region, as proposed in reference S3, then we would assume that both HV_4^{X} and HV_4^{Y} are most likely in the

amorphous region of the HB polymer; and since the HV_4^{X} has a slightly longer $^1\text{H } T_{1\rho}$ value, this species may be near the interface of the amorphous/crystalline region. If the whole area is a combination of various arrangements of the side chain in the crystalline HB copolymer lattice, as well as some amorphous HV/HB copolymer^{S4}, then it is difficult to separate out those components with this experiment since only some average $T_{1\rho}$ value would be seen. At this low level of HV in the copolymer, where only small amounts of HV are normally seen in the crystalline HB lattice^{S3,S5}, it seems more likely that the peaks HV_4^{X} and HV_4^{Y} are mostly due to species in amorphous areas with slightly different mobilities.

The third HV_4 side chain group peak, HV_4^{Z} , also fits better to an I-S model and has a similar $^1\text{H } T_{1\rho}$ value between the two other HV_4 species, but the T_{CH} time is longer. This would imply significant local motion which reduces the dipolar C-H interaction of the HV side chain CH_2 group. This observation would rule out this peak being due to any crystalline HV seen previously around 29 ppm^{S3}, since this would be expected to have a strong C-H dipolar interaction and thus a fast T_{CH} rise time. It also indicates that it is most likely not within the amorphous HB domain since the HV_4^{X} and HV_4^{Y} species in this domain have lower, similar, T_{CH} values. Therefore we propose that this peak around 30-31 ppm may be due to some very small amounts of amorphous HV homopolymer; and when the amount of HV increases, more crystalline HV forms in a HV lattice— giving the characteristic peak around 29 ppm, as seen for high HV content copolymers^{S3}.

Unfortunately, the signal-to-noise for the HV_4 peaks is quite low and the fits to the cross polarisation models rather poor. Therefore, further experiments with better signal-to-noise ratios need to be carried out to confirm the tentative proposals made here.

Supplementary references:

- S1) “Kinetics of Cross-Polarization in Solid-State NMR: A Guide for Chemists”,
Waclaw Kolodziejski and Jacek Klinowski, Chemical Reviews 2002 102 (3), 613-628
- S2) Fitted using the python curve_fit routine, version 2.7, Python Software Foundation.
www.python.org
- S3) “Studies of Cocrystallization of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by Solid-State High-Resolution ^{13}C NMR Spectroscopy and Differential Scanning Calorimetry”, Naoko Kamiya, Minoru, Sakurai, Yoshio Inoue, Riichiró Chújó and Yoshiharu Doi, Macromolecules 1991, **24**, 2178-2182.
- S4) “ ^{13}C NMR Determination of the Degree of Cocrystallization in Random Copolymers of Poly(β -hydroxybutyrate-co- β -hydroxyvalerate)” David L. VanderHart, William J. Orts and Robert H. Marchessault, Macromolecules 1995, 28, 6394-6400
- S5) “The biological and physical chemistry of polyhydroxyalkanoates as seen by NMR spectroscopy”, K.M. Bonthron, J. Clauss, D.M. Horowitz, B.K. Hunter and J.K.M. Sanders, Microbiology Reviews 103 (1992) 269-27

