Effect of Adding Organic Acid Salts on the Biosynthesis of PHBV by Ralstonia eutropha in a Nitrogen-limiting Condition

吳尚訓、?瑞澤 ; 余世宗

E-mail: 9510865@mail.dyu.edu.tw

ABSTRACT

In this study, organic acid salts were added in a nitrogen limiting medium as the second carbon source (glucose as the primary carbon source) to cultivate Ralstonia eutropha to produce PHBV (polyhydroxybutyrate-co-valerate). The method of one-factor-at-a-time was used to investigate the effects of cultivating conditions, including timing of adding organic acid salts (sodium acetate, sodium propionate, sodium butyrate, sodium valerate and sodium lactate) and their concentrations (sodium propionate, sodium valerate and sodium lactate) on the biosynthesis of PHBV. After the one-factor-at-a-time analysis was performed, results showed that the HB biosynthesis could be maximized (HB 1.34 g/L, about 53.5% of the biomass) if sodium acetate was added at the beginning of cultivation; adding sodium propionate after 24 h of cultivation, the HV biosynthesis could be maximized (0.72 g/L, about 12.6% of the biomass); when sodium butyrate was added after 24 h of cultivation, the HB biosynthesis could be maximized (1.81 g/L, about 72.5% of the biomass); added sodium valerate after 24 h of cultivation, the HV biosynthesis could be maximized (0.80 g/L, about 12.7% of the biomass); added sodium lactate after 24 h of cultivation, the HB biosynthesis could be maximized (5.66 g/L, about 59.0% of the biomass). The effect of organic acid salts concentration (between 2 and 10 g/L) at an appropriate timing of adding organic acid salts in a nitrogen limiting condition was examined. The results showed that the HV biosynthesis could be maximized (0.18 g/L) if 2 g/L sodium propionate was added; adding 8 g/L sodium valerate was the best to maximize the HV biosynthesis (0.11 g/L); the HB biosynthesis could be maximized (4.98 g/L) when 8 g/L sodium lactate was added. Based on the results obtained from the one-factor-at-a-time method, further investigation for the appropriate concentration of mixed organic acid salts was also performed with the aid of a central composite design.

Keywords : Ralstonia eutropha ; PHBV organic acid salts ; central composite design

