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Fast Method for the determination of Short-chain-length Polyhydroxyalkanoates (scl-PHAs) in Bacterial Samples by In Vial-Thermolysis (IVT)

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Abstract

A new method based on the GC-MS analysis of thermolysis products obtained by treating bacterial samples at high temperature (above 270°C) has been developed. This method, here named "In-Vial-Thermolysis" (IVT), allowed for the simultaneous determination of short-chain-length polyhydroxyalkanoates (scl-PHA) content and

composition. The method was applied to both single strains and microbial mixed cultures (MMC) fed with different carbon sources.
The IVT procedure provided similar analytical performances compared to previous Py-GC-MS and Py-GC-FID methods, suggesting a similar application for PHA quantitation in bacterial cells. The results from the IVT procedure and the traditional methanolysis method were compared; the correlation between the two datasets was fit for the purpose, giving a R^2 of 0.975. In searching for further simplification, the rationale of IVT was exploited for the development of a "field method" based on the titration of thermolyzed samples with sodium hydrogen carbonate to quantify PHA inside bacterial cells. The accuracy of the IVT method was fit for the purpose.
These results open up the possibility for the on-line measurement of PHA productivity. Moreover, they allow for the fast and inexpensive quantification/characterization of PHA for biotechnological process control, as well as investigation over various bacterial community and/or feeding strategies.

Keywords

Polyhydroxyalkanoates, Thermolysis-GC-MS, Thermolysis-titration, Crotonic acid, Mixed Microbial Cultures (MMC)

Highlights

- In-Vial Thermolysis (IVT) allows for GC-MS determination of scl-PHA amount and composition.
- IVT is fast, simple, and does not require special lab equipment.
- Direct titration of the IVT products allows for scl-PHA quantification without a GC instrument.

1 Introduction

Polyhydroxyalkanoates (PHAs) are a family of intracellular polyesters. They are synthesized by a wide variety of prokaryotic microorganisms, as carbon and energy storage material, under stress conditions including nitrogen and/or phosphate restriction, as well as excess carbon [1, 2]. Biodegradable PHAs have a broad range of applications; in particular, the copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate (P(3HB-3HV)) has a high potential for the substitution of conventional plastics because it has thermoplastic properties comparable to those of petroleum-based polyolefins, such as polypropylene and polyethylene [3, 4].

Single strains and, more recently, mixed microbial cultures (MMC) have been increasingly investigated in order to reduce PHA production costs using feedstocks originating from various wastes, such as: fermented molasses [5], glycerol [6], cheese whey [3, 7, 8], olive oil mill wastewater [9], pyrolysis oil [10] and slaughterhouse by-products [11]. Efficient PHA-storing organisms have been selected in these processes applying feast and famine (FF) cycles [12, 13], where organic carbon is provided to the biomass during a short feast phase, followed by a long period of starvation (famine phase).

In order to reduce the research cost in bioreactors production related with adaptation of the process regime and the changes in feeding strategy, an accurate and fast monitoring of PHA amount and composition is crucial [14]. Many analytical techniques are under investigation and some improvements have been achieved: from optical and electronic microscopy to spectroscopy and biosensors [15, 16, 17, 18]. It has also been demonstrated that the pyrolysis products derived from PHA can be used as molecular

markers to identify and quantify PHA in bacterial biomass [19]. However, this approach coupled with GC analysis (Py-GC) has not yet been fully-exploited in this field.

The present study aims at developing a new analytical approach that could simplify and accelerate the real-time monitoring of bacterial cultures able to produce short chain length-PHA (scl-PHA, hereafter named PHA). The goal was to improve the previous pyrolysis-based method developed by Torri et al (2014) [19]. The method presented here, following the QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, **S**afe) philosophy [20, 21, 22, 23, 24], focused on the simplification of the quali/quantitative analysis of PHA in bacterial biomass. Moreover, since bacterial lipid metabolism is closely related to the synthesis of reserve macromolecules such as PHA [25], the developed protocol could also be used for the qualitative estimation of long chain fatty acids (LCFA) within bacterial membranes.

Specifically, the method presented here, termed "IVT" (In-Vial Thermolysis) was based on the GC-MS analysis of the thermolysis products obtained from high temperature treatment (above 270°C) of both single strain bacteria and MMC. This method allows for the simultaneous determination of PHA content and PHA-monomer composition.

2 Materials and Methods

All solvents and chemicals used in this study were obtained from Sigma-Aldrich (purities ≥ 98%) and used without purification. Standard poly(3-hydroxybutyrate) P(3HB) was purchased from Biomer® (DE). The copolymer P(3HB-3HV) (3HB/3HV 80 mol%/20 mol%) was obtained as previously reported in the literature [26].

2.1 Microbial samples (Single strain and Microbial Mixed Cultures)

Cupriavidus necator DSM 545 was grown in shake-flasks according to Samorì et al 2015 [26] with four different feeding substrates: i) glucose; ii) glucose and valeric acid; iii) glucose, valeric acid and γ-butyrolactone; iv) acetic and propionic acid (Table 1).

