以Candida subtropicalis進行二階段醱酵產製木糖醇之研究

曾昭維、張德明

E-mail: 9417956@mail.dyu.edu.tw

摘 要

中文摘要 近年來食品的機能性已經廣為大眾所重視，包括均衡的營養需求，以及其他對人體有正面影響的功能。而木糖醇具有類似薄荷的清涼口感、低熱量、與蔗糖相等的甜度、以及不需胰島素代謝及可被人體消化吸收等特性，因此在新－代食品之要求中逐漸得到重視。而農林廢料中含有豐富纖維素、半纖維素，及木質素。將半纖維素以酸水解即可得豐富之木糖、葡萄糖，及其他少量糖類（半乳糖、甘露糖、阿拉伯糖）。近年來許多研究皆利用半纖維素水解液作為醱酵基質生產木糖醇，不僅可以降低成本更可以達到環保減費之目的。 本研究主要探討利用水解液中兩種主要糖類（木糖與葡萄糖）為碳源時，由於菌體雙期生長特性與其所產生的兩階段醱酵過程，藉由在不同階段裡控制不同溶氧量，以期有效提升木糖醇的產量及產率。研究結果顯示在雙碳源的環境下有利於提升木糖醇的產量及產率。以單一碳源溶氧量對菌體生長之影響，在葡萄糖醱酵時溶氧量範圍為5 ~ 10%菌體的生長情況最佳（μ = 0.356 h-1）；而在木糖醱酵產製木糖醇時，固定通氣量0.25 vvm、轉速為130 rpm時有較佳的產量（0.649 g g-1）及產率（0.263 g L-1 hr-1）。綜合上述之結果，串聯饋料批次及批次的兩個階段醱酵之操作，實驗證實以此方式之操作方式確實能有效地提升木糖醇的產率（0.246 g L-1 hr-1）。 關鍵字：兩階段醱酵、木糖醇、溶氧、半纖維素水解液、雙期生長。

關鍵詞 : 木糖醇 ; 溶氧量 ; 雙期生長 ; 半纖維素水解液 ; 兩階段醱酵

目錄

目 錄 封面內頁 中文摘要……i 英文摘要……ii 目錄……iii 圖目錄……viii 表目錄……x 第一章 研究目的……1 第二章 文獻回顧……3 2.1 木糖醇的介紹……3 2.1.1 木糖醇的來源……3 2.1.1.1 農業副產品簡介……3 2.1.1.2 半纖維素……6 2.1.1.3 木糖製備生產的過程……9 2.1.1.3.1 水解液之製備……9 2.1.1.3.2 水解液之分離純化……11 2.1.2 木糖醇的性質……12 2.1.3 木糖醇於人體內的代謝……15 2.1.4 木糖醇之應用……15 2.1.4.1 預防齲齒……18 2.1.4.2 食品添加劑……19 2.1.4.3 糖尿病患者之糖類補充劑……19 2.1.4.4 其他應用……20 2.2 生產木糖醇方法……20 2.2.1 傳統化學合成法……20 2.2.2 微生物醱酵法……22 2.2.2.1 生產木糖醇之酵母菌種類……22 2.2.2.2 酵母菌代謝木糖之途徑……24 2.2.2.3 固定化生產木糖醇……26 2.3 酵母菌的雙期生長……27 2.4影響酵母菌轉化木糖醇的因子……28 2.4.1 碳源……28 2.4.1.1 單一碳源……28 2.4.1.2 多碳源……29 2.4.1.3 天然半纖維素水解液……30 2.4.2 氮源……31 2.4.3 pH值……32 2.4.4 溫度……33 2.4.5 鹽類……34 2.4.6 溶氧量……34 2.4.7 菌體量……35 2.4.8 副產物乙醇……36 第三章 材料與方法……38 3.1 菌株……38 3.2 實驗藥品……38 3.3 實驗儀器設備……38 3.4 菌株培養……39 3.4.1 培養基……39 3.4.2 菌種保存……41 3.4.3 菌種活化……41 3.4.4 前培養……41 3.5 醱酵槽培養……42 3.5.1 批次醱酵培養……42 3.5.1.1 碳源的組成對於產製木糖醇的影響……43 3.5.1.2 溶氧量對於菌體生長之影響……43 3.5.1.3 通氣量對於產製木糖醇之影響……44 3.5.2 兩階段醱酵培養……44 3.5.2.1 兩階段醱酵……45 3.6 分析方法……45 3.6.1 生質量之菌重測定……45 3.6.1.1 菌體離心秤乾重測定……46 3.6.1.2 菌體分光光度計測定……46 3.6.2 糖類、木糖醇、乙醇之分析……

