

Study on the Production of Xylitol by Candida subtropicalis in Two-stage Fermentation

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ABSTRACT

Abstract Recently, it was cared by the most population that the functionality in food including the nutrient balance, the promotion in body, and so on. Xylitol, because of its taste of dew like peppermint, low quantity of heat, the sweetening equals to the sucrose, and anti-cariogenic properties, has been appreciated gradually in the food of new generation. Otherwise, the agricultural residual production abounds with cellulose, hemicellulose, and lignin. When the hemicellulose was hydrolyzed, abundant raw materials like xylose, glucose, and few other sugars (e.g., galactose, mannose, and arabinose) was productive. Hence, the subject of several investigates on the fermentation process for productive of xylitol was discussed the liquid of the lignocellulose hydrolyzed as the stock. Therefore, it not only reduces cost but also exploits nature resource by the sufficient disposal. In this study, in order to increase the yield and productivity of xylitol in ferment process by yeast, a two-stage fermentation that employ difference concentrations of dissolved oxygen was proposed due to the phenomenon of the diauxic growth when two kinds of main sugar (xylose and glucose) as substrate was used. Results explained that it is increased in xylitol's yield and productivity when the two composite substrates were applied. According to the object of increasing the biomass yield at first-stage fermentation, the dissolved oxygen was 5~10% for glucose metabolism ($\mu = 0.356 \text{ hr}^{-1}$). Moreover, at second-stage, metabolism of D-xylose into xylitol, the conditions of the fermentation were 0.25vvm and 130 rpm for the highest yield (0.649 g g⁻¹) and productivity (0.263 g L⁻¹ hr⁻¹) of the xylitol. Based on above of results, an operation procedure that is series connection the fed-batch culture and batch culture was designed. Results of the experiment displayed that xylitol's yield (0.246 g L⁻¹ hr⁻¹) had been increased effectively. Key word: two-stage fermentation, xylitol, dissolved oxygen, lignocellulose hydrolyzates, and diauxic growth.

Keywords : two-stage fermentation ; xylitol ; dissolved oxygen ; lignocellulose hydrolyzates ; diauxic growth

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REFERENCES

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。高密度微生物細胞醣酵培養。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醣酵。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。溶氧對木糖酒精醣酵之研究。大葉大學食品工程研究所碩士論文。彰化。8. 陳玉青。2004。酵母菌醣酵木糖生產木糖醇-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。木糖醣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 guilliermondii*. 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. Applied 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., Strehaino, 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 Strehaino, 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., Yuichi, Y., Kazhiro, T., Keiichi, K., Tohru, S., and Noriyasu, W. 1992. Production of xylitol from D-xylose by *Candida tropicalis*: optimumization of production rate. Biotechnol. Bioeng., 25: 85-102. 39. Hollmann, S. and Touster, O. 1956. An enzymatic pathway from L-xyulose to D-xyulose. 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 shehatae*. 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 -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., Mancilha, 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 hemicellulose 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. Bioconversion 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., Giro, 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 Gorhamann, 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 *Pichia 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.