

REFERENCES

- Abe, H.; Doi, Y.; Hori, Y.; and Hagiwara, T. Physical properties and enzymatic degradability of copolymers of (*R*)-3-hydroxybutyric acid and (*S,S*)-lactide. Polymer 39 (1997) : 59-67.
- Abe, H.; Matsuraba, I.; Doi, Y.; Hori, Y.; Yamaguchi, A. Physical Properties and Enzymatic Degradability of Poly(3-hydroxybutyrate) Stereoisomers with Different Stereoregularities. Macromolecules 27 (1994) : 6018-6025.
- Abe, H.; Doi, Y.; Kumagai, Y. Synthesis and Characterization of Poly[(*R,S*)-3-hydroxybutyrate-*b*-6-hydroxyhexanoate] a. Compatibilizer for a Biodegradable Blends of Poly[(*R*)-3-hydroxybutyrate and Poly(6-hydroxyhexanoate)]. Macromolecules 27 (1994) : 6012-6017.
- Anderson, A.J.; and Dawes E.A. Occurrence, Metabolism, Metabolic Role and Industrial Uses of Bacterial Polyhydroxyalkanoates Microbiological Reviews 54 (1990) : 450-472.
- Avella, M.; and Martuscelli, E. Poly-D(-)(3-hydroxybutyrate)/poly(ethylene oxide) blends: phase diagram, thermal and crystallization behavior Polymer 29 (1988) : 1731-1737.
- Avella, M.; Martuscelli, E.; and Greco, P. Crystallization behaviro of poly(ethylene oxide) from poly(3-hydroxybutyrate)/poly(ethylene oxide) blends: phase structuring, morphology and thermal behavior Polymer 32 (1991) : 1647-1653.
- Avella, M.; Martuscelli, E.; Orsello, G.; and Raimo, M. Poly(3-hydroxybutyrate)/poly(methylene oxide) blends: thermal, crystallization and mechanical behavior Polymer 38 (1997) : 6135-6143.
- Azuma, Y.; Yoshie, N.; Sakurai, M.; Inoue, Y.; and Chujo, R. Thermal behavior and miscibility of poly(3-hydroxybutyrate)/poly(vinyl alcohol) blends Polymer 33 (1992) : 4763-4767.
- Barham, P.J.; and Keller, A. The Relationship between Microstructure and Mode of Fracture in Polyhydroxybutyrate Journal of Polymer Science: Polymer Physics Edition 24 (1986) : 69-77.

- Beaucage, G. Crystallinity: Polymer Morphology[online]. 1998. Available from:
<http://www.eng.uc.edu/~gbeaucag/Classes/Morphology/Chapter2html/Chapter2.html> [2002, April 26].
- Bibers, I.; Tupureina, V.; Dzene, A.; and Kalnins, M. Improvment of The Deformative Characteristics of Poly- β -hydroxybutyrate by Plasticization. Mechanics of Composite Material 35 (1999) : 357-364.
- Bibers, I.; Tupureina, V.; Dzene, A.; Savenkava, L.; and Kalnins, M. Biodegradable Materials from Plasticized PHB Biomass Macromolecular Symposia. 170 (2001) 61-71.
- Billmeyer, F.W. Textbook of Polymer Science. New York: John Wiley & Sons, 1984.
- Bleoembergen, S.; Holden, D.A.; Hamer, G.K.; Bluhm, T.L.; and Marchessault, R.H. Studiesof Composition and Crystallinity of Bacterial Poly(β -hydroxybutyrate-co- β -hydroxyvalerate) Macromolecules 19 (1996) 2865-2871.
- Byrom, D. Polymer synthesis by microorganisms: technology and economics TIBTECH 5 (1987) : 246-250.
- Carraher, C.E. Polymer Chemistry: An Introduction. New York: Marcel Dekker, 1996.
- Ceccoruli, G.; Pizzoli, M.; and Scandola, M. Effect of a Low Molecular Weight Plasticizer on the Thermal and Viscoelastic Properties of Miscible Blends of Bacterial Poly(3-hydroxybutyrate) with Cellulose Acetate Butyrate) Macromolecules 26 (1993): 6722-6726.
- Ceccoruli, G.; Pizzoli, M.; and Scandola, M. Plasticization of Bacterial Poly(3-hydroxybutyrate) Macromolecules 25 (1992) 3304-3306.
- Cheremisinoff, N.P. Product design and testing of polymeric materials. New York: Mercel Dekker, 1990
- de Koning, G.J.M.; and Lemstra, P.J. Crystallization phenomena in bacterial poly[(R)-3-hydroxybutyrate]: 2. Embrittlement and rejuvenation Polymer 34 (1993): 4089-4094.
- de Koning, G.J.M.; Lemstra, P.J.; Hill, D.J.T.; Carswell, T.G.; and O'Donnell, J.H. Ageing phenomena in bacterial poly[(R)-3-hydroxybutyrate] 1. A study on the mobility in poly[(R)-3-hydroxybutyrate] powders by monitoring the radical decay with temperature after γ -radiolysis at 77K Polymer 33 (1992): 3295-3297.

- de Koning, G.J.M.; Scheeren, A.H.C.; Lemstra, P.J.; Peeters, M.; and Reynaers, H. Crystallization phenomena in bacterial poly[(R)-3-hydroxybutyrate]: 3. Toughening via texture changes Polymer 35 (1994): 4598-4605.
- Doi, Y. Microbial Polyesters. New York: VCH Publishers, 1990.
- Doi, Y.; Tmaki, A.; Kunioka, M.; and Soga, K. Production of copolyester of 3-hydroxybutyrate and 3-hydroxyvalerate by *Alcaligenes eutrophus* from butyric and pentanoic acid Applied Microbiology and Biotechnology 28 (1988): 330-334
- Dolarom, K. Extraction of Poly- β -hydroxybutyrate from Alcaligenes eutrophus NCIMB 11599 Using Sodium Hypochlorite Solution and Chloroform. Master's Thesis, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, 1999.
- Dubini Paglia, E.; Beltrame, P.L.; Canetti, M.; Seves, A.; Marcandalli, B.; and Martuscelli, E. Crystallization and thermal behavior of poly (D(-) 3-hydroxybutyrate)/poly (epichlorohydrin) blends Polymer 34 (1993): 996-1001.
- Eggink, E.; Smegen, J.; Ongen-baysal, G.; and Huijberts, G.N.M. Bacterial poly(hydroxyalkanoates). Food Packaging and Preservation. Ed. Mathlouthi, M. New York : Blackie Academic and Professional, 1994.
- Galego, N.; Rozsa, C.; Sanchez, R.; Fung, J.; Vazquez, A.; and Tomas, J.S. Characterization and application of poly(β -hydroxyalkanoates) family as composite biomaterials Polymer Testing 19 (2000): 485-492.
- Gassner, F. and Owen, A.J. Physical properties of poly(β -hydroxybutyrate)-poly(ϵ -caprolactone) blends Polymer 35 (1994): 2233-2236.
- George Mason University Scanning Electron Microscope—Theory[online] Available from: <http://www.gmu.edu/departments/SRIF/tutorial/semsem.htm> [2002, April 26]
- Grassino, S.B. Polymer Solutions[online]. 2000. Available from: <http://www.psrc.usm.edu/macrog/property/solpol/ps5.htm> [2002, April 26].
- Griffin, G.J.L. Chemistry and Technology of Biodegradable Polymer. New York: Blackie Academic and Professional, 1994.

