Biosynthesis of PHB by Bacillus megaterium in a Nitrogen-limiting Condition

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ABSTRACT

In this study, Bacillus megaterium was cultivated either in a flask or in a batch fermenter under a nitrogen-limiting condition. For a flask culture, the concentration of glucose (as the primary carbon source) was initially set at 20.0 g/L, and NH4NO3 (as the nitrogen source) was limited to be 0.6 g/L. In addition, one of the organic acid salts (sodium acetate, sodium propionate, sodium butyrate, and sodium valerate) was used as a second carbon source. Exploration of the optimal concentration was performed in order to obtain the highest yield of PHB. For the cultivation in a batch fermenter, the concentration of glucose (as the primary carbon source) was initially set at 40.0 g/L, and NH4NO3 (as the nitrogen source) was limited to be 0.6 g/L. In addition, sodium propionate or sodium valerate was used as a second carbon source to explore the effect of organic salt with odd number of carbon chains on the biosynthesis of PHB(V). Experimental results showed that the biosynthesis of HB (57.2% of the biomass) was maximized, if the concentration of sodium acetate in the medium was 2.0 g/L. Similarly, the biosynthesis of HB (27.6% of the biomass) was maximized at 0.5 g/L of sodium propionate. Adding 0.5 g/L sodium butyrate maximized the HB yield (39.18% of the biomass), and adding 1.0 g/L sodium valerate maximized the HB yield (31.0% of the biomass). For cultivation in a flask, no HV was detected even though an organic acid salt was added into the medium as a second carbon source. For cultivation in a fermenter, adding 3.0 g/L sodium propionate maximized the production of biomass and HB, being 3.96 and 0.72 g/L, respectively, at 12 h, and thereafter, both biomass and HB production decreased with the culture time. Adding sodium valerate resulted in the increase of HB production from 0.73 g/L (without adding valerate) to 1.70 g/L (adding valerate 3.0 g/L). However, adding either sodium propionate or sodium valerate as a second carbon source did not make the strain to synthesize HV.

Keywords : Bacillus megaterium ; PHB(V)