An assortment of MMC samples (MMC1-14, Table 1) were obtained from different sequencing batch reactors adapted to a feast/famine feeding regime, as described in various studies [5, 6, 12, 26], with different feeding substrates: glycerol, acetic acid, propionic acid, fermented cheese whey, bio-oil and triethyl citrate.

The amount of PHAs in each bacterial sample was determined according to the standard methanolysis procedure reported in literature [27].

Table 1. Microbial samples used in the present work: single strain or MMC, feeding substrates and intracellular PHA content.

Entry	Sample name	Single strain /MMC	Feeding substrate	3HB mol% : 3HV mol%	PHA in CDM (Wt%)[a]
1	Sing 1	*C. necator*	Glucose	100:0	74.0 ± 2.0
2	Sing 2	*C. necator*	Glucose + Valeric acid	80:20	54.0 ± 2.0
3	Sing 3	*C. necator*	Glucose + Valeric acid + γ-butyrolactone	90:10	64.0 ± 1.2
4	Sing 4	*C. necator*	Acetic+ Propionic acid	81:19	64.0 ±3.2
5	MMC 1	MMC	Glycerol	100:0	20.5 ± 2.2
6	MMC 2	MMC	Glycerol + Methanol	95:5	23.4 ± 2.5
7	MMC 3	MMC	Fermented bioil	94:6	17.5 ± 1.8
8	MMC 4	MMC	Fermented cheese whey	89:11	5.0 ± 0.4
9	MMC 5	MMC	Fermented cheese whey	85:15	5.0 ± 0.6

10	MMC 6	MMC	Fermented cheese whey	90:10	8.0 ± 0.9
11	MMC 7	MMC	Fermented cheese whey	88:12	13.0 ± 1.5
12	MMC 8	MMC	Acetic+ Propionic acid	91:9	44.0 ± 9.0
13	MMC 9	MMC	Acetic+ Propionic acid	89:11	43.0 ± 8.2
14	MMC 10	MMC	Acetic+ Propionic acid	90:10	3.0 ± 0.4
15	MMC 11	MMC	Acetic+ Propionic acid	89:11	37.0 ± 8.0
16	MMC 12	MMC	Acetic+ Propionic acid	90:10	40.0 ± 8.7
17	MMC 13	MMC	Acetic+ Propionic acid + Triethyl citrate	98:2	24.0 ± 6.0
18	MMC 14	MMC	Acetic+ Propionic acid + Triethyl citrate	99:1	8.0 ± 0.7

a Determined by methanolysis

2.2 IVT of microbial samples

Freeze dried microbial samples (5 mg) or standard P(3HB) (2-3 mg) were transferred to in a screw cap vial (4 ml volume, 50 mm high). The vial was left with the cap off for 5 min in order to reduce the electrostatic behavior of lyophilized powder, allowing for the gentle deposition of all powder to the bottom of the vial. Subsequently, as shown in Figure 1, the vial was closed and then placed on a hot plate at 350°C. During this procedure, the bottom of the vial reached a temperature of 300-320°C, whereas the upper part of the vial and the cap did not exceed 80°C. For safety reasons and in order to avoid cap damage or the release of thermolysis vapors, the temperature was carefully monitored during method setup (e.g. by means of an infrared (IR) thermometer). Different treatment times (20, 30, 60 and 120 min) were tested, in order to investigate the thermolytic process kinetics. Since thermolysis was completed within 20 min, this time was chosen for the final set up of the IVT procedure.

After 20 min, the vials were removed from the hot plate and left to cool down to room temperature before adding the internal standard (2-ethyl-butanoic acid, 0.25 mg dissolved in water). The sample was then diluted with CH_3CN (4 ml) and subjected to silylation-GC-MS analysis (see below). In the titration method, the sample was directly subjected to titration with 5 μM $NaHCO_3$ solution containing 1 mg/ml of bromothymol blue.

Finally, an aliquot of the CH_3CN-solution (0.1 ml) was withdrawn and subjected to silylation and GC-MS analysis for the determination of PHAs and fatty acid profiles and amount. For this purpose, *N,O* bis(trimethylsilyl)trifluoroacetamide containing 1% of trimethylchlorosilane (0.1 ml) and pyridine (0.02 ml) were added to the sample and the solution was heated at 60°C for 30 min. The identification of trimethylsilylated products

was confirmed by direct GC-MS analysis with the polar GC column and method previously used for Py-GC-MS [19]. The GC-MS analyses were performed with a 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer. The injection port temperature was 280°C. Analytes were separated by a HP-5 fused-silica capillary column (stationary phase poly[5% diphenyl/95% dimethyl]siloxane, 30 m, 0.25 mm i.d., 0.25 mm film thickness), with helium as carrier gas (at constant pressure, 33 cm s^{-1} linear velocity at 200°C). Mass spectra were recorded under electron ionization (70 eV) at a frequency of 1 scan s^{-1} within the 12-600 m/z range. The following thermal program was used: 50°C hold for 10 min, and then increased up to 325°C at 10°C min^{-1}.

