THE RESEARCH OF PRODUCTION PHBV BY A CONTINUOUS STIRRED TANK REACTOR

林家慶、涂瑞澤,張德明

E-mail: 9127890@mail.dyu.edu.tw

ABSTRACT

PHB (POLY-HYDROXYBUTYRATE) AND PHBV (POLY-HYDROXYBUTYRATE- CO-HYDROXY-VALERATE), HAVING PHYSICAL PROPERTIES SIMILAR TO POLY PROPYLENE,IS A KIND OF POLYESTERS PRODUCED BY MICROORG -ANISMS.THE APPLICATION OF PHBV IS QUITE EXTENSIVE BECAUSE IT CAN BE EASILY PROCESSED BY CURRENT TECHNIQUES.PHB AND PHBV CAN BE PRODUCED BY MANY STRAINS INCLUDING RALSTONIA EUTROP -HA,ALCALIGENES LATUS,AZOTOBACTER VINELANDI, METHYLOTROPHS SP.,PSEUDOMONAS SP.,AND RECOMB -INANT DNA'S ESCHERICHIA COLI. A CONTINUOUS STIRRED TANK REACTOR (CSTR) IS USED TO STUDY THE EFFECT OF LIMITING NUTRIEN -TS ON THE MICROBIAL GROWTH AND PRODUCT ACCUMULATION.THE RALSTONIA EUTROPHA WAS CULTIVATED BY USING A CONTINUOUS STIRRED TANK REACTOR WITH VARIOUS DILUTION RATES AND CONCENTRATIONS OF SODIUM PROPIONATE IN THE FEEDING SUBSTRATES IN ORDER TO EXPLORE THE MICROBIAL GROWTH,TH -E PHBV ACCUMULATION,THE CONSUMPTION OF GLUCOSE,NITROGEN AND SODIUM PROPIONATE SUBSTRATES DURING FERMENTATION.RESULTS SHOW THAT THERE IS NO HYDROXYVALERATE (HV) ACCUMULATION IF THE CONCENTRATION OF SODIUM PROPIONATE IN THE FEEDING SUBSTRATES IS LESS THAN 1 G/L.THE HIGHER THE SODIUM PROPIONATE CONCENTRATION IS,THE HIGHER THE HV MOLE FRACTION IN THE PHBV THE BIO -MASS POSSESSES.WHEN SODIUM PROPIONATE WAS NOT YET FED,ONLY PHB IS ACCUMULATED IN THE BIOM -ASS,AND THE AVERAGE PERCENTAGE OF PHB IN THE BIOMASS RANGES FROM 35 TO 41%,THE HIGHEST PE -AK OCCURRING AT THE DILUTION RATE OF 0.102 /H.IN THE CASE OF FEEDING SUBSTRATE CONTAINING 1G/L OF SODIUM PROPIONATE,ONLY PHB APPEARED IN THE PRODUCT DUE TO THE LOW CONCENTRATION OF PROPIONATE.FOR THIS CASE,THE PERCENTAGE OF PHB IN THE BIOMASS RANGES FROM 42 TO 55%, AND THE HIGHEST OCCURRED AT THE DILUTION RATE OF 0.1 /H.IN THE CASE OF FEEDING SUBSTRATES CONT -AINING 5 G/L OF SODIUM PROPIONATE, HV BEGAN TO ACCUMULATE IN THE PRODUCT, AND THE HIGHEST PERCENTAGE (ABOUT 78 WT.