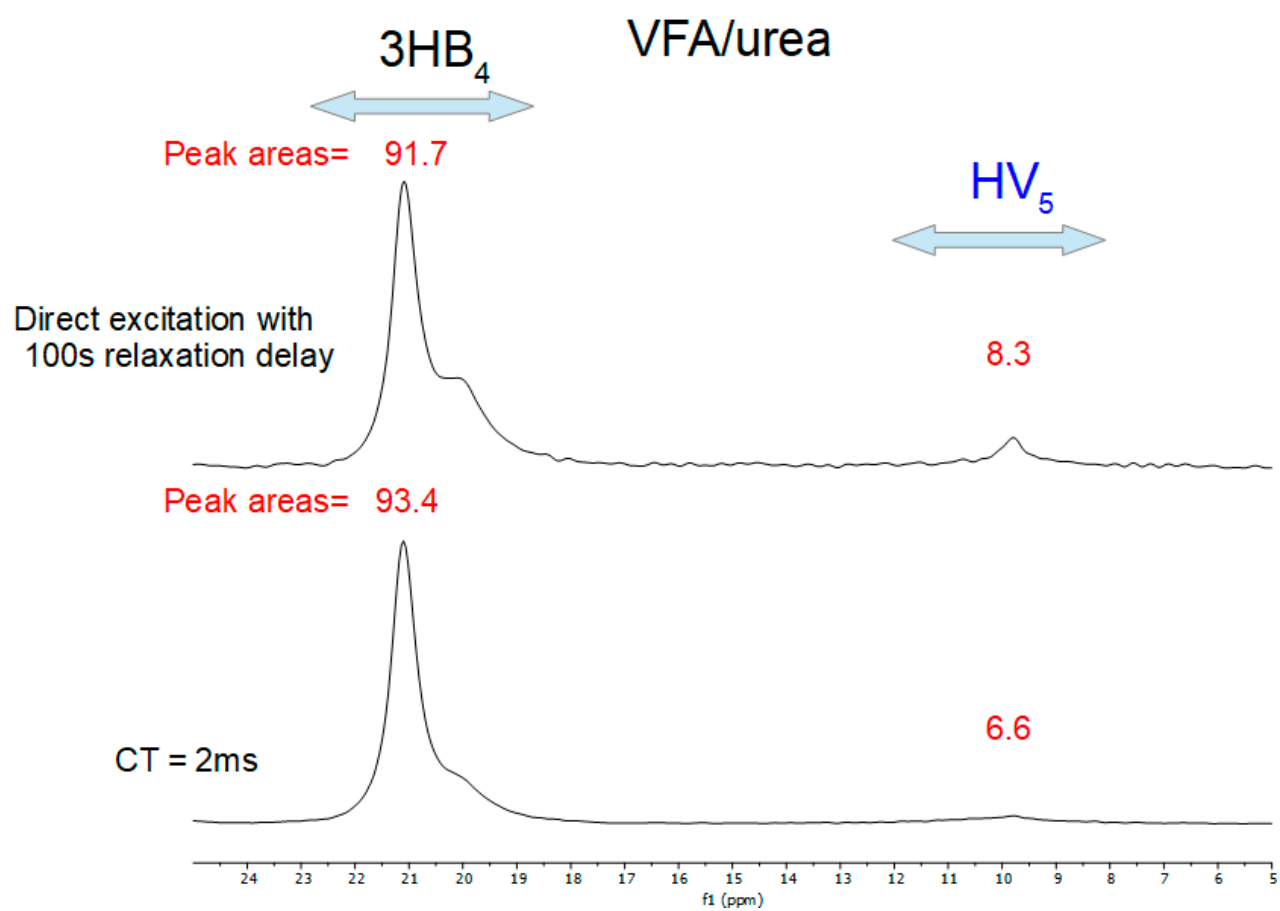


Figure S1. Comparison of PHB and PHV methyl areas in the sample “VFA/urea” using cross polarisation (contact time 2ms) and direct excitation.

