APPENDIX

I. Appendix 1 – Experimental set up of Sturm test

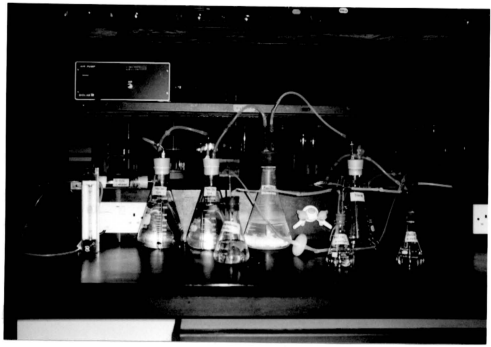

Figure A-1: A typical set up of Sturm-test for Run 2. (The apparatus was reset up after the actual experiment has completed. Only one reactor is shown. The volume of CO_2 scrubber was not the actual volume used in the experiment)

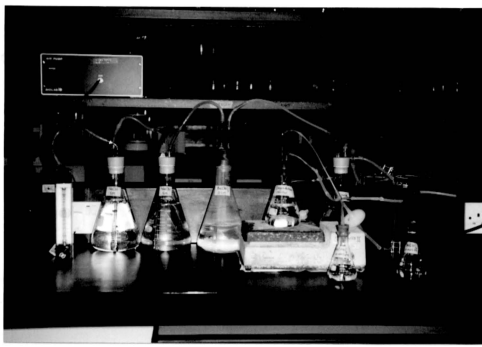

Figure A-2: A typical set up of Sturm-test for Run 3. (The apparatus was reset up after the actual experiment has completed. Only one reactor is shown. The volume of CO_2 scrubber was not the actual volume used in the experiment).

Appendix 2 – Calculation of % CO_2-biodegradability

i) $PHA + O_2 \xrightarrow{\text{microorganisms}} CO_2 + H_2O$

Theoretical CO_2 released from the 100% degradation of PHA;

$$W_{CO2} = \frac{W_{ci}}{12} \times 44 \quad ; \text{ where } W_{ci} = \text{initial weight of carbon in PHA sample}$$

(Detailed calculation to derive W_{ci} is shown in Appendix 5)

ii) Reaction in CO_2 absorber: $Ba(OH)_2 + CO_2 \longrightarrow BaCO_3 + H_2O$

Titration of CO_2 absorber: $Ba(OH)_2 + 2HCl \longrightarrow BaCl_2 + 2\,H_2O$

The remaining *m*mole of $Ba(OH)_2$ = ½ mmole of HCl

= ½ (0.05M)(V_{titre} in ml)

= 0.025 V_{titre}

mmole CO_2 reacted = mmole initial $Ba(OH)_2$ - mmole remaining $Ba(OH)_2$

= $M_i V_i$ - 0.025 V_{titre}

mg CO_2 released = 44 ($M_i V_i$ - 0.025 V_{titre})

iii) For the following calculation, please refer to Table 3.3*** (Result & discussion)

(1) CO_2 due to biodegradation = CO_2 of (Test-C1-C2+C3)

(2) CO_2 due to physical &/chemical degradation = CO_2 of (C2-C3)

(3) CO_2 due to degradation (biological, physical &/ chemical) = (1)+(2)

iv) Percentage carbon dioxide evolved due to biodegradation of PHA:

$$= \frac{mg\ \ CO_2\ \ produced\ \ due\ \ to\ \ biodegradation}{mg\ \ theoretical\ \ CO_2,\ \ (W_{\omega 2})} \times 100$$

(Note: When the mg of CO_2 produced was calculated as an average of CO_2 produced from a few reactors, then the theoretical CO_2 (mg) will be the average of the theoretical CO_2 of the PHA films tested in these reactors.)

Appendix 3 – Standard Curves for methyl ester of 3-hydroxyalkanoic acid

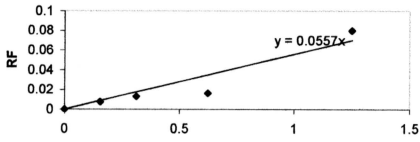

Figure A-3: Standard curve for methyl ester 3-hydroxyoctanaoic acid (C8).

