Biosynthesis of PHBV by Burkholderia sp. Yu-4 under a Nitrogen-Limited condition

李建泓、瑞澤、余世宗

E-mail: 321377@mail.dyu.edu.tw

ABSTRACT

Burkholderia sp. Yu-4 was cultured to produce PHAs by using glucose (as the first carbon source) and organic salts (sodium propionate or sodium valerate as the second carbon source) in a nitrogen-limited medium. A one-time-one-factor method was used to explore the effects of types of nitrogen and carbon sources, organic salts and their concentrations on PHBV biosynthesis and to search for optimal conditions for batch fermentation. From the results of one-time-one-factor experiments using various carbon sources (based on the same amount of carbon in glucose) and ammonium sulfate as the nitrogen source in a flask culture, glucose was the best carbon source, and yields of biomass and PHB reached 4.53 and 1.96 g/L, respectively. When Burkholderia sp. Yu-4 was cultured in various nitrogen sources (based on the same amount of nitrogen in ammonium sulfate) and glucose (as the carbon source), ammonium sulfate was the best nitrogen source to yield the highest biomass (4.90 g/L) and PHB (1.63 g/L). If sodium valerate was added as the second carbon source, the best timing was at 6 h to add this salt. For sodium propionate, the biomass and PHBV production reached 6.02 and 2.38 g/L (HB 2.33 g/L and HV 0.05 g/L), respectively. For sodium valerate, the biomass and PHBV production reached 3.95 and 1.76 g/L (HB 1.52 g/L and HV 0.24 g/L), respectively. When the concentration of organic salt was considered as a factor, the results show that the most appropriate concentration for sodium propionate was 1 g/L, and the biomass and PHBV reached 4.95 and 2.09 g/L (HB 1.95 g/L and HV 0.14 g/L), respectively. For sodium valerate, the most appropriate concentration was 4 g/L, the biomass, and PHBV were 4.93 and 1.98 g/L (HB 1.57 g/L and HV 0.41 g/L), respectively. If no organic salt was added, the optimal PHB production reached 3.95 g/L, and the biomass was 6.47 g/L in a batch fermenter.

Keywords : Burkholderia sp. Yu-4、PHB、PHBV、sodium propionate、sodium valerate

Table of Contents

REFERENCES

1.李吉祥，1997。分解性塑膠之回顧與展望。清潔生產資訊，10:41-56。 2.吳宇，2008。中國治理白色污染與可降解塑膠。漯河職業技術學院院報。生態經濟，5:28-30。 3.張市新，謝關林，羅遠嬋，2006。洋蔥伯克氏菌在農業上應用的利弊探討。中國農業科學，39(6):1166-1172。 4.董崇民，2007。由細菌生產的「生物可分解塑膠」之開發。行政院國家科學委員會專題報告，26。 5.Ali, A. Q., Kannan, T. P. , Ahmad, A., Samsudin, A. R. 2008. In vitro genotoxicity tests for polyhydroxybutyrate-a synthetic biomaterial. Toxicology. in Vitro. 22: 57-67. 6.Alexander, S., Tina, L. E. 2003. Metabolic engineering and pathway construction for biotechnological production of relevant polyhydr-oxyalkanoates in microorganisms. Biochem. Eng. J. 16: 81-96. 7.Anderson, A. J., Dawes, E. A. 1990. Microbiol. Rev. 54: 450-472. 8.Bengtsson, S., Werker, A., Christensson, M., Welander, T. 2008. Production of polyhydroxyalkanoates by activated sludge treating a paper mill