Figure 1.

2.3 Calibration

The amount of PHA in the biomass samples was determined by GC-MS from the quantity (Q_{AC}) of the most abundant 2-alkenoic acid (AC) derived from the thermolysis of the corresponding monomer in the polymer chain. The following 2-alkenoic acids were used for quantitation:

(*E*)-but-2-enoic acid (crotonic acid)

(*E*)-pent-2-enoic acid (pentenoic acid)

The quantity Q_{AC} in the thermolysate was calculated from the following formula:

$$Q_{AC} = Q_{IS} \cdot A_{AC} / A_{IS}$$

where Q_{IS} is the mass of the internal standard (2-ethylbutanoic acid) expressed in mg, while A_{AC} and A_{IS} are the GC-MS peak areas of the AC and internal standard, respectively.

The relationship between the quantity Q_{AC} of the selected 2-alkenoic acid and the corresponding quantity of the monomer in the sample ($X_{HA} \cdot Q_{PHA}$) was determined using the calibration curve obtained by treating standard polymers of known monomer composition, where X_{HA} is the mass fraction of the monomer in the polymer.

The ratio Q_{AC}/Q_{PHA} corresponds to the yield (Y_{AC}) of AC of the entire analytical procedure. The best linear relationship was determined by the least square fitting method:

$$Q_{PHA} = a\,(Q_{AC}) + b$$

For the fatty acid determination, a semi-quantitative analysis was performed by calculating the relative area of each fatty acid in comparison to the total area of all fatty acids.

3 Results and Discussion

3.1 IVT of purified PHA samples

The chromatogram obtained by applying low temperature thermolysis to the homopolymer P(3HB) and the copolymer P(3HB-3HV)) showed the typical alkenoic acid markers already found during pyrolysis (Figure 2) [19]. The mass spectra of the main peaks obtained from P(3HB) were characterized by the base peak at *m/z* 143 (loss of methyl radical from the molecular ion), an intense peak at *m/z* 69 ($C_4H_5O^+$) and a peak at *m/z* 99 ($C_5H_{11}Si^+$), in agreement with literature data [14]. The most abundant peak was attributed to the *trans* isomer according to previous studies pertaining to the analytical

pyrolysis of P(3HB) [17, 18]. Similar to pyrolysis, when P(3HB-3HV) was submitted to thermolysis, an additional peak was detected, likely identified as (*E*)-pent-2-enoic acid. A relevant yield of a homologue series with intense peaks at *m/z* 69 ($C_4H_5O^+$), 143 and 155 was also found, likely identified as P(3HB) oligomers. For P(3HB-3HV), four peaks were attributed to dimers whereas several peaks were attributed to various oligomers, in agreement with the possible monomeric sequence expected in the polymer.

The same yield of 2-butenoic acid and 2-pentenoic acid from low temperature IVT was obtained (27±5% w/w_{PHB} or w/w_{PHV}, determined by 20 replicates), significantly lower than that obtained through pyrolysis (40-50% w/w_{PHA}). This was probably related to an increased production of PHA oligomers due to specific reaction conditions (e.g. slow heating). Different amounts of P(3HB) were subjected to IVT in order to evaluate the linearity between the quantity of the 2-alkenoic acid produced (Q_{AC}) and the amount of the treated sample in the 0.2 – 5 mg range. Q_{AC} of 2-butenoic acid was approximately linear with the quantity of P(3HB) subjected to IVT (n=12, R^2 = 0.956).

Figure 2.

3.2 IVT of microbial samples

The typical PHA markers obtained by treating P(3HB-3HV) – enriched microbial biomass with IVT were not qualitatively influenced by the non-PHA cellular material, as already observed with pyrolysis (Figure 3).

Moreover, it was reasonable to assume that the low temperature of thermolysis treatment minimized the depolymerization of proteins and carbohydrates, thus avoiding significant analytical interferences. The chromatograms of IVT-treated microbial

204 biomass were qualitatively similar to those of IVT-treated PHA, with a lower
205 contribution of PHA oligomers.

206 In addition, a noticeable amount of long chain fatty acids (LCFA) could be detected in
207 the 20-25 minute portion of chromatogram (Figure 3). The mass spectra of each
208 trimethylsilyl (TMS) fatty acid peak was characterized by the base peak corresponding
209 to each molecular ion (a part from C14:1, C14:0-OH and C17:1), an intense peak
210 corresponding to the loss of a methyl group from the molecular ion and a peak at *m/z*
211 117, typical of all fatty acids (Table 2).

212

213 Figure 3.

214

215 Table 2. TMS derivatives of carboxylic acids (fatty acids and alkenoic acids) from the
216 IVT of the microbial biomass. Bold characters refer to the most abundant peaks.