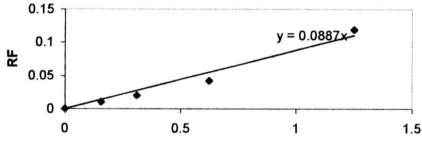

Figure A-4: Standard curve for methyl ester 3-hydroxydecanoic acid (C10).

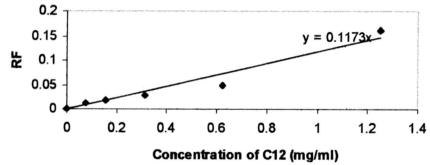

Figure A-5: Standard curve for methyl ester 3-hydroxydodecanoic acid (C12).

- Response factor (RF) = $\frac{S\tan dard \quad methyl \quad ester \quad alkanoic \quad acid}{Internal \quad S\tan dard}$

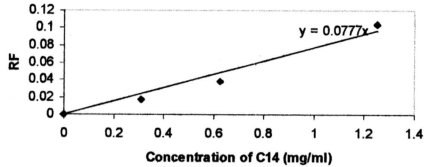

Figure A-6: Standard curve for methyl ester 3-hydroxytetradecanoic acid (C14).

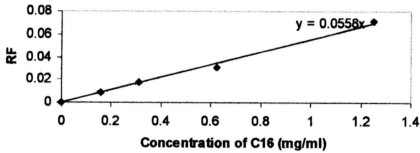

Figure A-7: Standard curve for methyl ester 3-hydroxyhexadecanoic acid (C16).

- Response factor (RF) = $\frac{S\tan dard \quad methyl \quad ester \quad alkanoic \quad acid}{Internal \quad S\tan dard}$

- Internal standard = 0.5 mg/ml methyl benzoate

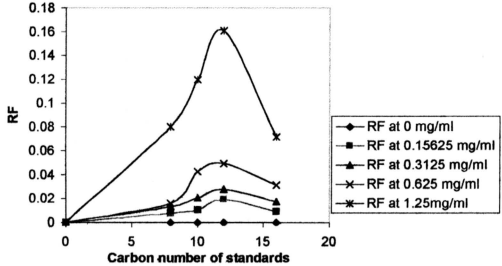

Figure A-8: Response factor (RF) of column versus carbon number at various standard concentration of methyl ester 3-hydroxyalkanoic acid.

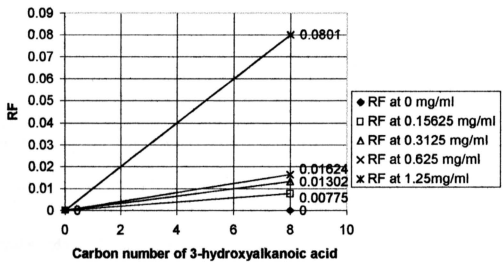

Figure A-9: RF of column versus carbon number at various standard concentration of methyl ester 3-hydroxyalkanoic acid.

Note. The graph of RF versus carbon number for every concentration shows a particular trend (Figure A-8 & A-9). Hence the RF of C4 and C6 at the specific concentrations can be extrapolated from the graph (FigureA-9) for plotting the standard curve of C4 (Figure A-10) and C6 (Figure A-11) (Theanmalar, 1996).

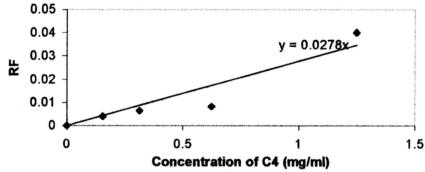

Figure A-10: RF versus concentration of methyl ester 3-hydroxybutyric acid (C4).

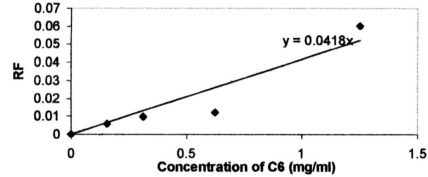

Figure A-11: RF versus concentration of methyl ester 3-hydroxyhexanoic acid (C6).