wastewater. Bioresource Technol. 99: 509-516. 9.Bramer, C. O., Silva, L. F., Gomez, J. G. C., Priefert, H., Steinb?佟hel, A. 2002. Identification of the 2-methylcitrate pathway involved in the catabolism of propionate in the polyhydroxyalkanoate-producing strain Burkholderia sacchari IPT101(T) and analysis of a mutant accumulating a copolyester with higher 3-hydroxyvalerate content, Appl. Environ. Microbiol. 68: 271-279. 10.Braunegg, G., Lefebvre, G., Genser, K. F. 1998. Polyhydroxyalkanoates, biopolyesters from renewable resources: physiological and engineering aspects. J. Biotechnol. 44: 127-61. 11.Byrom, D. 1992. Production of poly- β -hydroxybutyrate: poly- β -hydroxyvalerate copolymers, FEMS Microbiol. Lett. 103:247-250. 12.Cavalheiro, J. M. B. T., de Almeida, M. C. M. D., Grandfils, C., da Fonseca, M.M.R. 2009. Poly(3-hydroxybutyrate) production by Cupriavidus necator using waste glycerol Process Biochem. 44: 509-515. 13.Cai M., Chua H., Zhao, Q., Sin, N. S., Ren, J. O. 2009. production of polyhydroxyalkanoates (PHA) in activated sludge fed by volatile fatty acids (VFAs) generated from alkaline excess sludge fermentation. Bioresource Technol. 100: 1399-1405. 14.Chung, C. W., Kim, Y. S., Kim, Y. B., Bae, K. S., Rhee, Y. H. 1999. Isolation of a Pseudomonas sp. strain exhibiting unusual behavior of poly(3-hydroxyalkanoates) biosynthesis and characterization of synthesized polyesters. J. Microbiol. Biotechnol. 9: 847-853. 15.Dui, G., and Yu, J. 2002. Green technology for conversion of food scraps to biodegradable thermoplastic polyhydroxyalkanoates. Environ. Sci. Technol. 36: 5511-5516. 16.Gu, G., Smith, L., Wangc, N., Wang, H., Lu S. E. 2009. Biosynthesis of an antifungal oligopeptide in Burkholderia contaminans strain MS14. Biochemical and Biophysical Research Communications. 380: 328-333. 17.Galindo, E., Pena, C., Nunez, C., Segura, D. and Espin, G. 2007. Molecular and bioengineering strategies to improve alginate and polydydroxyalkanoate production by Azotobacter vinelandii. Microb. Cell Fact. 6: 1182-1202. 18.Gupta, R. C. 1994. Carbofuran toxicity. J Toxicol Environ Health. 43: 383-418. 19.Hazer, B., Steinbuchel, A. 2007. Increased diversification of polyhydroxyalkanoates by modification reactions for industrial and medical applications. Appl. Microbiol. Biotechnol. 74: 1-12. 20.Holme, P. A., Wright, L. F., Collins, S. H. 1981. Eur. Pat. Appl. 52-459. 21.Hsieh, W. C., Wada, Y., Chang, C. P. 2009. Fermentation, biodegradation and tensile strength of poly(3-hydroxybutyrateco-4-hydroxybutyrate) synthesized by Delftia acidovorans. Journal of the Taiwan Institute of Chemical Engineers. 40: 143-147. 22.Jiang, Y., Songa, X., Gonga, L., Li, P., Dai, C., Shao, W. 2008. High poly (β -hydroxybutyrate) production by Pseudomonas fluorescens A2a5 from inexpensive substrates. Enzyme Microb. Technol. 42: 167-172. 23.Khanna, S, Srivastava, A. K. 2004. Recent advances in microbial polyhydroxyalkanoates, Process Biochem. 40: 19-607. 24.Koller, M., Bona, R., Chiellini, E., Fernandes, E.G., Horvat, P., Kutschera, C., Hesse, P., Braunegg, G. 2008. Polyhydroxyalkanoate production from whey by Pseudomonas hydrogenovora. Bioresour. Technol. 99: 4854-4863. 25.Koller, M, Bona, R., Braunegg, G., Hermann, C., Horvat, P., Kroutil, M. 2005. Production of polyhydroxyalkanoates from agricultural waste and surplus materials. Biomacromolecules. 6: 5-561. 26.Lenz, R. W., Marchessault, R. H. 2005. Bacterial polyesters: biosynthesis, biodegradable plastics and biotechnology. Biomacromolecules. 6: 1-8. 27.Li, R., Hanxing, Z., Qingsheng, Q. 2007. The production of polyhydroxyalkanoates in recombinant Escherichia coli. Bioresource Technology. 98: 2313-2320. 