…47 3.6.3 酚硫酸法……....47 3.6.4 實驗數據整理……48
第四章 實驗結果與討論……50 4.1 搖瓶結果……50
4.2 醱酵槽批次實驗……53 4.2.1 消泡劑對菌體生長之影響……53 4.2.2 雙碳源
基質對產製木糖醇的影響……53 4.3 兩階段醱酵條件探討……56 4.3.1 兩階段醱酵
操作設計……56 4.3.2 菌體代謝葡萄糖時溶氧量的影響……59 4.3.2.1 葡萄糖的
消耗速率……59 4.3.2.2 菌體量……62 4.3.2.3 產物乙醇……
……62 4.3.3 木糖產製木糖醇之條件探討……64 4.3.3.1 轉速對於產製木糖醇的影響…
……64 4.3.3.2 不同通氣量對木糖醇產量及產率的影響…64 4.3.4 兩階段醱酵產製木糖醇……
…69 4.3.5高菌體濃度醱酵（第一階段葡萄糖代謝）……72 第五章 結論與展望……
……74 5.1 總結……74 5.2 未來展望……
……76 參考文獻……78 圖 目 錄 圖2-1 以化學催化法生產木糖醇…
……5 圖2-2 半纖維素之結構之一…7 圖2-3 木糖醇於人體內代謝之途徑…
……17 圖2-4 傳統化學合成法製造木糖、木糖醇的流程圖…21 圖2-5 酵母菌代謝木糖之途徑
…25 圖4-1 單一碳源之搖瓶培養，菌體、木糖醇、乙醇對木糖之時間 曲線……
……51 圖4-2 雙碳源之搖瓶培養，菌體、木糖醇、乙醇對木糖之時間曲 線……
……52 圖4-3 葡萄糖單一碳源下，添加不同比例之消泡劑對於菌體生…. 長之影響……
……54 圖4-4 混合碳源與單一碳源對於生產木糖醇之影響……55 圖4-5 兩
階段醱酵.....58 圖4-6 葡糖濃度70 g/L之醱酵情形……60
圖4-7 葡萄糖單一碳源下，不同溶氧量的環境下，菌體醱酵生. 長情形.....61
圖4-8 葡萄糖單一碳源下，溶氧量對於產量、產率、比生長速率 的影響……
…63 圖4-9 固定通氣量為1 vvm，轉速對於產製木糖醇的影響……65 圖4-10 葡萄糖及木糖雙碳源下，不
同通氣量對於醱酵產製木糖醇 的影響………66 圖4-11兩階段醱酵酵..
……70 圖4-12高菌體濃度醱酵，不同氮源濃度對於菌體生長之影響……73 表 目 錄 表2.1
木糖醇於蔬果中之含量……4 表2.2 各類植物之纖維素組成及其含量…8
表2.3 各種糖類甜度之比較……13 表2.4 木糖醇之物理及化學性質……
……14 表2.5 各種五碳糖臨床代謝實驗……16 表2.6 微生物從木糖轉化成木糖醇結果一覽表……
……23 表3.1 YMPX(G)營養培養基之組成……40 表 3.2實驗係數之整理……
……49 表4.1雙碳源與單一碳源對產製木糖醇之影響.....57 表4.2搖瓶培養與不同通氣量批次培
養對於產製木糖醇的影響..68 表4.3兩階段醱酵與批次醱酵對於產製木糖醇的影響……71