- Figure A-10 and A-11 are extrapolated from Figure A-9
- Response factor (RF) = $\frac{S\,\text{tan}\,dard \quad methyl \quad ester \quad alkanoic \quad acid}{Internal \quad S\,\text{tan}\,dard}$
- Internal standard = 0.5 mg/ml methyl benzoate

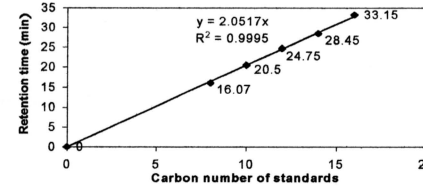

Figure A-12: Gas chromatogtaphy retention time of standards versus carbon number of methyl ester 3-hydroxyalkanoaic acid.

Note. The retention times for each β-methyl 3-hydroxyalkanoates standard were obtained from separate runs. The plot of the retention time versus carbon number of standards (Figure A-12) gives a linear regression line with R-square of 0.9995. From the equation of the plot (y=2.0517x), the retention time of C4 = 8.2min and the retention time of C6 = 12.3min

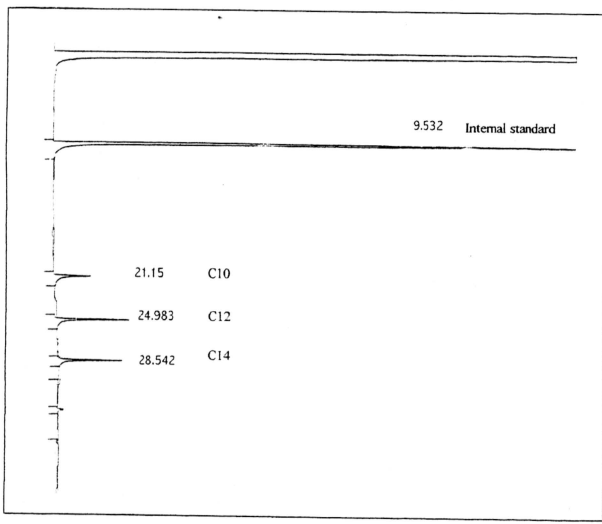

Figure A-13: Standard chromatogram of β-methyl 3-hydroxyalkanoates.

Note. Concentration of β-methyl 3-hydroxyalkanoate standards = 1mg/ml each.

Concentration of methyl benzoate (internal standard) = 0.5mg/ml.

The peaks for the C6 and C8 standards were not detected. This could be due to a lower RF values for C6 and C8 standards as compared to the rest of the standards tested (Figure A-8). A standard concentration higher than 1mg/ml may be more suitable in order to show peaks of all the standards.

Appendix 4 – Calculation of %w/w and %mole of monomer composition in PHA from GC of PHA sample

From the chromatogram of the PHA sample:

- The monomers present were identified with reference from the standard curve of Retention time versus carbon number of methyl-ester hydroxyalkanoic acids (Figure A-11 & Table A-1.).

- Response factor (RF) of the monomer was calculated:

$$RF = \frac{S\,tandard \quad methyl \quad ester \quad alkanoic \quad acid}{Internal \quad S\,tandard}$$

Internal standard = 0.5 mg/ml methyl benzoate

Subsequent calculations:

- The concentration of each monomer was obtained from the standard curve of RF versus methyl ester alkanoic acids of the same carbon number (Figure A1-Figure A6, Figure A9-Figure A10)

Table A-1: Calculation of %w/w and %mole of monomer composition in PHA

Monomer	C4	C6	C8	C10	C12	C14	C16
Retention time (min)	8.2	12.3	16.1	20.5	24.8	28.5	33.2
Concentration (mg/ml)	non	*v*	*w*	*x*	*y*	*z*	non
%w/w	0	*v*/*T*	*w*/*T*	*x*/*T*	*y*/*T*	*z*/*T*	0
Monomer molecular weight, M_w	104	132	160	188	216	244	272
*m*mole of monomer	0	*v*/132	*w*/160	*x*/188	*y*/216	*z*/244	0
mole %	0	*v*/132 T_m	*w*/160T_m	*x*/188 T_m	*y*/216 T_m	*z*/244 T_m	0

- The total PHA (mg/ml), T = sum of the monomer concentrations

$$= v + w + x + y + z$$

- The % w/w of PHA monomer = $\dfrac{Concentration\ \ of\ \ monomer}{T}$

- The monomer molecular weight can be calculated from the basic molecular formula, $C_nH_{2n}O_3$

- The *m*mole of monomer = $\dfrac{mg/ml\ \ monomer}{monomer\ \ molecular\ \ weight}$

- The total mmole of all monomer in the PHA sample, $T_m = \dfrac{v}{132} + \dfrac{w}{160} + \dfrac{x}{188} + \dfrac{y}{216} + \dfrac{z}{244}$

- The mole % of monomer = $\dfrac{mmole\ \ of\ \ monomer}{T_m}$

Appendix 5 – Calculation of PHA molecular weight & theoretical carbon dioxide released from 100% degradation of PHA

- From chromatogram of the original PHA sample, the initial %w/w of monomers were obtained as described in Appendix 4.