Source	Compound[a]	Retention time (min)	Main peaks (m/z)
P(3HB), P(3HB-3HV)	2-butenoic acid	8.51	**143**, 99, 75
P(3HB-3HV)	2-pentenoic acid	10.28	**117**, 113, 75
P(3HB), P(3HB-3HV)	(E)-3-(but-2-enoyloxy)butanoic acid	15.80	**143**, 75, 69
P(3HB-3HV)	(E)-3-(pent-2-enoyloxy)butanoic acid	16.75	157, 143, **69**
Lipids	C14:0	21.41	300, **285**, 228
Lipids	C14:1	21.60	**283** , 143, 129
Lipids	C15:0	22.04	314, **299**, 143
Lipids	C16:1	22.80	326, **311**, 254
Lipids	C14:0-OH	22.86	**373** , 331, 233
Lipids	C16:0	22.98	328, **313**, 256
Lipids	C17:0	23.64	342, **327**, 145
Lipids	C17:1	23.78	**325** , 129, 117
Lipids	C18:2	24.52	352, **337**, 229
Lipids	C18:1	24.60	354, **339**, 129
Lipids	C18:0	24.77	356, **341**, 145

	Lipids	C19:1	24.82	368, **353**, 229

[a] trimethyl silylated derivates. The tentative identification was based on a combination of MS library and fragment mass.

The IVT method gave similar analytical performances compared to the Py-GC-MS and Py-GC-FID methods [19], suggesting the same application for PHA quantification in bacterial cells. In order to quantitatively evaluate the matrix effect, the results from the IVT procedure were compared to those from the traditional methanolysis approach (Figure 4). The correlation between the two datasets was slightly lower than that observed with Py-GC-FID (R^2 0.986, [19]), giving a R^2 of 0.975 over 14 samples (bacterial samples and purified polymers) with known amounts of PHA (Figure 4). As previously observed for the Py-GC-FID method, the relative percentage of 2-butenoic acid and 2-pentenoic acid from IVT was the same relative percentage of the corresponding monomers in P(3HB-3HV) found using methanolysis.

Figure 4.

Concerning the replicability, the standard deviation of duplicates ranged from 15% to 30%, obtained by different operators during a two-year study on MMC. Therefore, a precision comparable to that of the pyrolysis-based method could be expected. The overall figures of merit had a slightly lower quality compared to the pyrolysis-based methods. Nevertheless, given the procedural advantages (ease, speed, operational

238 reliability) of IVT, they can be considered acceptable for the purpose of PHA
239 monitoring in complex mixed culture system.

240

241 **3.3 Titration method for determination of PHA amount.**

242 Despite the increase in speed and simplicity of the IVT procedure, the need for a GC
243 system, and more in general, the need for laboratory instruments can represent an
244 important bottleneck for an extensive monitoring of MMC in bioreactors or plants.
245 Moreover, the time needed for GC runs implies that it would be difficult to perform
246 near-real time measurements of PHA content (in bacteria or in solutions), which is a
247 mandatory operation for real-time monitoring. Titration is a well-established and
248 potentially automated procedure that can be applied with low cost consumables.

249 For this purpose, 2-alkenoic acids (AC) produced by IVT (markers of PHA thermolysis)
250 are the main source of weak acidity in the sample subjected to this procedure. Thus the
251 amount of PHAs can be quantified by simple titration of AC equivalent with alkali
252 solution.

253 To validate this approach, several bacterial samples (listed in Table 1) and PHA
254 amounts were subjected to IVT and subsequently titrated to neutrality by adding a
255 solution of $NaHCO_3$. Figure 5 shows the results from titration of pure crotonic acid,
256 IVT-treated P(3HB) and PHA-containing bacteria.

257

258 Figure 5.

As expected, the equivalents (meq) obtained by titration of crotonic acid were very close to the theoretical values (0.0116 meq/mg CA), with minor variability due to volumetric uncertainty. Titration of IVT-treated P(3HB) samples gave a linear correlation between polymer amount and meq produced; on average, 0.005 meq of acid per mg of P(3HB) was obtained. The intercept of correlation was negligible, indicating low matrix effect. Considering a yield of 0.27 g $_{\text{Crotonic acid}}$ g_{PHB}^{-1} (0.31 meq g_{PHB}^{-1}) and a significant production of dimers and trimers (contributing to acidity but to a lesser extent due to higher molecular weight), this is in agreement with the expected theoretical values.

Titration of IVT-PHA enriched bacterial samples produced 0.004 meq of acid per mg of PHA inside the bacterial cells, slightly lower than IVT-treated P(3HB) samples. This finding is in agreement with the expected production of 2-pentenoic acid from the monomer HV, characterized by a lower specific acidity (0.010 meq/mg 2-pentenoic acid); and probably also by the production of a minor amount of ammonia during IVT of proteins in the sample. The correlation, although affected by a certain variability and influenced by the type of polymer (lower meq for longer AC), can be considered good enough for a preliminary screening of PHA content. This method allowed for a reliable PHA quantification in the range 10-50% on 5 mg microbial samples.

Despite the loss of information for PHA monomer composition, IVT-titration method can be directly applied in PHA producing plants without the need of special equipment. Furthermore, it can also be used when specific analytical instrumentation is not available in close proximity to bacteria production plants.