- Gruenwald, G. Plastics: How Structure Determines Properties. New York: Hanser, 1993.
- Gross, R.A.; DeMello, C.; Lenz, R.W.; Brandl, H.; and Fuller, R.C. Biosynthesis and Characterization of Poly(β -hydroxyalkanoates) produced by *Pseudomonas oleovorans* Macromolecules 22 (1989): 1106-1115.
- Hadad, D.K.; Missiles, L.; and May, C.A. Physical, chemical, and thermal analysis of thermoset resins. Engineered Materials Handbook Volume 2 Engineering Plastics. pp. 517-532. Ohio: ASM International, 1988.
- Hahn, S.W.; Chang, Y.K.; Kim, B.S.; and Chang, H.N. Communication to the editor optimization of microbial poly(3-hydroxybutyrate) recovery using dispersions of sodium hypochlorite solution and chloroform. Biotechnology and Bioengineering. 44 (1994): 256-261.
- Hahn, S.W.; Chang, Y.K.; Kim, B.S.; Lee, K.M.; and Chang, H.N. The recovery of poly(3-hydroxybutyrate) by using dispersions of sodium hypochlorite solution and chloroform. Biotechnology Techniques 7 (1993): 209-212.
- Hahn, S.W.; Chang, Y.K.; and Lee S.Y. Recovery and Characterization of poly(3-hydroxybutyric acid) synthesized in *Alcaligenes eutrophus* and recombinant *Escherichia coli* Applied and Environmental Microbiology 61 (1995): 34-39.
- Haung, J.C. Mechanical properties. Engineered Materials Handbook Volume 2 Engineering Plastics. pp. 433-438. Ohio: ASM International, 1988.
- Hay, J.N.; and Sharma, L. Crystallization of Poly(3-hydroxybutyrate) /polyvinyl acetate blends Polymer 41 (2000): 5749-5757.
- Holmes, P.A. Applications of PHB – A microbially produced biodegradable thermoplastics. Physical Technology. 16 (1985) : 32-26
- Ikejima, T.; and Inoue, Y. crystallization behavior and environmental biodegradability of the blend films of poly(3-hydroxybutyric acid) with chitin and chitosan Carbohydrate Polymers 41 (2000): 351-356.
- Jogdand, S.N. Bioplastics[online]. 1999. Available from:
<http://members.rediff.com/jogns/index.html> [2002, April 26].
- Juttner, R.R.; Lafferty, R.M.; and Knackmuss, H.J. A simple method for the determination of poly- β -hydroxybutyric acid in microbial biomass European Journal of Applied Microbiology. 1 (1975): 233-237.

- Kim, B.S.; Lee, S.C.; Lee, S.Y.; Chang, H.N.; Chang, Y.K.; and Woo, S.I. Production of poly(3-hydroxybutyric acid) by fed-batch culture of *Alcaligenes eutrophus* with glucose concentration control Biotechnology and Bioengineering 43 (1994): 892-898.
- Koch, P.E. Polymer Chains[online]. 2000. Available from:
<http://eetsg27.bd.psu.edu/~pkoch/polymerconformation.html> [2002, April 26].
- Kunioka, M.; Nakamura, Y.; and Doi, Y. New bacterial copolymers produced in *Alcaligenes eutrophus* from organic acids Polymer Communications. 29 (1988): 174-176.
- Lee, J.C.; Nakajima, K.; Ikehara, T.; and Nishi, T. Miscibility in blends of poly(3-hydroxybutyrate) and poly(vinylidene chloride-co-acrylonitrile) Journal of Polymer Science: Part B: Polymer Physics 35 (1997): 2645-2652.
- Lee, S.Y. Review Bacterial polyhydroxyalkanoates Biotechnology and Bioengineering 49 (1996): 1-14.
- Lundgren, D.G.; Alper, R.; Schnaitman, C.; and Marchessault, R.H. Characterization of poly- β -hydroxybutyrate extracted from different bacteria. Journal of bacteriology 89 (1965): 245-251.
- Luzier, W.D. Materials derived from biomass/biodegradable materials Proceeding of the national academy of sciences of the United states of American 89 (1992): 839-842.
- Maekawa, M.; Pearce, R.; Marchessault, R.H.; and Manley, R.S.J. Miscibility and tensile properties of poly(β -hydroxybutyrate)-cellulose propionate blends Polymer 40 (1999): 1501-1505.
- Marand, H. Crystalline state[online]. 2001. Available from: <http://chem.vt.edu/chem-dept/marand/Lecture11.pdf> [2002, April 26].
- Mendenhall, W.; and Sincich, T. A second course in statistics: regression analysis. 5th ed., New Jersey: Prentice-Hall International, 1996.
- Mitomo, H.; Hsieh, W.C.; Nishiwaki, K.; Kasuya, K.; and Doi, Y. Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) produced by *Comamonas acidovorans* Polymer 42 (2001): 3455-3461.

Noda, I.; Marchessault, R.H.; and Terada, M. Poly(hydroxybutyrate) Polymer Data Handbook. London: Oxford University press, 1999

Orts, W.J.; Ramansky, M.; and Guillet, J.E. measurement of the crystallinity of Poly(β -hydroxybutyrate-co-hydroxyvalerate) copolymers by inverse gas chromatography. Macromolecules 25 (1992): 949-953.

Painter, P.C.; and Coleman, M.M. Fundamentals of polymer science. Lancaster: Technomic, 1984.

Paustian, T. Bacterial Plastics[online]. 1998. Available from:

[http://www.bact.wisc.edu:81/ScienceEd/stories/storyReader\\$10](http://www.bact.wisc.edu:81/ScienceEd/stories/storyReader$10) [2002, April 26].

