# Effect of Temperature on the Biosynthesis of PHB by Ralstonia eutropha in Nitrogen-limiting Fermentation


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**ABSTRACT**

Abstract Polyhydroxyalkanoates, with properties similar to conventional plastics—polypropylene, are completely biodegradable plastic materials that can be biosynthesized by various microbes in the excess of carbon sources and a limitation of some nutrients. In this study, PHB (polyhydroxybutyrate) was produced by the microbe of the Ralstonia eutropha that was cultivated in a nitrogen-limited medium at various temperatures (26, 30 and 35°C). The biomass, the yield of PHB and the utilization of carbon source and nitrogen source were examined. The highest yield of PHB, 0.099 g/ L． h, was obtained when the microbes were cultivated at 35°C. The maximum average yield of PHB was 0.20 gPHB/gGlucose at 26°C. The metabolic acids were changing during cultivation. The yields of citric acid were 0.136 and 0.157 g/ L． h at 26 and 30°C, respectively. The yield of acetic acid, 0.109 g/ L． h, was the highest under a cultivation at 35°C. For a nitrogen-limited continuous fermentation at 30°C, the microbes were first cultured in a batch mode until the microbial growth reached its log-growth phase and then switched to a continuous mode. Four dilution rates (D), 0.3028, 0.2335, 0.1918 and 0.1213 h-1, were used to explore the effect of the dilution rate on the microbial growth and the yield of PHB. The results show that the yield of PHB, 0.028 g/ L． h, was highest at D = 0.1213 h-1 , and the highest yield of biomass, 0.067 g/L． h, at D = 0.2335 h-1. Key words: PHB, Ralstonia eutropha, nitrogen-limited, metabolic acid, batch/continuous fermentation, dilution rate