Moreover, this rationale could be applied in an automated measurement system based on the thermolysis of known amounts of freeze-dried microbial biomass and the titration of evolved compounds. This system should include a reaction chamber connected to a titration vessel equipped with a pH-meter or a conductivity probe (both should be able to detect the onset point). In terms of complexity and cost, IVT coupled with titration could be considered comparable to the actual FOS/TAC determination

4 Conclusions

Low temperature thermolysis can be used as a depolymerization method for the purpose of PHA quantification and the determination of monomeric composition. In-vial thermolysis and quantification of 2-alkenoic acids by GC-MS allows for the quantification of PHA content in freeze-dried microbial biomass, as well as for the determination of the relative amounts of different monomers in PHA copolymers. This procedure was suited to conventional laboratory apparatus, thus making it simpler than the Py-GC method previously developed but providing similar analytical performances. Direct titration of IVT products with alkaline solution allowed for the fast quantification of PHA content without any lab apparatus, except a heating plate. Although less informative than the GC method (no information on the composition of PHA), IVT coupled with the titration procedure was suitable for fieldwork and can, in principle, be used as a basis for the on-line continuous measurement of PHA content in dry bacteria or bacterial slurries.

Glossary

- Crotonic acid (CA): 2-butenoic acid.

- FOS/TAC: this ratio is an indicator for assessing fermentation processes. The TAC (Totales Anorganic Carbonat) value is an estimation of the buffer capacity of the sample and the FOS (Flüchtige Organische Säuren) value corresponds to the volatile fatty acids content, calculated empirically.
- Gas Chromatography Mass Spectrometry (GC-MS).
- Long chain fatty acids (LCFA): fatty acids with aliphatic tails 13 to 21 carbons.
- In-Vial Thermolysis (IVT): high temperature treatment of bacterial biomass samples.
- Microbial Mixed Cultures (MMC): multiple strains and species of bacteria collected from a single sample.

Polyhydroxyalkanoates (PHA): linear polyesters generated by fermentation of sugars and lipids. They are generally made and used by bacteria to store energy and commercially to make bioplastics.scl-PHA: Short Chain Length Polyhydroxyalkanoate.

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Figure captions

Figure 1. Sequence of the IVT steps.

1. Figure 2: Chromatograms obtained from IVT and silylation of pure P(3HB) and P(3HB-3HV). 1. E)-trimethylsilyl but-2-enoate (2-butenoic acid); 2. (E)-4-oxo-4-((trimethylsilyl)oxy)butan-2-yl but-2-enoate; 3. (2E,6E)-4-oxo-4-((trimethylsilyl)oxy)butan-2-yl 5-oxoocta-2,6-dienoate; 4. (2E,6E)-1-oxo-1-((trimethylsilyl)oxy)pentan-3-yl 7-((E)-but-2-enoyloxy)-5-oxohepta-2,6-dienoate; 5. 2E,6E)-4-oxo-4-((trimethylsilyl)oxy)butan-2-yl 7-(((E)-3-((E)-but-2-enoyloxy)acryloyl)oxy)-5-oxohepta-2,6-dienoate); 6. (E)-trimethylsilyl pent-2-enoate (2-pentenoic acid); 7. (E)-trimethylsilyl 3-(but-2-enoyloxy)pentanoate; 8. (E)-4-oxo-4-((trimethylsilyl)oxy)butan-2-yl pent-2-enoate; 9. (E)-1-oxo-1-((trimethylsilyl)oxy)pentan-3-yl pent-2-enoate

Figure 3. Chromatogram of the microbial biomass treated with IVT followed by silylation. 1. E)-trimethylsilyl but-2-enoate (2-butenoic acid), TR: 8.55 min; IS: internal standard (2-ethyl butyrate), TR: 9.77 min; 2. (E)-trimethylsilyl pent-2-enoate (2-pentenoic acid), TR: 10.32 min, analytes used for the quantitation. LCFA: long-chain fatty acids

Figure 4. Correlation between PHA content (%, w/w of sample) determined through methanolysis vs IVT.

Figure 5. Correlation between meq titrated and amount of IVT derived alkenoic acids. Red line: 2-butenoic acid; green line: PHB and blue line: bacterial PHA.