Pearce, R.; Brown, G.R.; and Marchessault, R.H. Crystallization kinetics in blends of isotactic and atactic poly(β -hydroxybutyrate). Polymer 35 (1994): 3984-3989.

Pearce, R.; Jesudason, J.; Orts, W.; Marchessault, R.H.; and Bloembergen, S. Blends of bacterial and synthetic poly(β -hydroxybutyrate): effect of tacticity on melting behavior Polymer 33 (1992): 4647-4649.

Pizzoli, M.; Scandola, M.; and Ceccorulli, G. Crystallization and morphology of poly(3-hydroxybutyrate)/Cellulose ester blends Macromolecules 27 (1994): 4755-4761.

Ramsay, J.A.; Berger, E.; Ramsay, B.A.; and Chavarie, C. PHB recovery by hypochlorite digestion of non-PHB biomass. Biotechnology Techniques. 4 (1990): 221-226.

Papra Technology Limited Biodegradable Plastics[online]. 1999. Available from:

<http://www.rapra.net/absdocs/biodegrableplasticsalert.htm> [2002, April 26].

Rosato, D.V.; Dimattia, D.P.; and Rosato, D.V. Designing with Plastics and Composites a Handbook. New York: Van Nostrand Reinhold, 1991.

Scandola, M.; Ceccorulli, G.; and Pizzoli, M. Miscibility of bacterial poly(3-hydroxybutyrate) with cellulose ester. Macromolecules 25 (1992) 6441-6446.

Sperling, L.H. Introduction to physical polymer science 3rd Ed. New York: Wiley Interscience, 2001.

Sudesh, K.; Abe, H.; and Doi, Y. Synthesis, structure and properties of polyhydroxyalkanoates: Biological polyesters Progress in polymer science 25 (2000) 1503-1555.

University of Southern Mississippi Glass Transition[online]. 2000. Available from:

<http://www.psrc.usm.edu/macrog/tg.htm> [2002, April 26].

Wither, R.E.; and Hay, J.N. the effect of seeding on the crystallization of poly(hydroxybutyrate), and co-poly(hydroxybutyrate-co-valerate) Polymer 40 (1999): 5147-5152.

Xing, P.; Dong, L.; An, Y.; Feng, Z.; Avella, M.; and Martuscelli, E. Miscibility and crystallization of poly(β -hydroxybutyrate) and poly(*p*-vinylphenol) blends Macromolecules 30 (1997): 2726-2733.

Yoon, J.S.; Oh, S.H.; and Kim, M.N. Compatibility of poly(3-hydroxybutyrate)/poly(ethylene-co-vinyl acetate) blends Polymer 39 (1998): 2479-2487.

Yoshie, N.; Nakasato, K.; Fujiwara, M.; Kasuya, K.; Abe, H.; Doi, Y.; and Inoue, Y. Effect of low molecular weight additives on enzymatic degradation of poly(3-hydroxybutyrate) Polymer 41 (2000): 3227-3234.

Yuan, Y.; and Ruckenstein, E. Miscibility and transesterification of phenoxy with biodegradable poly(3-hydroxybutyrate) Polymer 39 (1998): 1893-1897.

Zhang, L.L.; Goh, S.H.; Lee, S.Y.; and Hee, G.R. Miscibility, melting and crystallization behavior of two bacterial polyester/poly(epichlorohydrin-co-ethylene oxide) blend systems Polymer 41 (2000): 1429-1439.

Zhang, L.L.; Deng, X.; and Haung, Z. Miscibility, thermal behavior and morphological structure of poly(3-hydroxybutyrate) and ethyl cellulose binary blends. Polymer 38 (1997a): 5379-5387.

Zhang, L.L.; Deng, X.; Zhao, S.; and Haung, Z. Biodegradable polymer blends of poly(3-hydroxybutyrate) and hydroxyethyl cellulose acetate Polymer 38 (1997b) 6001-6007.



APPENDICES

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A. Raw data on experimental results of mechanical property testing of Biomer/Modifying agent and f-PHB/modifying agent blends

Table A-1 Mechanical properties of pure Biomer

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
8	19.62	1.76	1.857
9	18.13	2.06	1.396
10	15.98	1.38	1.738
11	18.14	1.67	1.754
12	15.10	1.07	1.750
13	16.18	1.70	1.563
15	13.55	1.43	1.485
18	16.20	1.36	1.767
Average	16.61	1.55	1.664
Error bar	1.62	0.26	0.14
	16.61±1.62	1.55±0.26	1.664±0.14

Table A-2 Mechanical properties of Biomer/10%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	12.16	1.33	1.438
2	11.22	1.34	1.440
6	12.75	1.61	1.356
8	11.70	1.33	1.449
13	9.83	1.18	1.364
14	10.31	1.02	1.384
16	9.91	0.85	1.428
Average	11.12	1.24	1.408
Error bar	1.06	0.23	0.04
	11.12±1.60	1.24±0.23	1.408±0.04

Table A-3 Mechanical properties of Biomer/20%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	8.87	1.22	1.208
2	11.77	1.31	1.575
3	7.39	1.14	1.046
4	10.45	1.15	1.382
5	9.66	1.76	1.118
7	9.74	1.35	1.221
8	10.30	1.38	1.351
13	10.58	1.83	1.256
15	8.97	1.50	1.153
16	9.04	1.44	1.219
17	9.37	1.21	1.214
Average	9.65	1.39	1.249
Error bar	0.77	0.16	0.10
	9.65 ± 0.77	1.39 ± 0.16	1.249 ± 0.10

Table A-4 Mechanical properties of Biomer/30%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
3	8.57	2.52	0.890
5	8.49	2.42	0.850
7	8.54	2.14	0.847
8	8.49	2.40	0.766
9	8.67	2.75	0.912
14	8.33	2.31	0.902
17	8.11	3.05	0.842
18	6.73	2.31	0.658
20	8.20	2.92	0.818
23	8.55	2.67	0.878
Average	8.27	2.55	0.836
Error bar	0.41	0.21	0.05
	8.27±0.41	2.55±0.21	0.836±0.05

Table A-5 Mechanical properties of Biomer/40%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	2.37	6.97	0.193
6	2.38	7.99	-
7	2.20	7.08	0.280
8	2.35	5.70	0.270
10	2.38	5.17	0.232
12	2.44	6.50	0.187
14	2.33	8.35	0.165
Average	2.35	6.82	0.221
Error bar	0.07	1.06	0.04
	2.38 ± 0.07	6.82 ± 1.06	0.221 ± 0.04