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REFERENCES

1.王建龍與文湘華。2001。現代環境生物技術。清華大學出版社。北京。 2.向明。1998。微生物發酵的放大。生物技術的應用。151-163。九州圖書。台北。 3.朱惟君。2001。化作春泥更護花神奇的「生物可分解塑膠」。行政院環境保護署資源回收月刊。 4.李吉祥。1997。分解性塑膠的回顧與展望。清潔生產資訊，10:41-56。 5.胡恆達、陳建宏。1993。分解性塑膠市場/技術現況。化工資訊，7(8):68-70。 6.洪世淇。2001。生物分解性塑膠的技術與市場展望。化工資訊，61-65。 7.姜燮堂。2001。分解性塑膠。產業調查與技術，137:28-40。 8.徐惠美。2000。生物分解性塑膠。化工資訊，14(10): 81-84。 9.徐敬衡、胡長良、劉文佐。2002。探討氧氣供給對Kineosphaeralimosa生產PHB之關係。第七屆生化工程研討會論文集。台北。台灣。 10.張庭愷。1998。利用Alcaligenes eutrophus生產poly-β-hydroxybutyric acid之發酵與控制策略之研究。大同大學碩士論文。台北。 11.梁永芳。1991。環保新產品:可分解塑膠。科學月刊，22:771-778。 12.黃建銘。2001。生物可分解性塑膠對環境的益處與未來發展趨勢。環保月刊，2 (1): 176-181。 13.楊紹榮。1993。分解性塑膠在農業栽培之評估。台南區農業專訊，3:27-30。 14.簡志青。2003。淺談微生物系生物可降解性塑膠。化工資訊與商情，5: 66-71。 15.嚴群、堵國成、陳堅。2003。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，54(11):1580-1585。 16.Bailey, J. E. 1991. Towards a science of metabolic engineering, Science. 252: 1668-1675. 17.Beun, J. J., Dircks, K., Van Loosdrecht, M.C.M., and Heijnen, J.J. 2002. Poly-β-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures. Water Res., 36:1167-1180. 18.Brandl, H., Gross, R. A., Lenz, R.W. and Fuller, R.C. 1990. Plastics from bacteria and for bacteria. Biochem. Eng., 41:77-93. 19.Carmen, K., Hans, E.B., Rene, A., Claudia, N., Thomas, F., Sven, K., Burkhard, K., and Klaus-Peter, S. 2006. In vitro and in vivo studies on blends ofisotactic and atactic poly (β-hydroxybutyrate) for development of a dura substitute material. Biomaterials, 27:192 – 201. 20.Chen, Y., Chen, J., Yu, C., Du, G. and Lun, S. 1999. Recovery of poly-β-hydroxybutyrate from Alcaligenes eutrophus by surfactantchelate aqueous system. Process Biochem., 34:153-157. 21.Chen, Y., Yang, H., Zhou, Q., Chen, J. and Gu, G. 2001. Cleaner recovery of poly(β-hydroxybutyric acid) synthesized in Alcaligenes eutropha. Process Biochem., 36: 501-506. 22.de Koning, G. J. M. and Witholt, B. 1997. A process for the recovery of poly(hydroxyalkanoates) from Pseudomonads Part 1: Solubilization. Bioprocess Engineer., 17: 7-13. 23.Du, G. C., Yu, J. and Lun, S. 2001. Feeding strategy of propionic acid for production of poly(β-hydroxybutyrate-co-β-hydroxyvalerate) with Ralstonia eutropha. Biochem. Eng. J., 8:103-110. 24.Hahn, S. Y., Chang, Y. K., Kim, B. S. and Chang, H. N. 1994. Optimization of microbial poly(β-hydroxybutyrate) recovering using disruptions of sodium hypochlorite solution and chloroform. Biotechnol. Bioeng., 44:256-261. 25.Holmes, P. A. Wright, L. F., and Collins, S. H. 1985. Betahydroxybutyrate polymers, Eur. Pat., 0052459. 26.Kacser, H. and Burns, J. A. 1973. The control of flux. Symposia of the societyfor experimental Biology., 27: 65-104. 27.Leaf, T. A. and Srienc, F. 1998. Metabolic modeling of polyhydroxybutyrate biosynthesis. Biotechnol. and Bioeng., 57: 557-570. 28.Lemoigne, M. 1925. Etudes sur lautolyse microbie acidification par formation dacide β-oxybutyrique. Annales de L’Institut Pasteur (Paris). Microbiology 39:144-146. 29.Mahishi, L. H., Tripathi, G., and Rawal, S. K. 2003. Poly(β-hydroxybutyrate) (PHB) synthesis by recombinant Escherichia coli harbouring Streptomyces aureofaciens PHB biosynthesis genes: Effect of various carbon and nitrogen sources. Microbiol. Res., 158: 19 – 27. 30.Mallick, N., Sharma, L., and Kumar, S. A. 2007. Poly-β-hydroxybutyrate accumulation in Nostoc muscorum: Effects of metabolic inhibitors. J. PlantPhysiolo., 164:312-317. 31.Ramsay, J. A., Berger, E., Voyer, R., Chavarie, C. and Ramsay, B. A. 1994. Extraction of poly-β-hydroxybutyrate using chlorinated solvents. Biotechnol. Technol., 8:589-594. 32.Schubert, P., Steinbuchel, A. and Schlegel, H. G. 1988. Cloning of the Alcaligenes eutrophus genes for synthesis of poly-β-hydroxybutyric acid (PHB) and synthesis of PHB in Escherichia coli. J. Bacteriol., 170:5837-5847. 33.Shi, H., Shiraishi, M. and Shimizu, K. 1997. Metabolic flux analysis for biosynthesis of poly(β-hydroxybutyric acid) in Alcaligenes eutrophus fromvarious carbon sources. J. Fermen Bioeng., 84: 579-587. 34.Shimizu, H., Tamura, S., Shioya, S. and Suga, K. 1993. Kinetic study of poly-D-(-)-β-hydroxybutyric acid (PHB) production and its molecular distribution control in a fed-batch culture of Alcaligenes eutrophus. J. FermentBioeng., 76:465-469. 35.Slater, S. C., Voige, W. H. and Dennis, D. E. 1988. Cloning and expressionin Escherichia coli of the Alcaligenes eutrophus H16 poly-β-hydroxybutyrate biosynthetic pathway. J. Bacteriol., 170: 4431-4436. 36.Steinbuchel, A. and Valentin, H. E. 1995. Diversity of bacterial polyhydroxyalkanoic acid, FEMS Microbiol. Lett., 128: 219-228. 37.Wang, J. G. and Bakken, L. R. 1998. Screening of soil bacteria for poly-β-hydroxybutyric acid production and it’s role in the survival of starvation.Microbial. Ecology., 35:94-101. 38.Wong, H. H., van Weegen, R. J., Choi, J., Lee, S. Y. and Middelberg, A. P. J. 1999. Metabolic analysis of poly(β-hydroxybutrate) production by recombinant Escherichia coli. J. Microbio. Biotechnol., 9: 593-603.