參考文獻

參考文獻 1. 王三郎。1994。應用微生物學。高立圖書有限公司。台北。 2. 林偉彬。2000。以農業廢棄物生產木糖醇。大葉大學食品工程研究所碩士論文，彰化。 3. 李宗仁。1973。蔗渣之飼料利用價值。台糖通訊 51 (15): 18-19。 4. 李振綱、吳誌明、蔡有癸。2001。高密度微生物細胞醱酵培養。化工技術。9 (2): 163-170。 5. 吳俊彥。2003。半纖維素水解液中木糖之分離及其醱酵。大葉大學食品工程研究所碩士論文。彰化。 6. 苑永弘。1999。大蒜中之含硫胺基酸在肉類香味研發上之應用研究。大葉大學食品工程研究所碩士論文。彰化。 7. 莊正道。1994。溶氧對木糖酒精醱酵之研究。大葉大學食品工程研究所碩士論文。彰化。 8. 陳玉青。2004。酵母菌醱酵木糖生產木糖醇-培養基最適化朝陽科技大學應用化學研究所碩士論文。台中。 9. 陳齊聖、劉至一、王偉祺、涂瑞澤。1999。以鈣離子吸附之陽離子交換樹脂層析分離糖蜜色素與糖分。大葉學報8 (2): 121-126。 10. 陳榮耀、許清森。1986。纖維質廢棄物之生化組成及微生物分解。工業技術 142: 60-68。 11. 陳觀彬。2000。固定化生產木糖醇。雲林科技大學工業化學與災害防治研究所碩士論文。雲林。 12. 莫景棠。1988。各種新構想全靜脈營養液對肝臟功能不全病患之效益。榮民總醫院外科部。 13. 張為憲、李敏雄、呂政義、張永和、陳昭雄、孫璐西、陳怡宏、張基郁、顏國欽、林志城、林慶文。1996。食品化學。華香園出版社。台北 14. 趙士慶。1999。木糖醱酵生產木糖醇之研究。大葉大學食品工程研究所碩士論文，彰化。 15. 鄭錫霖。1976。木糖醇的生理作用。科學月刊7 (4): 60-62。 16. Antal, M. J. Jr., Leesomboon, T., Mok, W. S., and Riehards, G. N. 1991. Mechanism of formation of 2-furaldehyde from D-xylose. Carbohydr. Res., 217, 71-85. 17. Aranda-Barradas, J. S., Delia, M. L., and Riba, J. P. 2000. Kinetic study and modeling of the xylitol production using Candida parasilosis in oxygen-limited culture conditions. Bioprocess Eng., 22: 219-225. 18. Azuma, M., Ikeuchi, R., Kiritani, J., Kato and Ooshima K. 2000. Increase in xylitol production by Candida tropicalis upon addition of salt. Biomass and Bioenergy. 19: 129-135. 19. Barbosa, M. S. S., De Medeiros, M. B., De Mancilha, I. M., Schneider, H., and Lee. H. 1988. Screening of yeasts for production of xylitol from D-xylose and some factors which affect xylitol yield in Candida guillermondii. J. Ind. Microbiol., 3: 241-251. 20. Cao, N-J., Tang, R., Gong, C. S., and Chen, L. F. 1994. The effect of cell density on the production of xylitol from D-xylose by yeast. Appl. Biochem. Biotechnol., 45, 515-519. 21. Christophe, R., Jens, N., and Lisbeth, O. 2003. Metabolic engineering of ammonium assimilation in xylose-fermenting Saccharomyces cerevisiae improves ethanol production. Appliend and Environmental microb., 12(6): 4731-4736. 22. Curless, C., Swank, R., Fu, K., Menjares, A., Fieschko, J., and Tsai, L. Design and evaluation of two-stage, Cyclic, Recombinant Fermentation Process. Biotechnology and Bioengineering., 38: 1082-1090. 23. Dahiya, J.