%) OF PHBV IN THE BIOMASS OCCURRED AT THE DILUTION RATE OF 0.093/H .THE AVERAGE MOLAR RATIO OF HB TO HV IS ABOUT 70:30.IN THE CASE OF FEEDING SODIUM PROPIONA -TE AT A CONCENTRATION OF 7 G/L,THE BIOMASS CONTAINS ABOUT 68WT.% OF PHBV AT THE DILUTION RATE OF 0.016 /H,AND THE AVERAGE MOLAR RATIO OF HB TO HV APPROXIMATELY MAINTAINED AT 60:40. WHEN THE DILUTION RATE WAS ADJUSTED TO 0.058 /H,THE BIOMASS AND PHBV REDUCED SIGNIFICANTLY. IN THE CASE OF FEEDING A SUBSTRATE CONTAINING 15 G/L OF SODIUM PROPIONATE,THE AVERAGE MOLA -R RATIO OF HB TO HV IS MAINTAINED AT 40:60,WHILE THE DILUTION RATE WAS AT 0.016 /H. WHEN TWO CARBON SOURCES COEXIST IN THE FEED STREAM OF A CONTINUOUS FLOW SYSTEM, R. EUTRO -PHA CAN PRODUCE INTRACELLUAR PHBV.THE HIGHER THE CONCENTRATION OF SODIUM PROPIONATE IN THE FEED STREAM IS,THE HIGHER THE MOLAR FRACTION OF HV IN THE PRODUCT HAS.THE DILUTION RATE CO -ULD REACH 0.15 /H TO AVOID A POSSIBLE WASHOUT,WHEN THE SODIUM PROPIONATE CONCENTRATION IS LESS THAN 5 G/L.WHEN THE DILUTION RATE IS NEAR 0.1 /H,THE PHBV PRODUCED BY R.EUTROPHA REAC -HED THE HIGHEST CONCENTRATION.THE DILUTION RATE COULD BE AS HIGH AS 0.028 /H,AND THE SYST -EM COULD STILL MAINTAIN A STEADY STATE IF THE CONCENTRATION OF FEEDING SODIUM PROPIONATE WAS 7 G/L.IF THE CONCENTRATION REACHED 15 G/L, THE STEADY STATE WAS HARD TO MAINTAIN EVEN FOR A LOW DILUTION RATE OF 0.016 /H.ALTHOUGH A HIGHER FEEDING CONCENTRATION OF SODIUM PROP -IONATE CAN PROMOTE THE ACCUMULATION OF HV,IT MAY EXHIBIT THE GROWTH OF R. EUTROPHA.CONSEQ -UENTLY, THE BIOMASS AND THE PHBV PRODUCTION BECOME LOW.