28.Loo, C. Y., Kumar, S. 2007. Biosynthesis and native granule characteristics of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in Delftia acidovorans. Int. J. Biol. Macromol. 40: 466-471. 29.Lu, S. E., Woolfolk S., Caceres J. 2005. Isolation and identification and genetic analysis of rhizobacteria antagonistic to plant soilborne fungal pathogens. Phytopathology. 95: 62-63. 30.Mohee, R., and Unmar G. 2007. Determining biodegradability of plastic materials under controlled and natural composting environments, Was. Man. 27: 1486-1493. 31.Nicolas, J., Chi, W. L., Yu, H. W., Wu, H. S., Wang, S. S. 2007. Isolation and purification of bacterial poly(3-hydroxyalkanoates), Biochemical Eng. J. 39: 15-27. 32.Noanto, R. V., Mantelatto P. E., Rossell C. E. V. 2001. Integrated production of biodegradable plastic, sugar and ethanol, Appl. Microbiol. Biotechnol. 57: 1-5. 33.Verlinden, R. A. J., Hill, D. J., Kenward, MA., Williams, C. D. 2007. I. Radecka. Bacterial synthesis of biodegradable polyhydroxyalkanoates. J. Appl Microbiol. 102: 49-1437. 34.Plangklang, P., Reungsang, A. 2010. Bioaugmentation of carbofuran by Burkholderia cepacia PCL3 in a bioslurry phase sequencing batch reactor, Process Biochemistry. 45: 230-238. 35.Patnaik, P. R. 2005. Perspectives in the modeling and optimization of PHB production by pure and mixed cultures. Crit. Rev. in Biotechnol. 25(3): 153-171. 36.Pijuan, M., Carles, C., Antonio, B. J. 2009. Polyhydroxyalkanoate synthesis using different carbon sources by two enhanced biological phosphorus removal microbial communities. Process Biochem. 44: 97-105. 37.Shilpi, K., Ashok, K. S. 2004. Recent advances in microbial polyhydroxyalkanoates. Process Biochemistry. 40: 607-619. 38.Steinbuchel, A., Lutke-Eversloh, T. 2003. Metabolic engineering and pathway construction for biotechnical production of relevant polyhydroxyalkanotes in microoganisms. Biochem. Eng. J. 15: 81-96. 39.Steinbuchel, A., Fuchtenbusch, B. 1998. Bacterial and other biological systems for polyester production. Trends Biotechnol. 19: 27-419. 40.Steinbuchel, A., Aerts, K., Babel, W., Follner, C., Liebergesell, M., Madkour, M.H., Mayer, F., Pieper-Furst, U., Pries, A., Valentin, H.E., et al. 1995. Considerations on the structure and biochemistry of bacterial polyhydroxyalkanoic acid inclusions. Can. J. Microbiol. 41: 94-105. 41.Steinbuchel, A., Valentin, H. E., Schunebaum, A. 1994. Application of recombinant gene technology for production of polyhydroxyalkanoic acids: biosynthesis of poly(4-hydroxybutyric acid) homopolyester. J. Environ. Polym. Degr. 2: 67-74. 42.Steinbuchel, A., Debzi, E. M., Marchessault, R. H., Timm, A. 1993. Synthesis and production of poly (3-hydroxyvaleric acid) homopolyester by Chromabacterium violaceum. Appl. Microbiol. Biotechnol. 39: 443-449. 43.Yan, Q. X., Hong, Q., Han, P., Dong, XJ., Shen, YJ., Li, S.P. 2007. Isolation and characterization of a carbofuran-degrading strain Novosphingobium sp. FND-3, FEMS Microbiol Lett. 271: 207-213. 44.Yu, J., Stahl, H. 2008. Microbial utilization and biopolyester synthesis of bagasse hydrolysates. Bioresour. Technol. 99: 8042-8048. 45.Yu, D., Yuan, Z., Jack, K., Keller, J. 2007. Production of targeted poly(3-hydroxyalkanoates) copolymers by glycogen accumulating organisms using acetate as sole carbon source. J. Biotechnology. 129: 489-497. 46.Zha, C. Y., Dong F. B., Yang, Y., Jl X. L., Mu Z. M. 2009. Optimization of Medium Components and Cultural Conditions for Producing Antibacterial Substances From Burkholderia cepacia Lu10-1. Shandong Agricultural University. 35(2): 229-234. 47.Zhang, Y., Xin, J., Chen, L., Song, H., Xia, C. 2008. Biosynthesis of poly-3-hydroxybutyrate with a high molecular weight by methanotroph

from methane and methanol. Science Direct. 17: 103-109.