- The % weight of C atoms in each monomer,

$$(\%C\ w/w) = \frac{Carbon\ \ molecular\ \ weight\ \ of\ \ the\ \ monomer\ \ (C_n)}{Total\ \ molecular\ \ weight\ \ of\ \ the\ \ monomer\ \ (C_nH_{2n}O_3)}$$

- The % weight of C atoms in a PHA sample = Total % C w/w of each monomer in the PHA sample.

- The initial weight of C in the PHA sample, W_{ci} = Initial weight of PHA sample x Total %C w/w

- 100% degradation of PHA is $C_nH_{2n}O_3 + nO_2 \longrightarrow nCO_2 + nH_2O_{3/n}$

- Theoretical weight of CO_2 released from 100% PHA degradation:

$$W_{CO2} = \frac{W_{ci}}{12} \times 44$$

Table A-2: Calculation of % weight of carbon of a PHA sample

Monomer	Molecular formula	M_w			Total M_w	Run 1		Run 2		Run 3		Run 2 &3	
						PHA_1	PHA_1	PHA_2	PHA_2	PHA_r	PHA_r	PHB	PHB
	$C_nH_{2n}O_3$	C	H	O	(g/mol)	%w/w	%C w/w	%w/w	%C w/w	%w/w	%C w/w	%w/w	%C w/w
C4	$C_4H_8O_3$	48	8	48	104	0.0	0.0	0.0	0.0	0.0	0.0	87.6	40.4
C6	$C_6H_{12}O_3$	72	12	48	132	57.1	31.1	0.0	0.0	0.0	0.0	0.0	0.0
C8	$C_8H_{16}O_3$	96	16	48	160	30.8	18.5	44.2	26.5	48.1	28.9	0.0	0.0
C10	$C_{10}H_{20}O_3$	120	20	48	188	6.5	4.1	24.8	15.8	25.0	16.0	5.8	3.7
C12	$C_{12}H_4O_3$	144	24	48	216	0.9	0.6	9.6	6.4	17.4	11.6	1.4	0.9
C14	$C_{14}H_{28}O_3$	168	28	48	244	0.0	0.0	14.2	9.8	9.5	6.5	2.3	1.6
C16	$C_{16}H_{32}O_3$	192	32	48	272	4.7	3.3	7.3	5.2	0.0	0.0	3.0	2.1
	Total %C w/w						57.7		63.7		63.0		48.8

Table A-3: Calculation of theoretical CO_2 released from 100% degradation of a PHA sample film

Sample film	PHA type	PHA initial weight, W_i	Initial carbon weight, W_{ci}	Theoretical CO_2 weight, W_{CO2}
		(mg)	%C*W_i (mg)	(W_{ci}/12)*44 (mg)
P1	PHA 1	24.1	13.9	51.0
Cp2	PHA1	22.4	14.7	54.0
Sp+	PHB	36.6	21.2	77.8
T1	PHA2	20.4	14.4	52.7
T2	PHA2	17.5	12.3	45.2
T3	PHA2	17.8	12.5	45.9
T4	PHA2	15.8	11.1	40.8
T5	PHA2	19.4	13.7	50.1
C2	PHA2	21.0	14.8	54.2
S+	PHB	38.4	22.3	81.7
R1	PHAr	11.0	7.7	28.2
R2	PHAr	12.9	9.0	33.1
R3	PHAr	11.5	8.0	29.5
R4	PHAr	11.3	7.9	28.8
R5	PHAr	10.8	7.5	27.7
Cr2	PHAr	14.7	10.3	37.7
Sr+	PHB	38.6	22.4	82.1
Cr4	PHB	33.3	19.3	70.7