Table of Contents

REFERENCES

中文部份 1.王亦隆。1998。由 Alcaligenes eutrophus 生產生物可分解性塑膠的能量模式，大葉大學食品工程研究所碩士論文，彰化。台灣。 2.王鼎。2001。統計學。鼎茂圖書出版有限公司。台北。 3.李愛萍、李光吉。2004。聚羥基脂肪酸酯生物合成的研究進展。高分子通報 5:20-26。 4.沈明來。2000。生物檢定統計法。九州圖書文物有限公司，台北。 5.林東恩。2002。利用活性污泥合成可生物降解塑料-聚羥基脂肪酸酯的研究。華南理工大學博士論文。中國。 6.吳國雄。2003。食品廢棄物好氧生物降解(堆肥化)。高雄第一科技大學環境與安全衛生工程系碩士論文。高雄。台灣。 7.金大勇、陳堅、倫世儀。1999。Alcaligenes eutrophus 利用不同有機酸生產聚 β -羥基烷酸的比較研究。應用與環境生物學報5(2):199-202。 8.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。 9.陳清泉。1993。最適化實驗設計在食品工業產品開發上的應用食品工業 25 (2):50-62。 10.楊世民、林讚峰。1994。簡介利用回應曲面實驗設計法決定工業微生物的最佳培養基。製酒科技專論彙編 (16):135-150。 11.謝光健、李家洲。2005。利用有機廢水生產聚羥基烷酸 (PHAs) 的進展。廣東化工 7:34-36。 12.蘇濤。1995。真氧產鹼桿菌 Alcaligenes eutrophus 以不同碳源合成可降解塑料:發酵過程及產物種類確定。工程微生物25(3):38-42。 13.蘇濤、周河治、梁靜娟。1997。微生物合成可降解塑料聚羥基鏈烷酸 (PHA)。工應微生物 3(27):37-48。 14.嚴群、堵國成、陳堅。2002。真氧產鹼桿菌利用短鏈有機酸合成聚羥基烷酸酯。過程工程學報 2(5):454-458。 15.嚴群、堵國成、陳堅。2003。真氧產鹼桿菌利用混合有機酸合成聚羥基烷酸酯。過程工程學報 54(11):1580-1585。 英文部份 1.Bruce, A., Ramsay, K. L., Claude, C., Brigette, D., Pierre, B. and Juliana A. R. 1990. Production of poly-(p-hydroxybutyric-co-3-hydroxyvaleric) acids. Applied and Environmental Microbiology. 56(7):2093-2098. 2.Byrom, D. 1987. Polymer synthesis by microorganisms: technology and economics. Trends Biotechnol. 5:246-250. 3.Chen, G. Q., Wu, Q. and Zhao, K. 2000. Functional polyhydroxyalkanoates synthesized by microorganisms. Chinese J Polym Sci., 18:389-396. 4.Choi, J. and Lee, S. Y. 1997. Process analysis and economic evaluation for poly (3-hydrobutyrate) production by fermentation. Bioprocess Eng. 17:335-342. 5.Dayong, J., Jian, C. and Shiyi, L. 1999. Production of poly(hydroxyalkanoate) by a composite anaerobic acidification-fermentation system. Process Biochemistry. 34:829-833. 6.Douglas, D. A. and James E. B. 1995. Transport of lactate and acetate through the energized cytoplasmic membrane of Escherichia coli. Biotech Bioeng, 47:8-1. 7.Haywood, G. W., Anderson, A. J. and Dawes, E. A. 1989. Accumulation of a poly(hydroxyalkanoates) copolymer containing primarily 3-hydroxyvalerate from simple carbohydrate substrates by Rhodococcus sp. NCIMB 40126. Biotechnol. Letters. 11(7):417-476. 8.Holmes, F. A., Wright, L. F. and Collins, S. H. 1981. Eur. Pat. Appl., 0052459 and 0069497. 9.Holmes, P. A. 1985. Applications of PHB-A microbially produced biodegradable thermoplastic. Phys. Technol. 16:32-36. 10.Kim, B. S., Lee, S. C., Lee, S. Y. Chang, H. N., Chang, Y. K. and Woo, S. I. 1994. Biotechnol. Bioeng. 43:892. 11.Lawford, H. G. and Rousseau, J. D. 1993. Effect of pH and acetic acid on glucose and xylose metabolism by a genetically engineered ethanologenic Escherichia coli. Appl Biochem Biotech. 39:301-32. 12.Lee, I. Y., Kim, M. K., Kim, G. J., Chang, H. N. and Park, Y. H. 1995. Production of poly(β -hydroxybutyrate-co- β - hydroxyvalerate) from glucose and valerate in Alcaligenes eutrophus. Biotechnol. Lett. 17:571-574. 13.Lee, I. Y., Kim, G. J., Shin, Y. C., Chang, H. N. and Park, Y. H. 1995. Production of poly(β -hydroxybutyrate-co- β -hydroxyvalerate) by two-stage fed-batch fermentation of Alcaligenes eutrophus. J.Microbial.Biotechnol. 5:292-296. 14.Lee, I., Kim, M. K., Choi, D. K., Yeon, B. K. and Park, Y. H. 1996. Improvement of hydroxyvalerate fraction in poly (β -Hydroxybutyrate-co- β -Hydroxyvalerate) by a mutant strain of Alcaligenes eutrophus. J. Ferment Bioeng. 81(3):255-258. 15.Longan, S., Seong, C. Y., Hyun, G. P. and Ho, N. C. 2004. Sequential feeding of glucose and valerate in a fed-batch culture of Ralstonia eutropha for production of poly(hydroxybutyrate-co-hydroxyvalerate) with high 3-Hydroxyvalerate Fraction. Biotechnol. Prog. 20:140-144. 16.Peoples, O. P. and Sinskey, A. J. 1989. J. Bio. Chem. 264(26):15293-15297. 17.Qun, Y., Du, G. and Chen, J. 2003. Biosynthesis of polyhydroxyalkanoates (PHAs) with continuous feeding of mixed organic acids as carbon sources by Ralstonia eutropha. Process Biochemistry. 39:387-391. 18.Roe, A. J., McLaggan, D. and Davidson, I. 1998. Perturbation of anion balance during inhibition of growth of Escherichia coli by weak acids. J Bacteriol. 180(4):767-77. 19.Ruan, W., Jian, C. and Shiyi, L. 2003. Production of biodegradable polymer by A. eutrophus using volatile fatty acids from acidified wastewater. Process Biochemistry. 39:295-299. 20.Rusell, J. B. 1992. Another explanation for the toxicity of fermentation acids at low pH: Anion accumulation versus uncoupling. Bioresource Tech. 54(2):191-19. 21.Salmond, C. M., Kroll, R. G. and Booth, H. I. 1984. The efect of food preservatives on pH homeostasis in Escherichia coli. J Gen Microbiol. 130:2845-2850. 22.Shang, L., Doi,

J. H., Fan, D., Ming, J. and Chang, H. N. 2003. Optimization of propionic acid feeding for production of poly(3-hydroxybutyrate-co-hydroxyvalerate) in fed-batch culture of Ralstonia eutropha. Chinese J. Chem. Eng. 11(2):220-223. 23.Shi, H. D., Shiraishi, M. and Shimizu, K. 1997. Metabolic flux analysis for biosynthesis of poly(β - hydroxybutyric acid) in Acaligenes eutrophus from various carbon sources. J. Ferment Bioeng. 84(6):579-587. 24.Shilpi, K. and Ashok, K. S. 2005. Recent advances in microbial polyhydroxyalkanoates. Process Biochemistry. 40:607-619. 25.Shilpi, K. and Ashok, K. S. 2005. Statistical media optimization studies for growth and PHB production by Ralstonia eutropha. Process Biochemistry. 40:2173-2182. 26.Sudesh, K., Abe, H. and Doi, Y. 2000. Process in Polymer Science. 25:1503-1555. 27.Takeharu, T., Kenji, T., Mitsuya, S. and Ayaaki, I. 1999. Optimization of L-Lactic Acid Feeding for the Production of poly-D-3-Hydroxybutyric Acid by Alcaligenes eutrophus in Fed-Batch Culture. Journal of Bioscience and bioenigineering. 88(4):404-409. 28.Yu, J. and Wang, J. P. 2001. Metabolic flux modeling of detoxification of acetic acid by Ralstonia eutopha at slightly alkaline pH levels. Biotechnol. Bioeng. 73:458-464. 29.Yan, Q., Du, G. and Chen, J. 2003. Biosynthesis of polyhydroxyalkanoates (PHAs) with continuous feeding of mixed organic acids as carbon sources by Ralstonia eutropha. Process Biochemistry. 39:387-391.