Table A-6 Mechanical properties of Biomer/50%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	1.00	8.43	-
4	0.72	6.77	0.118
5	1.02	7.18	0.110
10	0.87	8.35	0.108
13	1.03	5.82	0.117
14	0.77	4.97	0.137
18	0.76	7.09	0.117
Average	2.447	6.94	0.118
Error bar	0.12	1.16	0.01
	2.447 ± 0.12	6.94 ± 1.16	0.118 ± 0.01

Table A-7 Mechanical properties of Biomer/10%PG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	12.13	1.01	1.582
3	13.28	1.20	1.673
5	16.92	1.30	1.849
6	14.34	1.49	1.564
7	14.98	1.15	1.730
8	17.68	1.87	1.664
9	13.37	1.13	1.743
11	16.41	1.58	1.643
13	16.09	1.37	1.820
14	15.89	1.26	1.804
Average	15.11	1.33	1.707
Error bar	1.28	0.18	0.07
	15.11±1.28	1.33±0.18	1.707±0.07

Table A-8 Mechanical properties of Biomeric/20%PG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
2	13.99	2.30	1.386
3	10.93	1.83	1.192
6	10.40	1.37	1.252
7	13.10	2.03	1.457
11	12.67	1.55	0.977
12	11.85	1.84	1.313
Average	12.16	1.72	1.238
Error bar	1.19	0.27	0.18
	12.16±1.19	1.72±0.27	1.238±0.18

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Table A-9 Mechanical properties of Biomer/30%PG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
3	5.71	3.39	0.739
4	6.68	4.45	0.627
6	6.77	4.11	0.762
7	6.67	6.44	0.744
9	6.09	3.87	0.679
11	6.87	3.73	0.764
12	6.78	3.53	0.693
13	6.84	5.44	0.730
16	6.78	6.21	0.737
17	6.56	4.27	0.665
Average	6.57	4.55	0.714
Error bar	0.27	0.79	0.03
	6.75 ± 0.27	4.55 ± 0.79	0.714 ± 0.03

Table A-10 Mechanical properties of Biomer/40%PG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	4.00	10.56	0.355
2	3.71	7.79	0.326
4	3.49	9.26	0.336
5	4.01	7.40	0.431
7	4.01	9.55	0.391
11	3.75	7.17	0.427
12	3.29	7.82	0.315
14	3.96	7.22	0.384
Average	3.78	8.35	0.371
Error bar	0.23	1.06	0.04
	3.78 ± 0.23	8.35 ± 1.06	0.371 ± 0.04

Table A-11 Mechanical properties of Biomer/50%PG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	2.68	2.13	0.414
2	2.39	1.51	0.388
3	2.74	2.41	-
4	3.40	2.46	0.373
5	2.35	2.78	0.387
7	2.66	1.80	0.387
8	2.70	3.84	0.370
Average	2.70	2.42	0.386
Error bar	0.32	0.70	0.01
	2.70 ± 0.32	2.40 ± 0.70	0.386 ± 0.01

Table A-12 Mechanical properties of Biomer/10%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	13.12	2.01	1.522
3	12.78	1.66	-
4	13.27	1.72	1.493
5	13.43	1.32	1.587
6	11.19	1.42	1.323
7	12.50	1.47	-
11	12.20	1.51	1.438
12	14.76	1.37	1.683
14	13.22	1.76	1.572
17	13.87	1.45	1.651
18	10.80	1.29	1.307
Average	12.83	1.54	1.508
Error bar	0.76	0.05	0.19
	12.83 ± 0.76	1.54 ± 0.05	1.508 ± 0.19

Table A-13 Mechanical properties of Biomer/20%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	7.93	2.91	0.892
2	7.08	2.14	0.830
3	8.27	3.98	0.868
5	10.64	4.49	1.072
6	10.67	2.80	1.193
7	8.99	4.97	0.878
8	10.89	3.43	1.413
9	9.75	3.66	1.085
11	7.96	3.36	0.955
14	9.87	3.06	1.066
15	10.86	3.47	1.037
16	10.45	4.62	1.119
17	9.99	2.24	1.033
19	6.85	3.69	0.790
Average	9.30	3.49	1.017
Error bar	0.83	0.49	0.10
	9.30±0.83	3.49±0.49	1.017±0.10

Table A-14 Mechanical properties of Biomer/30%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
7	8.41	5.58	1.015
8	8.86	5.99	0.913
11	10.17	4.76	1.041
14	11.50	5.56	1.064
15	10.90	5.27	1.058
17	10.75	5.07	1.077
19	10.24	5.07	1.010
20	11.13	5.98	1.075
23	9.70	4.55	1.082
Average	10.19	5.31	1.037
Error bar	0.80	0.39	0.04
	10.19 ± 0.80	5.31 ± 0.39	1.037 ± 0.04

Table A-15 Mechanical properties of Biomer/40%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
4	6.62	7.26	0.687
6	6.47	10.14	0.568
8	6.72	6.84	0.621
10	6.49	6.67	0.624
21	6.81	6.43	0.687
Average	6.62	7.47	0.638
Error bar	0.18	1.61	0.06
	6.62 ± 0.18	7.47 ± 1.61	0.638 ± 0.06

Table A-16 Mechanical properties of Biomer/50%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
2	4.87	3.35	0.523
3	4.75	3.16	0.472
4	4.53	2.65	0.422
5	4.52	2.53	0.507
6	4.07	2.58	0.434
7	4.31	2.84	0.469
8	4.83	3.09	0.516
9	4.22	2.12	0.488
10	4.07	2.03	0.427
Average	4.46	2.70	0.473
Error bar	0.24	0.35	0.03
	4.46 ± 0.24	2.70 ± 0.35	0.473 ± 0.03

Table A-17 Mechanical properties of pure f-PHB1

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	15.07	1.21	1.629
2	17.49	1.62	1.675
3	14.13	1.35	1.422
6	14.22	1.28	1.547
7	19.31	1.85	1.810
8	17.37	1.87	1.760
10	18.02	1.94	1.656
11	16.45	1.50	1.680
15	15.25	1.51	1.438
16	16.68	1.51	1.626
17	17.98	1.91	1.676
Average	16.54	1.59	1.629
Error bar	1.14	0.18	0.08
	16.54±1.14	1.59±0.18	1.629±0.08

Table A-18 Mechanical properties of f-PHB1/10%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
2	15.70	1.95	1.835
5	16.04	2.03	1.665
7	15.73	2.31	1.360
8	16.20	1.80	1.505
9	18.13	2.10	1.720
10	16.25	1.97	1.658
11	15.74	1.60	1.950
12	16.10	2.25	1.233
13	14.57	1.68	1.426
14	16.32	1.85	1.492
15	16.85	1.63	1.846
16	18.65	2.36	2.018
17	16.01	2.25	1.660
18	17.38	1.96	1.494
20	14.34	1.92	1.453
21	17.96	2.49	1.613
22	15.69	2.00	1.572
Average	16.33	2.01	1.618
Error bar	0.60	0.13	0.11
	16.33±0.60	2.01±0.13	1.618±0.11

