

Supporting information

Biosynthesis and Characterisation of Polyhydroxyalkanoates with Controlled Composition and Microstructure

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Table 1. Cell growth, PHBV production and composition for cultures grown in C2:0 to C6:0 at different carbon concentration and feeding strategy.

Exp.	Feeding strategy	Fatty acid	Carbon concentration (M)	Cultivation time (h)	$\mu_{\max}$ (h ⁻¹)	CDM (g/L)	PHBV (g/L)	$m_{\text{PHA/CDM}}$ (%)	3HV/PHA (mol %)
A1	batch	C2:0	0.10	94	0.034	2.5	0.4	16.7	19.0
A2	batch	C2:0	0.25	101	0.028	3.8	0.5	12.3	10.2
A3	batch	C2:0	0.50	149	0.025	3.4	0.4	12.9	7.3
A4	fed-batch	C2:0	0.50	144	0.035	3.7	0.4	11.4	8.2
B1	batch	C3:0	0.10	94	0.026	2.2	0.1	5.7	73.7
B2	batch	C3:0	0.25	119	0.023	3.2	0.2	6.2	72.1
B3	batch	C3:0	0.50	196	0.017	6.7	1.0	14.5	66.2
B4	fed-batch	C3:0	0.50	144	0.023	4.5	0.5	10.3	86.9
C1	batch	C4:0	0.10	149	0.013	2.6	0.4	14.8	1.5
C2	batch	C4:0	0.25	262	0.012	5.6	1.0	18.7	1.1
C3	batch	C4:0	0.50	335	0.010	5.6	1.3	23.3	0.9
C4	fed-batch	C4:0	0.50	246	0.012	5.3	1.3	24.2	0.0
D1	batch	C5:0	0.10	101	0.017	2.4	0.3	12.5	78.1
D2	batch	C5:0	0.25	189	0.012	4.7	0.8	16.0	93.1
D3	batch	C5:0	0.50	238	0.006	1.2	0.2	13.9	83.2
D4	fed-batch	C5:0	0.50	237	0.016	5.5	1.5	27.1	99.5
E1	batch	C6:0	0.10	189	0.011	2.9	0.3	10.6	0.2
E2	batch	C6:0	0.25	288	0.02	1.4	0.1	6.1	1.9
E3	batch	C6:0	0.50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
E4	fed-batch	C6:0	0.50	262	0.011	7.7	1.2	15.6	0.0

$\mu_{\max}$ maximum specific growth rate, CDM cell dry mass, $m_{\text{PHA/CDM}}$ PHA accumulation, n.d. not detected.

Table 2 Cell growth, PHBV production and composition for cultures cofed or sequentially fed with C4:0/C5:0 mixtures.

Exp.	Feeding strategy	Final C4:0/C5:0 ratio	$\mu_{\max}$ (h ⁻¹)	CDM (g/L)	PHBV (g/L)	$m_{\text{PHA/CDM}}$ (%)	3HV/PHA (mol %)	$Y_{\text{X/S}}$ (Cmol/ Cmol)	$Y_{\text{PHA/S}}$ (Cmol/ Cmol)
F1	Cofed C4:0:C5:0	29:71	0.014	5.8	1.5	25.0	71.5	0.33	0.14
F2	Cofed C4:0:C5:0	56:44	0.014	6.0	1.2	19.9	44.4	0.36	0.11
F3	Cofed C4:0:C5:0	79:21	0.015	5.5	1.2	20.7	20.6	0.33	0.11
G1	Sequential C4:0+C4:0+C5:0	65:35	0.014	5.9	1.3	21.8	25.9	0.35	0.12
G2	Sequential C5:0+C5:0+C4:0	45:55	0.015	5.1	1.0	19.4	60.5	0.31	0.10

$\mu_{\max}$ maximum specific growth rate, CDM cell dry mass, $m_{\text{PHA/CDM}}$ PHA accumulation, $Y_{\text{X/S}}$ biomass yield and $Y_{\text{PHA/S}}$ product yield.

Table 3. Molecular weight, mechanical and thermal properties of PHBV copolymers obtained from pure fatty acids (A4-E4), co-feeding or sequentially feeding C4:0/C5:0 mixtures (F1-F3 or G1, G2 respectively).

Exp.	HV (mol %)	<i>D</i> value	Molecular weight			Mechanical properties			Thermal properties			
			M_n (10 ⁶ g/mol)	M_w (10 ⁶ g/mol)	PDI	Young's modulus (MPa)	Tensile Strength (MPa)	Elongation at break (%)	T_g (°C)	T_c (°C)	T_m (°C)	T_d (°C)
A4	8.2	-	0.35	0.46	1.3	559.1	7.6	2.8	n.d.	99.2	141.0, 158.0	220.8, 261.9
B4	86.9	-	1.46	3.58	2.4	369.9	9.0	23.7	-9.9	n.d.	102.3	226.9, 351.7
C4	0.0	-	1.10	2.26	2.0	1176.4	20.8	6.3	n.d.	n.d.	164.4	231.2
D4	99.5	-	0.78	1.69	2.2	324.3	7.9	13.2	-17.8	56.0	108.0	253.6
E4	0.0	-	1.57	3.61	2.3	1558.6	22.7	2.5	n.d.	117.2	165.4	262.8
F1	71.5	1.8	1.26	3.07	2.4	336.5	9.8	6.1	-9.6	n.d.	73.7, 101.3	232.6, 373.1
F2	44.4	1.1	1.54	2.71	1.8	198.0	6.4	7.9	-5.5	n.d.	84.3, 134.3	267.4
F3	20.6	1.1	1.37	3.40	2.5	583.3	14.4	8.4	-0.5	64.7	128.4, 165.8	245.0
G1	25.9	21.0	1.49	2.82	1.9	298.5	7.9	9.5	n.d.	55.9	108.4, 174.0	265.8
G2	60.5	> 5000	1.11	3.08	2.8	782.3	14.5	7.4	n.d.	34.7	110.0, 167.8, 175.7	256.7

M_n number average molecular weight, M_w weight average molecular weight, PDI polydispersity index, T_g glass transition temperature, T_c crystallisation temperature, T_m melting temperature, T_d decomposition temperature, n.d. not detected.