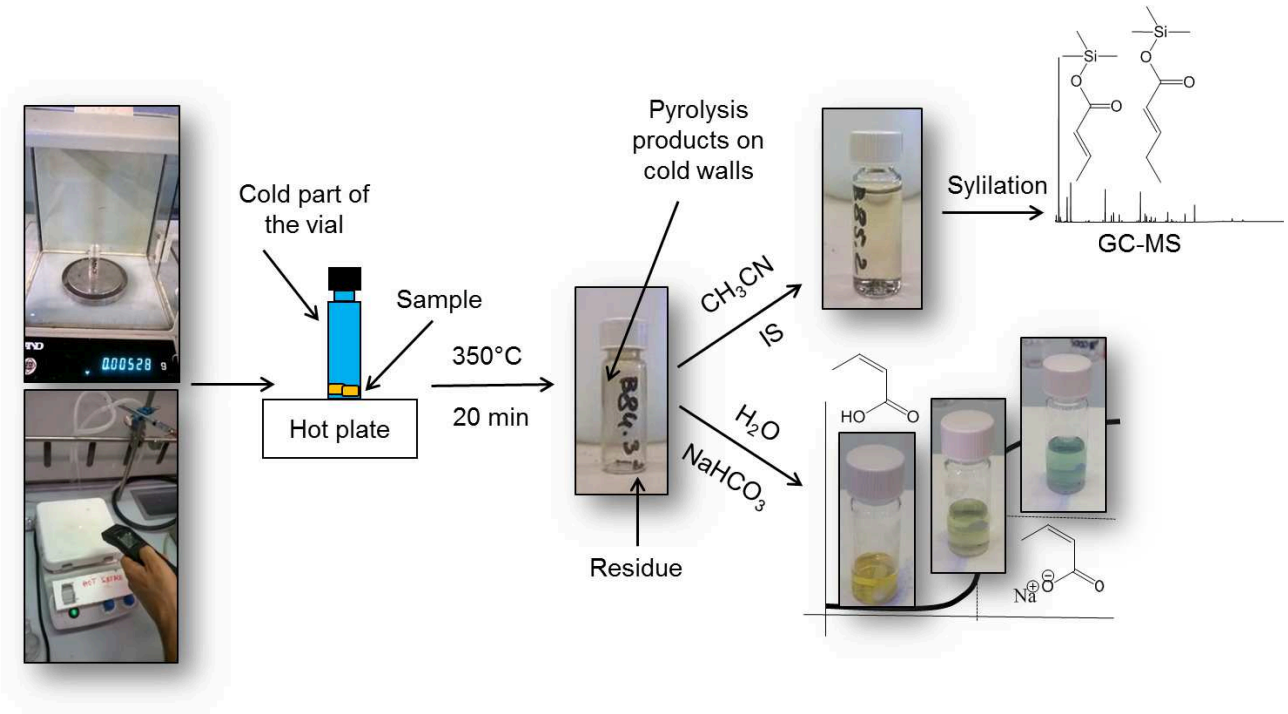

Cold part of
the vial
Sample
Hot plate
350°C
20 min
Pyrolysis
products on
cold walls
Residue
CH_3CN
IS
H_2O
$NaHCO_3$
Sylilation
GC-MS
HO
O
Na
O
O