S. 1991. Xylitol production by petromyces albertensis grown on medium containing D-xylose. Applied Microb., 37: 14-18. 24. Delgenes, J. P., Moletta, R., and Navarro, J. M. 1988. Fermentation of D-xylose, D-glucose, L-arabinose mixture by Picha stipitis Y-7124. Applied Microbiol. Biotechnol., 29: 155-161. 25. De Silva, S. S., and Afschar, A. S. 1994. Microbial production of xylitol from xylose using Candida tropicalis, Bioprocess Eng., 11: 129-134. 26. Domingues, J. M., and Gong, C. S., and Taso, G. T. 1996. Pretreatment of Sugarcane Bagasse hemicellulose hydrolysate for xylitol production by yeast. Appl. Biochem. Biotechnol., 57: 49-56. 27. Domingues, J. M., Ningjun, C., Gong, C. S., and TSao, G. T. 1997. Dilute acid hemicellulose hydrolysates from corn cobs for xylitol production by yeast, Bioresource Technol., 61: 85-90. 28. Du Toit, P. J., Olivier, S. P., and Van Biljon, P. L. 1984. Sugar cane bagasse with regard to monosaccharide, hemicellulose, and amino acid composition. Biotechnol. Bioeng., 26: 1071-1078. 29. Emodi, A. 1978. Xylitol: its properties and food applications. Food Technol., January, 28-32. 30. Fratzke, A. R., and Reilly, P. J. 1977. Uses and metabolic effects of xylitol. Process Biochem., 12: 27-29. 31. Furlan, S. A., Boutlloud, P., Strehaiano, P., and Riba, J. P. 1991: Study on xylitol formation from xylose under oxygen limiting conditions, Biotechnol. Lett., 13: 203-206. 32. Furlan, S. A., Dupuy, M. L., and Strehaiano, P. 1989. Bioconversion of D-xylose: aeration and kinetics. Biotechonol. Food Stuttgart Germany., Febuary 20-24. 33. Furlan, S. A., Boutlloud, A., and De Castro, H. F. 1994. Influence of oxygen on ethanol and xylitol production by xylose fermenting yeasts. Process Biochem., 29: 657-662. 34. Girio, F. M., Roseiro, J. C., Sa-Machado, P., Duarte-Reis, A. R., and Amaral-Collaco, M. T. 1994. Effect of oxygen transfer rate on levels of key enzymes of xylose metabolism in Debaryomyces hansenii. Enzyme Microbiol. Technol., 16: 1074-1078. 35. Gong, C. S., Chen, C. S., and Chen, L. F. 1993. Pretreatment of sugarcane bagasse hemicellulose hydrolysate for ethanol production by yeast. Appl. Biochem. Biotechnol., 19(40): 83-88. 36. Gurgel, P. V., Mancilha, I. M., Pecanha, R. P., and Siqueira, J. F. M. 1995. Xylitol recovery from fermented sugarcane bagasse hydrolyzate. Bioresource Technology., 52: 219-213. 37. Heikkila, H., Nurmi, J., Rahkila, L., and Toyryla, M. 1990. Method for the production of xylitol from mixtures containing xylose: Patent WO., 90(08): 913. 38. Hiroyuki, H., Yuuichi, Y., Kazhiro, T., Keiichi, K., Tohru, S., and Noriyasu, W. 1992. Production of xylitol from D-xylose by Candida tropicalis: optiumization of production rate. Biotechnol. Bioeng., 25: 85-102. 39. Hollmann, S. and Touster, O. 1956. An enzymatic pathway from L-xylulose to D-xylulose. J. Am. Chem. Soc., 78: 3544. 40. Horistu, H., Yahashi, Y., Takamizawa, K., Kawai, K., Suzuki, T., and Watanabe, N. 1992. Production of xylitol from D-xylose by Candida tropicalis: optimization of production rate rate. Biotechnol. Bioeng., 40: 1085-1090 . 41. Jaffe, G. M. 1978. Xylitol- a Specialty Sweetener. Sugar y Azucar. Biotechnol. Bioeng., 73 (4): 36-42. 42. Jeffries, T. W., and Sreenath, H. K. 1988. Fermentation of hemicellulosic sugar and sugar mixtures by Candida shehate. Biotechnol. Bioeng., 27: 302-307. 43. Jones, K. D., and Kompala, D. S. 1999. Cybernetic model of the growth dynamics of Saccharomyces cerevisiae in batch and continuous cultures. J. Biotech., 71: 105-131. 44. Kim, J. H., Ryu, Y. W., and Seo, J. H. 1992. Analysis and optimization of a two-substrate fermentation for xylitol production using Candida tropicalis. J. Industrial Microbiology and Biotechnology., 22: 181-186. 