Table A-19 Mechanical properties of f-PHB1/20%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
5	13.25	2.99	1.130
6	12.50	2.40	1.086
9	13.10	3.40	1.257
11	12.11	3.52	1.115
12	12.71	3.67	1.047
13	12.49	3.07	1.175
14	13.36	3.33	1.186
16	15.82	4.34	1.280
Average	13.17	3.34	1.160
Error bar	0.96	0.47	0.07
	13.17±0.96	3.34±0.47	1.160±0.07

Table A-20 Mechanical properties of f-PHB1/30%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	5.93	7.59	0.491
2	6.16	8.20	0.463
3	5.36	10.46	0.430
5	5.79	12.46	0.408
6	5.44	7.28	0.392
8	5.20	6.51	0.387
10	5.77	5.11	0.462
11	5.60	7.99	0.430
12	5.08	9.24	0.378
13	5.44	7.17	0.444
14	5.96	8.00	0.447
15	5.49	8.76	0.424
16	5.46	8.31	0.454
Average	5.59	8.24	0.432
Error bar	0.19	1.10	0.02
	5.59 ± 0.19	8.24 ± 1.10	0.432 ± 0.02

Table A-21 Mechanical properties of f-PHB1/40%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	3.53	10.24	0.347
2	3.40	4.73	0.299
3	4.16	9.41	0.424
4	3.70	5.87	0.408
7	3.69	5.66	0.525
8	4.07	6.46	0.331
9	3.22	6.17	0.391
10	3.15	7.46	0.428
12	4.02	11.90	0.571
13	4.12	12.21	0.549
14	3.84	11.29	0.429
Average	3.72	8.31	0.428
Error bar	0.04	1.86	0.06
	3.72±0.04	8.31±1.86	0.428±0.06

Table A-22 Mechanical properties of f-PHB1/50%PPG blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	1.44	60.02	0.172
2	1.37	52.90	-
11	1.87	54.20	0.153
13	1.62	55.52	-
18	1.41	63.04	-
19	1.31	51.30	0.144
22	1.39	74.78	0.144
Average	1.49	58.82	0.153
Error bar	0.18	7.53	0.02
	1.49 ± 0.18	58.82 ± 7.53	0.153 ± 0.02

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Table A-23 Mechanical properties of pure f-PHB2

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	21.23	3.20	1.819
2	19.32	3.00	1.644
4	19.79	3.92	1.754
5	20.22	3.09	1.639
6	20.62	3.12	1.886
8	19.23	3.84	1.394
9	20.46	2.76	1.979
10	19.69	3.16	1.734
11	18.76	3.44	1.540
Average	19.92	3.28	1.710
Error bar	0.60	0.30	0.14
	19.92±0.60	3.28±0.30	1.710±0.14

Table A-24 Mechanical properties of f-PHB2/10%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
3	17.54	3.17	1.581
5	16.42	3.99	1.423
6	15.89	2.97	1.811
7	15.85	3.55	1.928
8	17.61	3.65	1.631
9	17.23	2.17	1.721
10	17.17	3.48	1.766
11	15.13	2.74	1.821
12	16.36	3.19	1.789
16	16.55	3.47	1.681
17	15.79	2.71	1.644
18	14.68	2.26	1.470
20	16.34	2.09	1.457
Average	16.35	3.03	1.671
Error bar	0.54	0.37	0.09
	16.35±0.54	3.03±0.37	1.671±0.09

Table A-25 Mechanical properties of f-PHB2/20%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
1	14.61	6.51	1.330
2	14.43	6.64	1.382
4	15.13	5.09	1.424
9	14.36	8.97	1.317
11	15.29	8.61	1.653
13	14.84	6.01	1.504
14	14.69	6.66	1.194
15	13.62	6.09	1.234
16	15.11	6.32	1.385
Average	14.68	6.77	1.380
Error bar	0.39	0.93	0.11
	16.48±0.39	6.77±0.93	1.380±0.11

Table A-26 Mechanical properties of f-PHB2/30%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
6	13.70	8.88	1.313
7	12.92	9.23	1.158
8	14.07	7.55	1.305
9	13.03	11.59	1.338
10	14.00	8.13	1.360
11	13.51	13.34	1.288
13	13.74	8.74	1.374
14	12.59	11.96	1.175
Average	13.44	9.93	1.289
Error bar	0.45	1.74	0.07
	13.44 ± 0.45	9.93 ± 1.74	1.289 ± 0.07

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Table A-27 Mechanical properties of f-PHB2/40%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
4	8.67	7.26	1.396
6	8.94	10.14	0.877
7	9.02	6.84	1.317
10	8.44	6.67	1.304
12	8.44	6.43	1.321
14	8.89	6.04	1.143
17	8.65	6.48	1.576
20	8.58	6.94	1.298
23	8.53	6.42	1.408
Average	8.68	7.02	1.293
Error bar	0.18	1.02	0.16
	8.68±0.18	7.02±1.02	1.293±0.16

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Table A-28 Mechanical properties of f-PHB2/40%ESO blend

Sample	Maximum tensile strength (MPa)	%elongation at break (%)	Modulus of elasticity (GPa)
7	8.03	6.51	0.925
8	7.35	8.16	0.846
9	7.04	5.70	0.727
10	8.08	7.78	0.775
12	7.69	8.89	1.012
13	7.21	4.98	0.862
14	7.58	8.25	1.015
Average	7.57	7.18	0.880
Error bar	0.37	1.36	0.10
	7.57 ± 0.37	7.18 ± 1.36	0.800 ± 0.10

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B. Effect of the specimens storage day on the mechanical properties of PHB.

The effect of the specimen storage day on the mechanical properties of PHB is presented in this section. From the literatures, PHB sample were always kept to achieve the equilibrium for several weeks. The object of this section is to find the optimum samples storage day and to study the effect of the sample storage day on the mechanical properties of PHB. The results of the mechanical properties of PHB at various samples storage day are presented in Table B-1.