Keywords : PHBV,CONTINUOUS STIRRED TANK REACTOR,SODIUM PROPIONATE, DILUTION RATE,RALSTONIA EUTROPHA

Table of Contents

REFERENCES

1.王三郎，生物技術，高立圖書，台北 (2000)。 2.王奕隆，由ALCALIGENES EUTROPHUS 生產生物可分解塑膠的能量模式，大葉大學碩士論文，彰 化 (1998)。 3.王韻婷，高分子生物塑膠生產菌之分離，特性分析及其高分子合成基因之選殖，台灣大學農業化學研 究所碩士論文，台北 (2000)。 4.向明，微生物發酵的放大，IN"生物技術的應用" (田蔚城編彙) 151-163。九州圖書，台北 (1998)。 5.行政院環保署，廢塑膠回收再生策略報告，行政院環保署，台北(1990)。 6.何志煌，植物二次代謝物的生產，IN"生物技術的應用" (田蔚城編彙) 207-212。九州圖書，台北 (1998)。 7.吳欣達，聚-Ｐ-羥丁酸其衛生聚合物生化製程開發，大葉大學碩士論文，彰化 (1998)。 8.李吉祥，分解性塑膠之回顧與展望，清潔生產資訊，10:41-56 (1997)。 9.林碧洲，分解性塑膠之回顧與前瞻，清潔生產資訊，5:29-38 (1996)。 10.柯志強、邱政文，生物塑膠的經濟面觀及未來展望縱觀歐洲之市場需求，塑膠資訊，1(4):43-49 (1996)。 11.高銘木，高分子聚合物生物分解的探討，工業污染防治，5:138-145 (1986)。 12.陳怡如，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 (2001)。 13.陳國誠，微生物酵素工程學，藝軒圖書，台北 (1988)。 14.陳義融譯，日本開發生物分解性塑膠的國家計劃，化工資訊，6(2):84-96 (1992)。 15.張庭愷，利用 ALCALIGENES EUTROPHUS 生產POLY-Ｂ-HRDROXYBUTYRIC ACID 之發酵與控制策略之研 究，大同工學院碩士論文，台北 (1998)。 16.楊紹榮，生物分解性材料與廢棄物處理環境，塑膠工業，1 (15):74-82 (1998)。 17.蘇遠志、黃世佑，微生物化學工程學，華香園出版社，台北 (1993)。 18.ANDERSON, A. J., AND E. A. DAWES, OCCURRENCE, METABOLISM, METABOLIC ROLE, AND INDUSTRIA -L USE OF BACTERIAL POLY-HYDROXY- ALKONATES, MICROBIOL., 54:450-472 (1990). 19.BARHAM, P. J., AND A. SELWOOD, EXTRACTION OF POLY(BETA-HYDROXYBUTYRIC ACID), EUR. PAT. APPL., 58,480 (1982). 20.BERGER,E.,J.A.RAMSAY,B.A.RAMSAY,C.CHAVARIE AND G.BRAUNEGG, PHB RECOVERY BY HYPOCHLORITE DIGESTION OF NON-PHB BIOMASS, BIOTECH. TECH., 3:227-232 (1989). 21.BLOEMBERGEN, S., D. A. HOLDEN, G. K. HAMER AND T. L. BLUHUM, MARCHESSAULT RH STUDTES OF COMPOSITION AND CRYSTALLINITY OF BACTERIAL POLY (B HYDROXYBUTYRATE CO B HYDROXYVALERATE), MACROMOLECULES, 19:2865-2871 (1986). 22.BRANDL,H.,R.A.GROSS,R.W. LENZ AND R.C. FULLER, PLASTICS FROM BACTERIA AND FOR BACTERIA, BIOCHEM. ENG., 41:77-93 (1990). 23.BRAUNEGG G., B. SONNLEITNER AND R. M. LAFFERTY, EXTRACTION OF POLY (BETA-HYDROXYBUTYRIC ACID), EUR. J. APPL. MICROBIOL. BIOTECHNOL., 629 (1978). 24.COLLINS, S. H., CHOICE OF SUBSTRATE IN POLYHYDROXYBUTYRATE SYNTHESIS, SPEC. PUBL. SOC. GEN. MICROBIOL., 21:161-168 (1987). 25.DOI, Y., M. KUNIOKA, Y. NAKAMURA AND K.SOGA.,NUCLEAR MAGNETIC RESONANCE STUDIES ON POLY (B -HYDROXYBUTYRATE) AND A COPOLYMER OF B -HYDROXYBUTYRATE AND B -HYDROXYVALERATE ISOL -ATED FROM ALCALIGENES EUTROPHUS H16, MACROMOLECULES, 19:860-2864(1986). 26.GASSNER, F. AND A. J. OWEN,SOMEPROPERTIES OF POLY (3-HYDROXY -BUTYRATE)-POLY (3-HYDROXY -VALERATE) BLENDS, POLYMER INTERNATIONAL, 39:215-219 (1996). 27.GERNGROSS,T. U.,CAN BIOTECHNOLOGY MOVE US TOWARD SUSTAINABLE SOCIETY NATURE BIOTECHNOLO -GY, 17:541-544 (1999). 28.GOSTOMSKI, P. A., AND H. R. BUNGAY, EFFECT OF GLUCOSE AND NH4 LEVELS ON POLY (b-HYDROXY -BUTYRATE) PRODUCTION AND GROWTH IN A CONTINUOUS CULTURE OF ALCALIGENES EUTROPHUS,BIOTE -CHNOL. PROG., 12:234-239 (1996). 29.HENRY, E. V. AND D. DENNIS, PRODUCTION OF POLY (3-HYDROXY BUTRATE-CO-4-HYDROXYBUTRATE) IN RECOMBINANT ESCHERICHIA COLI GROWN ON GLUCOSE, J. BIOTECHNOL., 58:33-38 (1997).