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**REFERENCES**

參考文獻 1.王西華、王進琦（1992）食品微生物學，藝軒圖書出版社，台北。 2.王建龍、文湘華（2001）現代環境生物技術，清華大學出版社，北京。 3.王進琦編著（2000）基礎微生物學，藝軒圖書出版社，台北。 4.行政院環保署（1999）減少廢棄塑膠袋，行政院環保署，台北， http://www.epa.gov.tw/news/en881123.htm。 5.任凌波、辛思規、任曉蕾（2002）生物化工產品生產工藝，化學工業出版社，北京。 6.李吉祥（1997）分解性塑膠的回顧與展望，清潔生產，10: 41-56。 7.何志煌（1998）生物技術的發展與應用（田蔚城彙編），眾光出版社，台北，207-212。 8.李振綱、吳誌明、蔡有癸（2001）高密度微生物細胞發酵培養，化工技術，9(2): 163-175。 9.林家慶（2002）以連續式發酵槽生產PHBV之研究，大葉大學碩士論文，彰化。 10.洪哲穎、陳國誠（1992）回應曲面實驗設計法在微生物酵素生產上之應用，化工，39(2): 3-18。 11.姜燮堂（2001）分解性塑膠，產業調查與技術，137: 28-40。 12.徐惠美（2000）生物分解性塑膠，化工資訊，14(10): 81-84。 13.徐敬衡、胡長良、劉文佐（2002）探討氧氣供給對Kineosphaera limosa生產PHB之關係，第七屆生化工程研討會論文集，台北，台灣。 14.梁永芳（1991）環保新產品:可分解塑膠，科學月刊，22: 771-778。 15.陳怡如（2001）酸類基質對Alcaligenes eutrophus生產PHBV組成影響之研究，大葉大學碩士論文，彰化。 16.梅東和、姚善涇、林東強（2000）生化生產工藝學，科學出版社，北京。 17.張庭愷（1998）利用Alcaligenes eutrophus生產poly-β-hydroxy- butyric acid之發酵與控制策略之研究，大同大學碩士論文，台北。 18.曾義雄（1993）細菌代謝，藝軒圖書出版社，台北。 19.楊正昌、陳俊男、陳聯泰（2002）《生化工程技術》在聚酯纖維產業之應用，化工資訊，16(11): 43-53。 20.董崇民、邱文英、陳信龍、劉小萍、廖茗名（2002）生物可分解性塑膠PHA的結構分析、摻合及應用，NSC-90-2621-Z-032-002-，永續發展科技與政策研討會，台北。 21.蔡宏明（1998）線上學習神經網路控制器在程序控制上之應用，大葉大學碩士論文，彰化。 22.劉英俊編著（1996）最新微生物應用工業（Applied Industrial Microbiology 4th Ed.），中央圖書出版社，台北。 23.Bailey, J. E. (1991) Towards a science of metabolic engineering, Science, 252: 1668-1675. 24.Brauegg, G., B. Sonnleitner and R. M. Lafferty (1978) A Rapid Gas Chromatoraphic Method for the Determination of Poly-β-hydroxybutyric acid in Microbial Biomass, European Journal of Applied Microbiology and Biotechnology, 6: 39-37. 25.Burdon, K. L., J. C. Stokes and C. E. Kimbrouge (1942) Studies of The Common Aerobic Spore-Forming Bacilli, I. Staining for Fat with Sudan Black B-Safranin, Journal of Bacteriology, 43: 717-724. 26.Chen, Y., J. Chen, C. Yu, G. Du and S. Lun (1999) Recovery of poly-3-hydroxybutyrate from Alcaligenes eutrophus by surfactant－chelate aqueous system, Process Biochemistry, 34: 153-157. 27.Clesceri, L. S., A. E. Greenberg and A. D. Eaton Ed. (2001) Standard Methods for the Examination of Water and Wastwater, pp. 409-421, American Public Health Association, Washington, D. C. 28.Curley, J. M., R. W. Lenz, Fuller, R. Clinton, Browne, Sheila Ewing and Gabriel, Chelvanaya B. (1997) 13C n.m.r. spectroscopy in living cells of Pseudomonas oleovorans, Polymer, 38: 5313-5319. 29.de Koning, G. J. M. and B. Witholt (1997) A process for the recovery of poly(hydroxyalkanoates) from Pseudomonads Part 1: Solubilization, Bioprocess Engineering, 17: 7-13. 30.de Koning, G. J. M., M. Kellerhals, C. van Meurs and B. Witholt (1997) A process for the recovery of poly(hydroxyalkanoates) from Pseudomonads Part 2: Process development and Engineering, Bioprocess Engineering, 17: 15-21. 31.Doi, Y., M. Kunioka, Y. Nakamura and K. Soga (1986) Nuclear magnetic resonance studies on poly(β-hydroxybutyrate) and a copolymer of β-hydroxybutyrate and β-hydroxyvalerate isolated from Alcaligenes eutrophus H16, Macromolecules, 19: 2860-2864. 32.Du, G. C., J. Yu and S. Lun (2001) Feeding strategy of propionic acid for production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with Ralstonia eutropha, Biochemical Engineering Journal, 8: 103-110. 33.Gorenflo, V., A. Steinbuchel, S. Marose, M. Rieseberg and T. Scheper (1999) Quantification of bacterial polyhydroxyalkanoic acids by Nile red staining, Applied Microbiology and Biotechnology, 51: 765-772. 34.Haywood, G. W., A. J. Anderson, L. Chu and E. A. Dawes (1988) Characterization of two 3-ketothiolases possessing differing substrate specificities in the polyhydroxy-alkanoate synthesizing organism Alcaligenes eutrophus, FEMS Microbiology Letters, 52: 91-96. 35.Houmiel, K. L., S. Slater, D. Broyles, L. Casagrande, K. Gonzalez, T. A. Mitsky and S. E. Reiser (1999) Poly(hydroxybutyrate) production in oilseed leucoplasts of Brassica napus, Planta, 209: 547-550. 36.Huisman, L. A. (1982) Isolation and identification of the reserve material of Bacillus megaterium, In: Sourcebook of experiments for the teaching of microbiology, pp. 233-241, S. B. Primrose and A. C. Wardlaw Ed., Academic Press, London. 37.James, S., R. Legge and H. Budman (2002) Comparative study of black-box and hybrid estimation methods in fed-batch fermentation, Journal of Process Control, 12: 113-121. 38.Jan, S., C. Roblot, J. Courtois, B. Courtois, J. N. Barbotin and J. P. Seguin (1996) 1H NMR spectroscopic determination of poly 3-hydroxybutyrate extracted from microbial biomass, Enzyme and Microbial Technology, 18: 195-201. 39.Kacser, H. and J. A. Burns (1973) The control of flux, Symposia of the Society for Experimental Biology, 27: 65-104. 40.Kim, Y. B. and R. W. Lenz (2001) Polyesters from Microorganisms, Advances in Biochemical Engineering/Biotechnology, 71: 51-79. 41.Lafferty, R. M., B. Korstko and W. Korsatko (1988) Microbial production of poly(β-hydroxybutyric acid), In: Biotechnology, 6: 135-176, H. J. Rehm and G. Reed Ed., Verlagsgesellschaft, Weinheim,