Table B-1 The mechanical properties of PHB at various samples storage day

Sample storage day	Maximum tensile strength (MPa)	%Elongation at break (%)	Modulus of elasticity (GPa)
3	10.9±2.05	4.37±0.95	1.098±0.20
9	15.89±1.11	1.87±0.24	1.522±0.13
10	16.61±1.28	1.55±0.26	1.664±0.14
21	18.69±2.25	1.85±0.25	1.678±0.121
30	16.41±2.21	1.25±0.15	1.658±0.127

The effect of the sample storage day on the maximum tensile strength, the %elongation at break and the modulus of elasticity is presented in Figure B.1, B.2 and B.3, respectively. It is shown that the mechanical properties of PHB are continuously changed when the sample storage day is from 3 days to 10 days. The mechanical properties of PHB reach the steady state in the sample kept for 10 days. From this result, all PHB samples in this work are kept for 10 days before the mechanical properties testing.

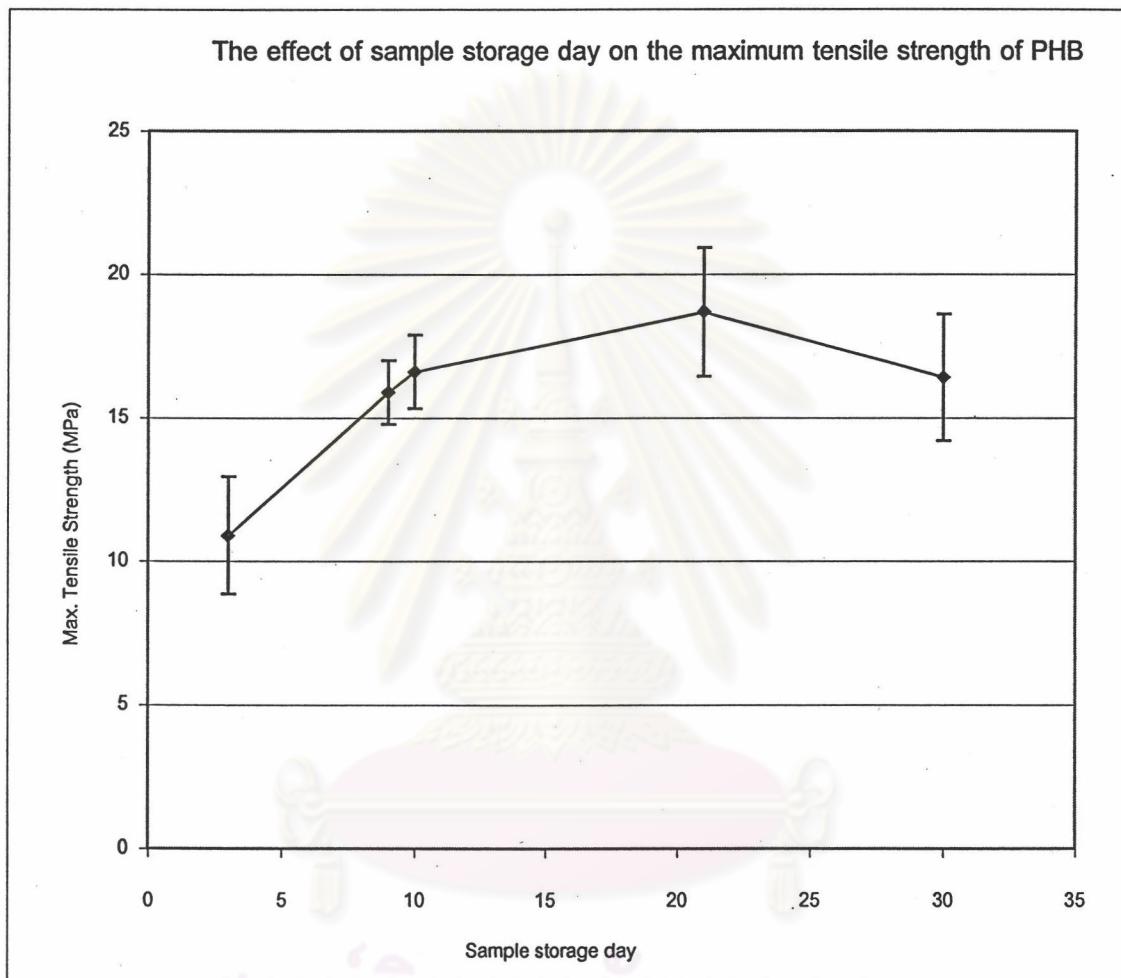


Figure B-1 the effect of the sample storage day on the maximum tensile strength of PHB