45. Kind, V. B., Vyglazov, V. V., and Kholkin, Y. J. 1987. Use of cationic surfactants for clarification of pentose hydrolyzates in xylitol production. Gidroliz. Lesokhim. Prom-st., 3: 11-12. 46. Kontula, P., Wright, A., and Mattila-Sanholm, T. 1998. Oat bran β-gluco- and xylo-oligosaccharides as fermentative substrates for lactic acid bacteria. Food Microb., 45: 163-169. 47. Kretzl, K., Silbernael, H., and Bassler, K. 1963. Naturwize., 50: 154. 48. Laplace, J. M., Delgenes, J. P., Moletta, R., and Navarro, J. M. 1991. Alcoholic fermentation of glucose and xylose by Picha stipitis, Candida shehatae, Caccharomyces cerevisiae and Zymomonas mobilis: oxygen requirement as a key factor. Appl. Microbiol. Biotechnol., 36: 158-162. 49. Law ford, H. G., and Rousseau, J. D. 1992. Effect of acetic acid on xylose conversion to ethanol by genetically engineered E. coli. Appl. Biochem. Biotechnol. 34: 185-216. 50. Leathers Timonthy D. and Dien Bruce S. 2000. Xylitol production from corn fibre hydrolysates by a two stage fermentation process. Process Biochemistry. 35: 765-769. 51. Lee, Y. Y., Yue, T., and Tarrer, A. R. 1976. Acid hydrolysis of oak sawdust. AIChE National Meeting. 52. Lee, Y. Y., Lin, L. M., Johnsin, T., and Chamber, R. P. 1978. Selective hydrolysis of hardwood hemicellulose by Acid. Biotechnol. Bioeng., 8: 75-88. 53. Meyrial, V., Delgenes, J. P., Moletta, R., and Navarro, J. M. 1991. Xylitol production from D-xylose by Candida guilliermondii: fermentation behavior. Biotechnol. Lett., 11: 281-286. 54. Monod, J. 1942. Reserches sur la croissance des cultures bacteriennes. Hermann and Cie., Paris. 55. Nigam, P., and Singh, D. 1995. Processes for fermentative production of xylitol: a sugar substitute. Process Biochem., 30: 117-124. 56. Nikolaev, D. I., Chernikova, L. P., Glazman, B. A., Kostyuk, L. N., Rutskaya, M. S., and Chivyaga, A. A. 1983. New ionchange resins in xylitol production. Gidroliz. Lesokhim. Prom-st., 2: 16-18. 57. Nolleau, V., Preziosi-Belloy, L., Delgenes, J. P., and Navarro, J. M. 1993. Xylitol production from xylose by two yeast strains: sugar tolerance. Current Microbiol., 27: 191-197. 58. Nolleau, V., Preziosi-Belloy, L., and Navarro, J. M. 1995. The reduction of xylose to xylitol by Candida guilliermondii and Candida parapsilosis: incidence of oxygen and pH. Biotechnol. Lett., 17: 417-422. 59. Preziosi-Belloy, L., Nolleau, V., and Navarro, J. M. 1997. Fermentation of hemicellulosic sugars and sugar mixtures to xylitol by Candida parapsilosis. Enzyme and Microbial Technology., 21: 124-129. 60. Roberto, I. C., Sato, S., Manciha, I. M., and Taqueda, M. E. S. 1995. Influence of media composition on xylitol fermentation by Candida guilliermondii using response surface methodology. Biotechnol. Lett., 17 (11): 1223-1228. 61. Roberto, I. C., Sato, S., and De Mancilha, I. M (1996).: Effect of inoculum level on xylitol production from rice straw hemicullulose hydrolysate by Candida guilliermondii. J. Ind. Microbiol., 16: 348-350. 62. Roberto, I. C., Silva, Silvo S., Felipe Maria, G. A., Mancilha, Ismael M. D., and Sunao, S. 1996. Bioconverson of rice Straw hemicellulose hydrolysate for the production of xylitol-effect of pH and Nitrogen source. Appl. Biotechnol., 57(58): 339-347. 63. Roseiro, J. C., Peito, M. A., Girio, F. M., and Amaral-Collaco, M. T. 1991. The effects of oxygen transfer coefficient and substrate concentration on the xylose fermentation by Debaryomyces hansenii. Arch. Microbiol., 156: 484-490. 64. Sai Ram, M., and Seenayya, G. 1991. Production of ethanol from straw and bamboo pulp by primary isolates of clostridium thermocellum. World Journal of Microb and Biotechnol 7: 372-378. 65. Schinin, A., and Mackinen, K. K. 1975. Turku sugar studies I-XII Acta Odontologia Scandinavica 33, supplementum 70: 28. 66. Sirisansaneeyakul, S., Staniszewski, M., and Rizzi,