Germany. 42.Leaf, T. A. and F. Srienc (1998) Metabolic Modeling of Polyhydroxybutyrate Biosynthesis, Biotechnology and Bioengineering, 57: 557-570. 43.Lee, I. Y., G. Y. Choi, G. J. Kim, S. W. Nam, Y. C. Shin, H. N. Chang and Y. H. Park (1994) Optimization of fed-batch formentation for production of poly- β -hydroxy-butyrate in Alcaligenes eutrophus, World Journal of Microbiology and Biotechnology, 4: 146-156. 44.Lee, S. Y. (1996) Bacterial Polyhydroxyalkanoates, Biotechnology and Bioengineering, 49: 1-14. 45.Lemoigne, M. (1925) Etudes sur lautolyse microbie acidification par formation dacide β -oxybutyrique, Annales de L ' Institut Pasteur (Paris), Microbiology, 39: 144-146. 46.Mckee, T. and J. R. Mckee (2003) Biochemistry: The Molecular Basis of Life, 3rd Ed., McGraw-Hill Co., Inc., New York. 47.MEROPS—the Protease Database (2003) http://merops.sanger.ac.uk/speccards/peptidase/SP000854.htm. 48.Monod, J. (1949) The growth of bacterial cultures, Annual Review of Microbiology, 3: 371-394. 49.Ostle, A. and J. G. Holt (1982) Nile Blue A as a Fluorescent Stain for Poly- β -Hydroxybutyrate, Applied and Environment Microbiology, 44: 238-241. 50.Peoples, O. P. and A. J. Sinskey (1989a) Poly- β -hydroxybutyrate biosynthesis in Alcaligenes eutrophus H16: identification and characterization of PHB polymerase gene (phbC), The Journal of Biological Chemistry, 264: 15293-15297. 51.Peoples, O. P. and A. J. Sinskey (1989b) Poly- β -hydroxybutyrate biosynthesis in Alcaligenes eutrophus H16: characterization of the genes encoding β -ketothiolase and acetoacetyl-CoA reductase, The Journal of Biological Chemistry, 264: 15298-15303. 52.Poirier, Y., D. Dennis, K. Klomparens, C. Nawrath and C. Somerville (1992) Prespectives on the production of polyhydroxyalkanoates in plants, FEMS Microbiology Letters, 103: 237-246. 53.Preusting, H., R. V. Houten and A. Hoefs (1993) High Cell Density Cultivation of Pseudomonas oleovorans: Growth and Production of Poly(3-hydroxyalkanoates) in Two-Liquid Phase Batch and Fed-Batch Systems, Biotechnology and Bioengineering, 41: 550-556. 54.Riesenberg, D. and R. Guthke (1999) High-cell-density cultivation of microorganisms, Applied Microbiology and Biotechnology, 51: 422-430. 55.Riis, V. and W. Mai (1988) Gas chromatographic determination of poly- β -hydroxybutyric acid in microbial biomass after hydrochloric acid propanolysis, Journal of Chromatography, 445: 285-289. 56.Schubert, P., A. Steinbuchel and H. G. Schlegel (1988) Cloning of the Alcaligenes eutrophus Genes for Synthesis of Poly- β -Hydroxybutyric Acid (PHB) and Synthesis of PHB in Escherichia coli, Journal of Bacteriology, 170: 5837-5847. 57.Shi, H., M. Shiraishi and K. Shimizu (1997) Metabolic Flux Analysis for Biosynthesis of Poly( β -Hydroxybutyric Acid) in Alcaligenes eutrophus from Various Carbon Sources, Journal of Fermentation and Bioengineering, 84: 579-587. 58.Shimizu, H., S. Tamura, S. Shioya and K. Suga (1993) Kinetic study of poly-D-(-)-3-hydroxybutyric acid (PHB) production and its molecular distribution control in a fed-batch culture of Alcaligenes eutrophus, Journal of Fermentation and Bioengineering, 76: 465-469. 59.Slater S. C., W. H. Voige and D. E. Dennis (1988) Cloning and Expression in Escherichia coli of the Alcaligenes eutrophus H16 Poly- β -Hydroxybutyrate Biosynthetic Pathway, Journal of Bacteriology, 170: 4431-4436. 60.Slater S., K. L. Houmiel, M. Tran, T. A. Mitsky, N. B. Taylor, S. R. Padgette and K. J. Gruys (1998) Multiple β -ketothiolases mediate poly ( β -hydroxyalkanoate) copolymer synthesis in Ralstonia eutropha, Journal of Bacteriology, 180: 1979-1987. 61.Slater, S., T. A. Mitsky, K. L. Houmiel, M. Hao, S. E. Reiser, N. B. Taylor, M. Tran, S. R. Padgette, G. Kishore and K. J. Gruys (1999) Metabolic engineering of Arabidopsis and Brassica for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer production, Nature Biotechnology, 17: 1011-1016. 62.Taguchi, K., T. Tsuge, K. Matsumoto, S. Nakae, S. Taguchi and Y. Doi (2001) Investigation of metabolic pathways for biopolyester production, RIKEN Review: Focused on Ecomolecular Science Research, 42: 71-74. 63.Wong, H. H., van Weegen R. J., Choi J., Lee S. Y. and Middelberg A. P. J. (1999) Metabolic analysis of poly(3-hydroxybutrate) production by recombinant Escherichia coli, Journal of Microbiology and biotechnology, 9: 593-603. 64.van Wegen, R., J., S. Y. Lee and A. P. J. Middelberg (2001) Metabolic and Kinetic Analysis of Poly(3-Hydroxybutyrate) Production by Recombinant Escherichia coli, Biotechnology and Bioengineering, 74: 69-80.