Figure 1

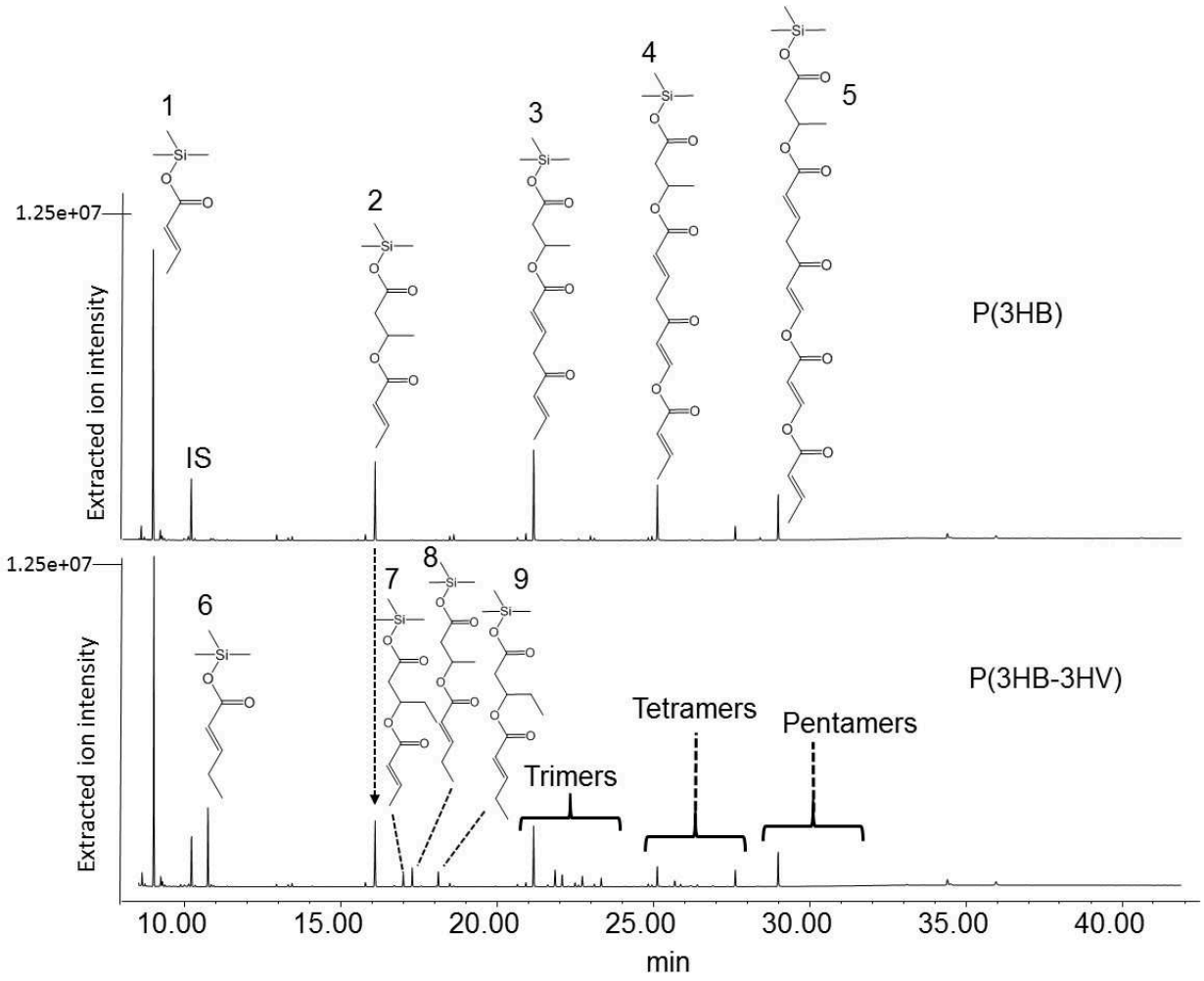

1
2
3
4
5
IS
1.25e+07
Extracted ion intensity
P(3HB)
6
7
8
9
Trimers
Tetramers
Pentamers
P(3HB-3HV)
10.00
15.00
20.00
25.00
30.00
35.00
40.00
min

Figure 2.

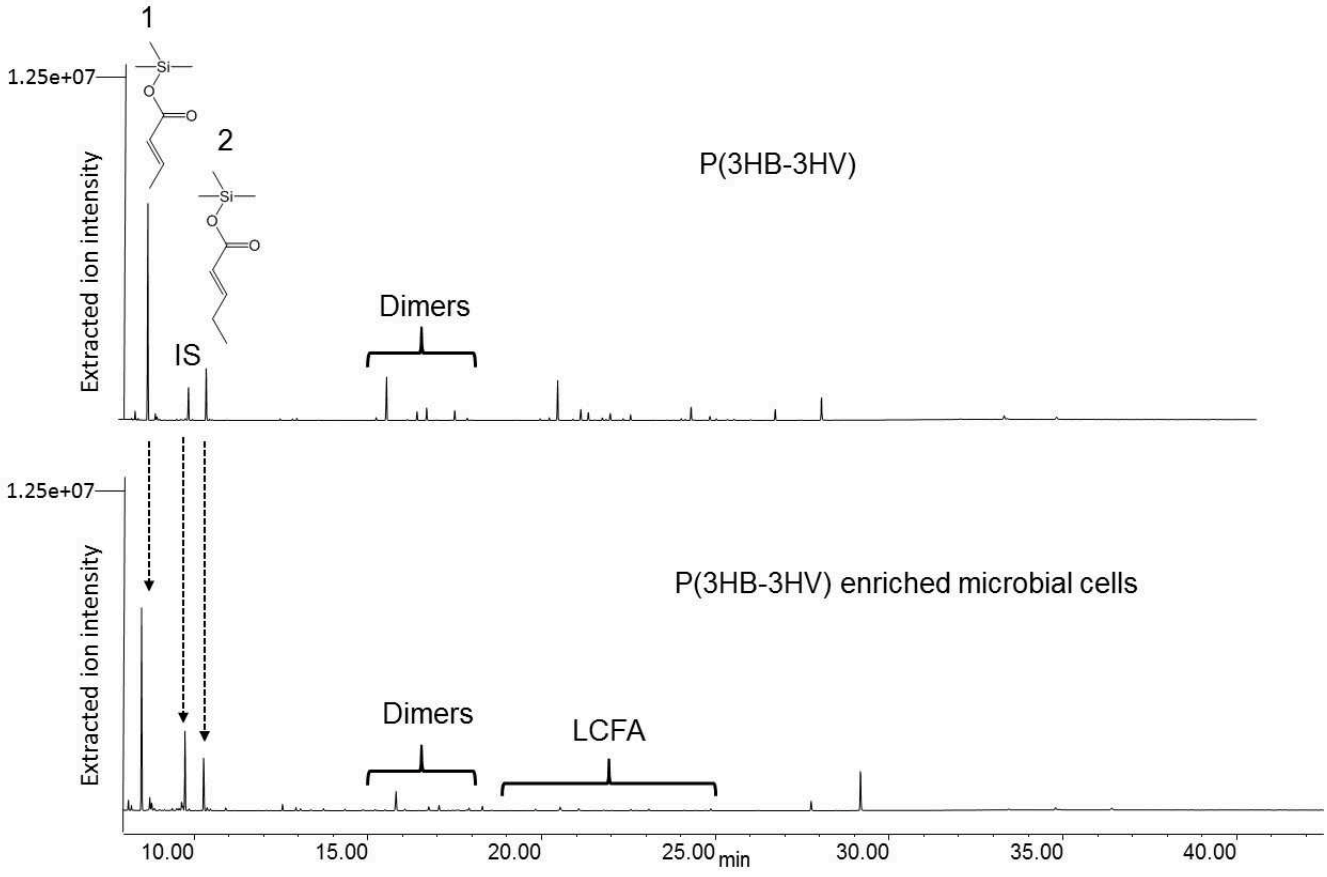
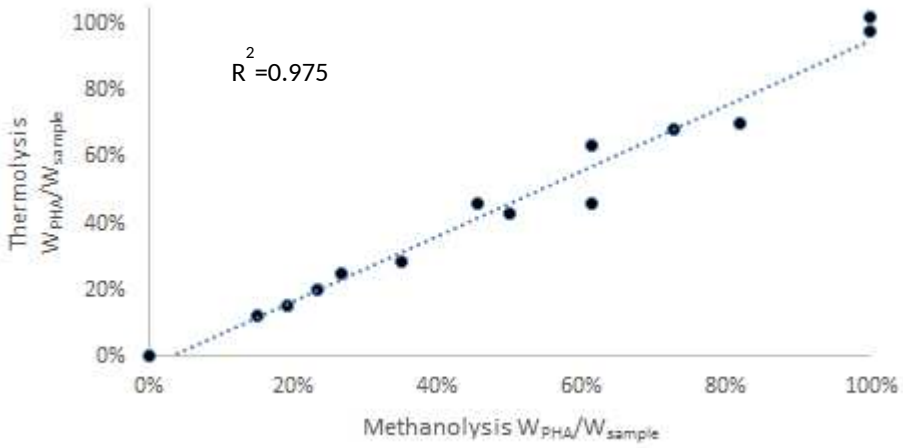