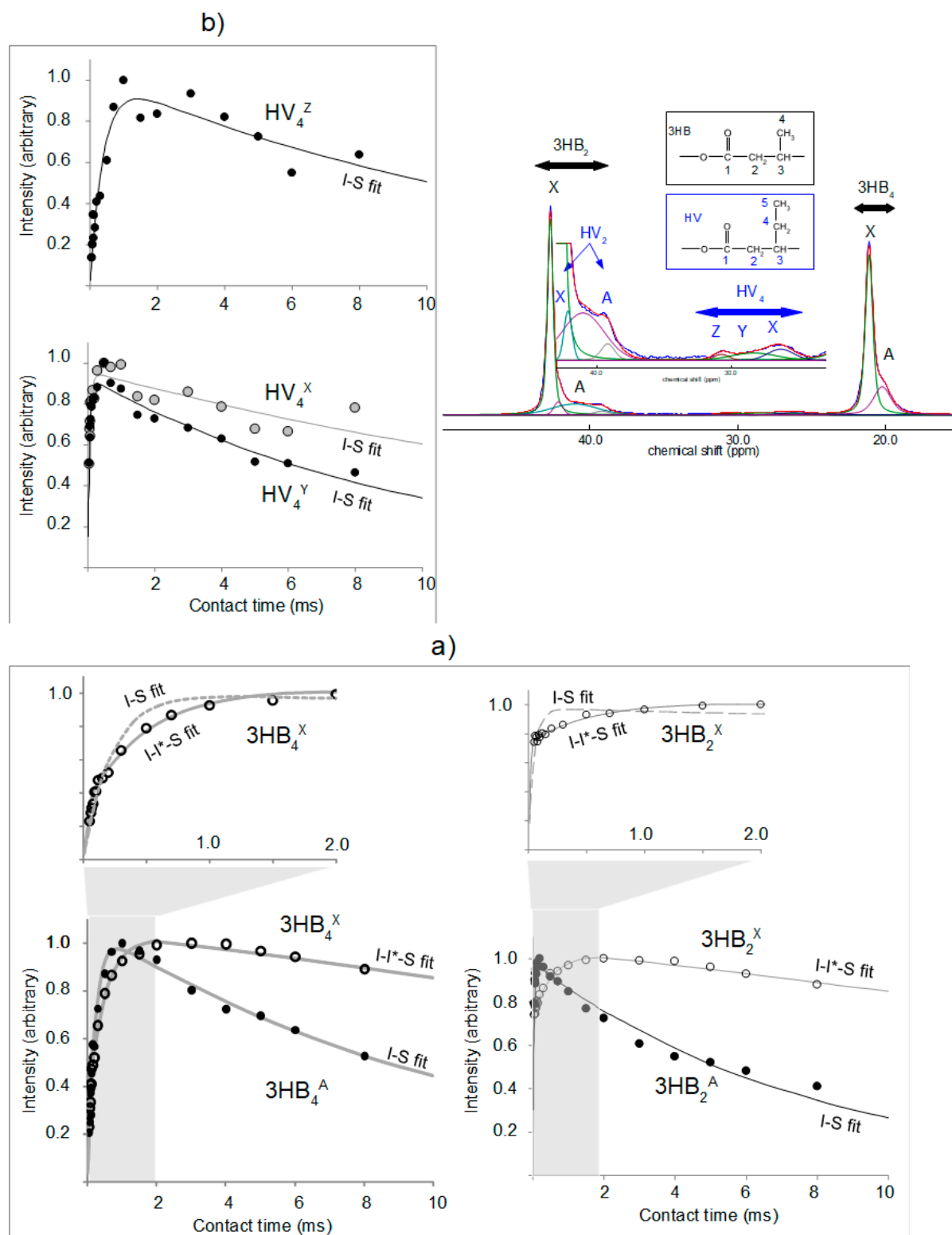


Figure S2. Fits of the I-I*-S and I-S cross polarisation models to the a) $3HB_4$ and $3HB_2$ peak areas, and b) the HV peaks. Peak intensities were scaled to 1.0 for the most intense peak. The peaks labelled as HV_2 were not used but can be identified in the variable contact experiment.

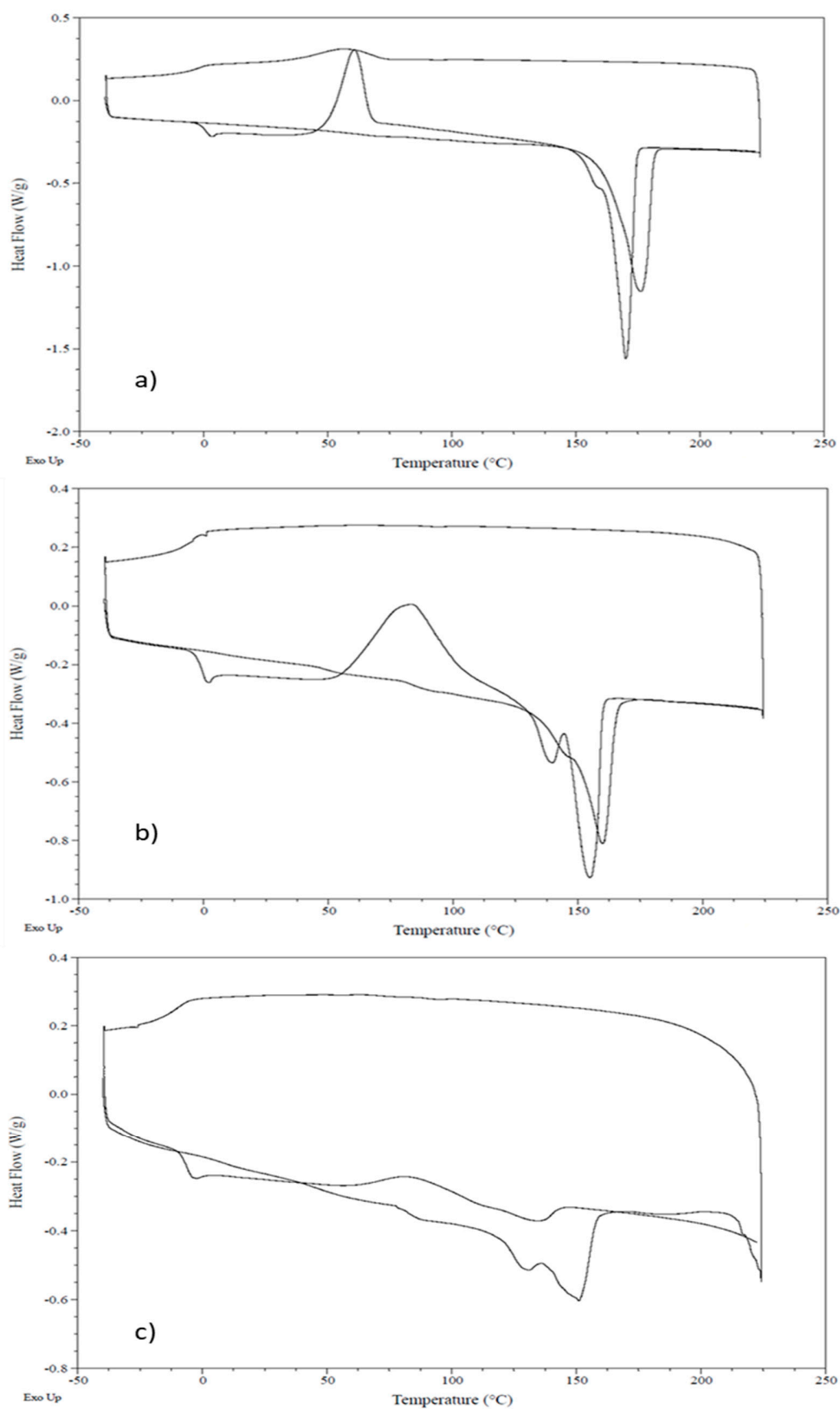


Figure S3. DSC profiles of extracted samples from biomass cultivated in different carbon sources of (a) glucose, (b) synthetic VFAs and (c) real VFA stream.