M. 1995. Screening of yeasts for production of xylitol from D-xylose. J. Ferment. Bioeng., 6: 564-570. 67. Slininger, P. J., Bolen, P. L., and Kurtzman, C. P. 1987. Pachysolen tannophilus: properties and process consideration for ethanol production from D-xylose. Enzyme Microb. Technol., 9: 5-15. 68. Torget, R., Walter, P., Himmel, M., and Gorhmann, K. 1991. Dilute-Acid Pretreatment of corn residues and short-rotation woody crops. Appl. Biotechnol., 28(29): 75-86. 69. Tran, A. V., and Chambers, R. P. 1985. Red oak wood derived inhibitors in the ethanol fermentation of xylose by Picha stipitis CBS 5776. Biotechnol. Lett. 7(33): 841-846. 70. Walther T., Hensirisak P., and Agblevor F .A. 2001. The influence of aeration and hemicellulosic sugars on Xylitol production by Candida tropicalis. Bioresource Technolo., 76: 213-220. 71. Wang, Y. M., and van Eyes, J. V. 1981. Nutritional significance of fructose and sugar alcohols. Ann. Res. Nutr., 1: 437. 72. Washuttle, J., Riederer, P., and Banchem, E. 1973. Agualitative and quantitative study of sugar-alcohols in sever foods. J. Food Sci. 38(35): 1262. 73. Winkelhausen. E., and Kuzmanova, S. 1998. Microbial conversion of D-xylose to xylitol. J. Ferm. Bioeng., 86: 1-14. 74. Yahashi, Y., Hatzu. M., Kawai, K., Suzuki, T., and Takamizawa, K. 1996. Production of xylitol from D-xylose by Candida tropicalis: the effect of D-Glucose Feeding. J. Ferment. Bioeng., 81: 148-152. 75. Yahashi, Y., Hatzu. M., Kawai, K., Suzuki, T., and Takamizawa, K. 1996. D-Glucose feeding for improvement of xylitol productivity from D-xylose using Candida tropicalis immobilized on a non-woven Fabric. Biotechanol. Lett., 18: 1395-1400. 76. Yamagata. T. 1965. Clinical effect of xylitol on carbohydrate and lipid metabolism in diabetes. Lancet. 2(36): 918-921. 77. Vandeska, E. 1995. Amartey, S.; Kuzmanova, S. and Jeffries, T. W.: Fed-batch Culture for Xylitol Production by Candida boidinii. Process Biochem., 31: 265-270. 78. Vandeska, E., Amartey, S., Kuzmanova, S., and Jeffries, T. W 1996. Fed-batch culture for xylitol production by Candida boidinii. Process Biochem., 31: 265-270.