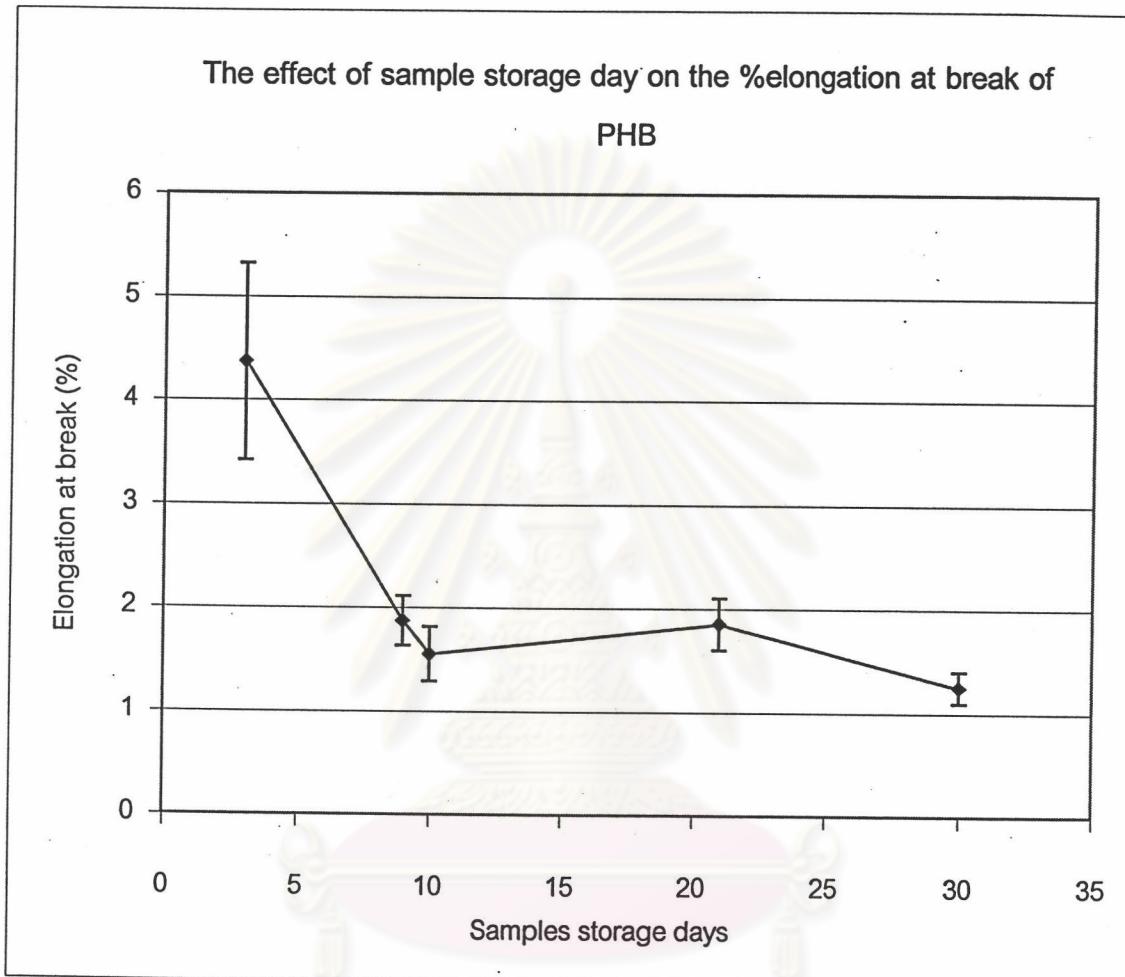


Figure B-2 the effect of the sample storage day on the %elongation at break of PHB

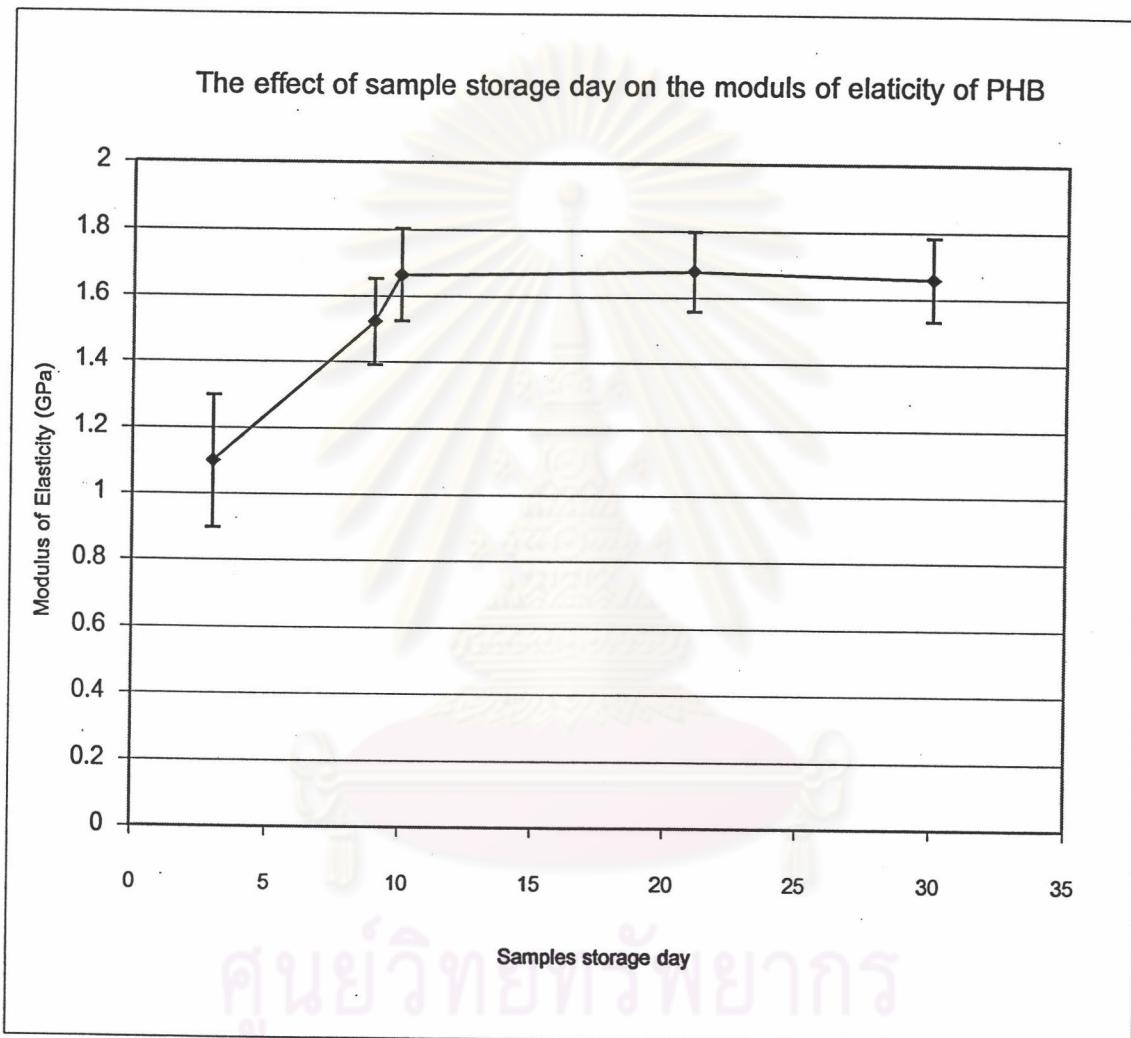


Figure B-3 the effect of the sample storage day on the modulus of elasticity of PHB

C.PHB fermentation, isolation and purification [Dolarom, 1999]

To obtain f-PHB, the bacteria fermentation, PHB isolation and purification were proceeded as follows:

Microorganism and media

The bacterial strain used in this work was *Alcaligenes eutrophus* NCIMB 11599, which was purchased from National Collections of Industrial, Food and Marine Bacteria, Scotland.

The medium used as nutrient source for bacteria can be divided into two recipes. The first one was used for normal growth which all of the nutrients was supplied adequately. The second recipe was used for unfavorable growth which nitrogen was limited.