1
2
1.25e+07
Extracted ion intensity
P(3HB-3HV)
Dimers
IS
1.25e+07
Extracted ion intensity
P(3HB-3HV) enriched microbial cells
Dimers
LCFA
10.00
15.00
20.00
25.00
min
30.00
35.00
40.00

Figure 3.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80%
60%
40%
20%
0%
Thermolysis WPHA/Wsample
0%
20%
40%
60%
80%
100%
Methanolysis WPHA/Wsample

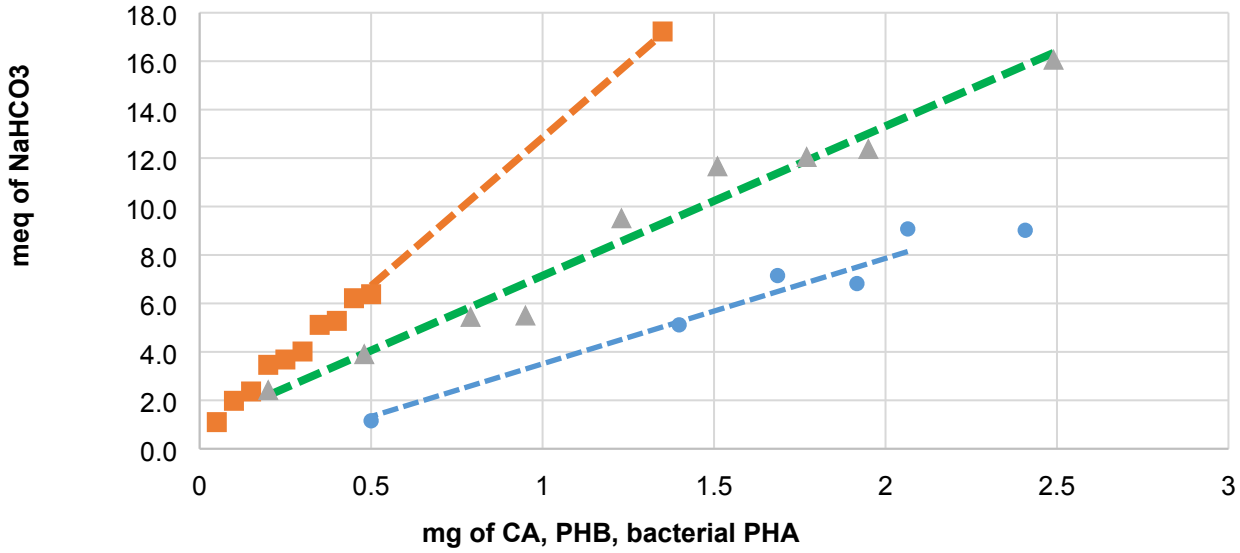
meq of NaHCO3
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
0.5
1
1.5
2
2.5
3
mg of CA, PHB, bacterial PHA

Figure 5.

Highlights

- In-Vial Thermolysis (IVT) allows GC-MS determination of PHA amount and composition.
- IVT is fast, simple, and does not require special lab equipment.
- Direct titration of IVT products allows to quantify PHA without GC instrument.