30.HOLMES, P. A., APPLICATIONS OF PHB-A MICROBIALLY PRODUCED BIODEGRADABLE THERMOPLASTIC, PHYS. TECHNOL., 16:32-36 (1993). 31.HOLMES, P. A., L. F. WRIGHT, AND S. H. COLLINS, BETA-HYDROXYBUTYRATE POLYMERS, EUROPEAN PATENT, 00,52,459 (1985). 32.JANES, B., J. HOLLAR, AND D. DENNIS, MOLECULAR CHARACTERIZATION OF POLY- B -HYDROXYBUTYR -ATE BIOSYNTHETIC PATHWAY OF ALCALIGENES EUTROPHUS H16, NEW BIOSYNTHETIC BIODEGRADABLE POLYMERS OF INDUSTRIAL INTEREST FROM MICROORGANISMS, 186:175-190 (1990). 33.JUETTNER R. R. , R. M. LAFFERTY AND H. J. KNACKMUSS, BETA-HYDROXYBUTYRATE POLYMERS,EUR. J. APPL. MICROBIOL. BIOTECHNOL., 1 (1975) 233. 34.LAFFERTY,R. M., B. KORSATKO AND W. KORSATKO, MICROBIAL PRODUCTION OF POLY b-HYDROXYBUT -YRIC ACID, BIOTECHNOL., 6B:135-176 (1988). 35.LEE, E. Y. AND C. Y. CHOI, STRUCTURAL IDENTIFICATION OF POLYHYDROXY ALKANOIC ACID (PHA) CONTAINING 4-HYDROXYBUTYRIC ACIDS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) AND ITS APPLICATION TO BACTERIA SCREENING, BIOTECHNOL. TECH., 11:167-171 (1997). 36.LEE, S. Y., BACTERIAL POLYHYDROXYALKANOATES, BIOTECHNOL. BIOENG., 49: 1-14 (1996). 37.OWEN, A. J., SOME DYNAMIC MECHANICAL PROPERTIES OF MICROBIALLY PRODUCED POLY B -HYDROXY -BUTYRATE/ B -HYDROXYVALERATE COPOLY- MERS, COLLOID POLYMER SCI., 263:799-803 (1985). 38.PAGE, W. J. AND O. KNOSP, HYPERPRODUCTION OF POLY-b-HYDROXYBUTYRATE DURING EXPONENTIAL GROWTH OF AZOTOBACTER VINELANDII UWD, APPL. ENVIRON. MICROBIOL., 55:1334-1339 (1989). 39.AGE, W. J., PRODUCTION OF POLY-b-HYDROXYBUTYRATE BY AZOTOBACTER VINELANDII UWD IN MEDIA CONTAINING SUGARS AND COMPLEX NITROGEN SOURCES, APPL. MICROBIOL. BIOTECHNOL.,38:117-121 (1992). 40.PARK, C. H. AND V. K. DAMODARAN, BIOSYNTHESIS OF POLY (3-HYDROXYBUTYRATE-CO- 3-HYDROXYV -ALERATE) FROM ETHANOL AND PENTANOL BY ALCALIGENES EUTROPHUS, BIOTECHNOL. PROG.,10: 615 -620 (1994). 41.REUSTING, H., R. V. HOUTEN, A. HOEFS, E. K. V. LANGENBERGHE, O. FAVRE-BULLE AND B.WITHO -LT, HIGH CELL DENSITY CULTIVATION OF PSEUDOMONAS OLEOVORANS: GROWTH AND PRODUCTION OF POLY (3-HYDROXYALKANOATES) IN TWO-LIQUID PHASE BATCH AND FED-BATCH SYSTEMS, BIOTECHNOL. BIOENG., 41:550-556 (1993). 42.RAMSAY, B. A., G. M. ZNOJ AND DAVID G. C, FORMAL KINETICS OF POLY- B HYDROXYBUTYRIC ACID (PHB) PRODUCTION IN ALCALIGENES EUTROPHUS H16 AND MYCOPLANARUBRA R14 WITH RESPECT TO TH -E DISSOLVED OXYGEN TENSION IN AMMONIUM-LIMITED BATCH CULTURES,APPL.ENVIRON. MICROBIOL, JULY :152-156 (1986). 43.RIIS V. AND W. MAI, GAS CHROMATOGRAPHIC DETERMINATION OF POLY- B -HYDROXYBUTYRIC ACID IN MICROBIAL BIOMASS AFTER HYDROCHLORIC ACID PROPANOLYSIS, ELSEVIER SCIENCE PUBLISHERS B.V., 445: 285-289 (1988). 44.WALKER, J., J. R. WHITTON AND B. ALDERSON, EXTRACTION OF POLY(BETA-HYDROXY- BUTYRIC ACI -D), EUR. PAT. APPL. , 46,017 (1982). 45.WANG, D. I. C., FERMENTATION AND ENZYME TECHNOLOGY. WILEY, NEW YORK, 1979. 46.WILLIAMS, J. P., PRODUCTION OF POLY- B -HYDROXYBUTYRATE BY AZOTOBACTER VINDLANDII STRAIN UWD DURING GROWTH ON MOLASSES AND OTHER COMPLEX CARBON SOURCES,APPL.MICROBIOL.BIOTECHNO -L., 31:329-333 (1989). 47.WILLIAM,J. P.,J.MANCHAK AND B. RUDY.,FORMATION OF POLY (HYDROXYBUTYRATE CO HYDROXYVALER -ATE) BY AZOTOBACTER VINELANDII UWD,APPL.AND ENVIRON.MICROBIOL.,SEPT.:2866-2873 (1992). 48.YIM, K.S., S.Y. LEE AND H.N. CHANG, SYNTHESIS OF POLY-(3-HYDROXY- BUTYRATE-CO-3-HYDROXY -BUTYRATE) BY RECOMBINANT ESCHERICHIA COLI, BIOTECHNOL. AND BIOENG., 49:495-503 (1996).