In one liter of the first recipe, it was comprised of

Glucose	20	gram
MgSO ₄ .7H ₂ O	0.2	gram
(NH ₄) ₂ SO ₄	1	gram
KH ₂ PO ₄	1.5	gram
Na ₂ HPO ₄ .12H ₂ O	9	gram
Trace element	1	mL

In one liter of trace element, it was comprised of

FeSO ₄ .7H ₂ O	10	gram
ZnSO ₄ .7H ₂ O	2.25	gram
CuSO ₄ .5H ₂ O	1	gram
MnSO ₄ .5H ₂ O	0.5	gram
CaCl ₂ .H ₂ O	2	gram
Na ₂ B ₄ O ₇ .7H ₂ O	0.23	gram

HCl 35% by weight solution	10	mL
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In one liter of the second recipe, it was comprised of

Glucose	20	gram
MgSO ₄ .7H ₂ O	1.2	gram
(NH ₄) ₂ SO ₄	4	gram
KH ₂ PO ₄	13.3	gram
Trace element	10	mL

The seed culture were prepared in 500 mL flask on a reciprocal shaker with a stir rate of 180 rpm at 30°C for 24 hours. One hundred milliliters of seed culture was used to inoculate the "Biostat®ED" batch fermentor equipped with a "DCU-system v.2.30" controller (Biochemical Engineering Laboratory, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University) The initial volume of seed culture is 5 liters. Temperature and pH were controlled at 30°C and 6.8, respectively. The pH was controlled with one molar of Ammonium Hydroxide solution which was replaced with one molar of Sodium Hydroxide solution during the period of nitrogen limitation. Dissolved oxygen concentration was maintained by manipulating the agitation speed and the aeration rate up to 400 rpm and 1 liter of air per liter of the seed culture per minute. Glucose concentration of the seed culture was monitored every 4 hours to maintain around 10 to 20 gram per liter of the seed culture. The fermentation was continuing proceeded until the bacterial cell concentration reached the constant value around 90 grams per liter of the seed culture. Around 76 hours were taken for one batch of fermentation

PHB isolation and purification

Once PHB have been synthesized and accumulated in bacterial cells, the cell walls must be broken apart and the polymer separated from cell debris. Care must be taken to avoid depolymerization during this process. Early measurements of physical properties of PHB isolated from various bacterial strains showed wide variation of molecular weight [Griffin, 1994], which was proved to be primarily due to the harsh methods of isolation used. There are three general approaches to isolating PHB: solvent extraction, chemical digestion by sodium hypochlorite and selective enzymolysis. (the solvent extraction is only used in this work) Review of each approach is presented as follows:

The solvent extraction method usually yields PHB with high molecular weight. PHB is extracted from the cell paste by dissolving in an organic solvent such as chloroform, methylene chloride or propylene carbonate. The solution is filtered to eliminate bacterial cell debris, then PHB is precipitated by slowly cooling the solution or by adding a non-solvent such as methanol, diethyl ether or hexane.

Sodium hypochlorite affects the PHB isolation by degrading and dissolving the cell wall and non-PHB component. PHB can be further purified by washing with diethyle ether or methanol to remove lipids. However, the high alkalinity of the system can cause chain cleavage, affecting the surface properties and molecular weight of polymer chains

Enzymatic digestion was developed by Merrick and Doudoroff which various enzymes were used to solubilize the peptidoglycans and nucleic acids of the cells. The weakened cell walls were the ruptured by ultrasonic treatment to liberate the PHB granules. The isolated PHB is either submitted to an extra purification step by solvent extraction or spray dried.

As mentioned earlier, The isolation method used in this research is solvent extraction. After the fermentation was finished, The culture broth was centrifuged to isolate the bacterial cells from the seed culture. The bacterial cells was washed with purified water and centrifuged for 2 times to remove anything which may cause the contamination in PHB purification process. At this stage, the bacterial cell was called "the wetted cell". The wetted cells can be stored for further PHB isolation by keeping in the refrigerator.

The wetted cells were dried in the oven at 40°C for 5 hours. At this stage, the bacterial cells were called "the dried cell". The PHB isolation by solvent extraction method was proceeded as follows. The dried cell was broken into the small pieces and then filled in the bag which was made from the No. 5 filter paper. The bags were added into the 500 mL flask which was filled with 100 mL of chloroform. The ratio of the chloroform to the dried cell is 50mL per 5 grams of the dried cell. The flasks were then shaken by the shaker for 48 hours to improve the PHB extraction process. After the extraction was finished, The solution of PHB in chloroform was kept in the glass bottle and divided in to many batches of purification. Each batch of the PHB solution was diluted and then filtered with the GF/A filter paper to remove any impurities. The filtered solution was added with the excess of hexane to precipitated PHB. The solution was filtered again with the No. 5 filter paper and left in the fume cupboard to evaporate the solvent. After the solvent was evaporated completely, PHB was ready for further experiments.

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D. Error analysis

Most experimental data in this work are reported with the error bar 95% confidence. It should be noted that the 95% confidence interval or 0.95 confidence coefficient in fact means if it is assumed that the distribution is the normal probability distribution, 95% of data fall within this region. The value can be defined as;

$$t_{.025} \left(\frac{\sigma}{\sqrt{n}} \right) \quad (D-1)$$

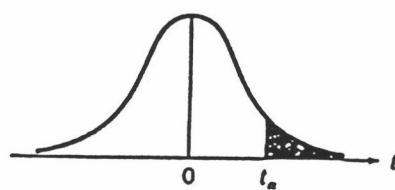
where $t_{.025}$ is the standard normal value of t0.025 at the degree of freedom of $(n-1)$, as can be seen in Figure D-1

σ is standard deviation

n is number of data

For example

From tensile test, it appears that the standard deviation of tensile modulus of pure Biomer is 0.162 and the number of specimen is eight. At the column of t.025 and the degree of freedom of 7 in Figure D-1, it gives 2.365. The value of 95% confidence therefore is 0.135.



ν	$t_{.100}$	$t_{.050}$	$t_{.025}$	$t_{.010}$	$t_{.005}$	$t_{.001}$	$t_{.0005}$
1	3.078	6.314	12.706	31.821	63.657	318.31	636.62
2	1.886	2.920	4.303	6.965	9.925	22.326	31.598
3	1.638	2.353	3.182	4.541	5.841	10.213	12.924
4	1.533	2.132	2.776	3.747	4.604	7.173	8.610
5	1.476	2.015	2.571	3.365	4.032	5.893	6.869
6	1.440	1.943	2.447	3.143	3.707	5.208	5.959
7	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	1.397	1.860	2.306	2.896	3.355	4.501	5.041
9	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	1.319	1.714	2.069	2.500	2.807	3.485	3.767
24	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	1.311	1.699	2.045	2.462	2.756	3.396	3.659
30	1.310	1.697	2.042	2.457	2.750	3.385	3.646
40	1.303	1.684	2.021	2.423	2.704	3.307	3.551
60	1.296	1.671	2.000	2.390	2.660	3.232	3.460
120	1.289	1.658	1.980	2.358	2.617	3.160	3.373
∞	1.282	1.645	1.960	2.326	2.576	3.090	3.291

Figure D-1 Critical value for student's t [Mendenhall, 1996]

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