

利用實驗設計最適化及模式化Candida tropicalis的比生長速率

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摘要

Candida tropicalis可用來生產木糖醇、酒精及，-dicarboxylic acid等具有經濟效益之產物；同時它也具有降解酚及甲酚之能力，可以用來做為污水處理之菌株。本研究目的在應用有效率之方法，尋求此菌株最佳之比生長速率，決定菌體醱酵時的最佳操作條件。所謂有效率的方法是指綜和二因子實驗、陡升路徑與中心混成實驗等實驗設計而成的回應曲面法。一般以批次醱酵搜尋最佳條件方法，實驗過程中各參數是隨時間不斷地變化。但本研究採用連續操作，使所識別出之比生長速率是確保於特定之測試條件。研究選定溫度及基質糖濃度作探討因子。其中為了維持連續醱酵操作能夠控制在設定的條件，並能在不穩定的區域中進行操作，故需要施以回饋控制。而控制器是以一個經過驗證的控制器諧調法則進行參數調諧。此組控制器可將連續式醱酵維持於化學恆常(chemostat)，使溫度及糖濃度控制在設計實驗的條件上。經由反應曲面實驗設計結果，C. tropicalis之比生長速率(μ)與溫度(T)及糖濃度(S)之關係為 $\mu = -240.31 - 8.15S + 15.88T - 0.296S^2 - 0.245T^2 + 0.317ST$ 。預測最大比生長速率大約出現在糖濃度在5.5 g L⁻¹以及溫度35.90°C之條件下，其預測值為0.687 h⁻¹，而同條件下實際操作之結果為0.701 h⁻¹，成功找到最適條件。而此條件下連續醱酵的產率為0.27 g/g glucose，與批次醱酵幾乎相同。

關鍵詞：熱帶假絲酵母菌；二階段醱酵；連續式醱酵

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參考文獻

- 1.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。
- 2.林偉彬。2000。以農林廢棄物生產木糖醇。大葉大學食品工程研究所碩士論文。彰化。
- 3.范瓊藝。2006。利用饋料醱酵生產納豆激酶。大同大學生物工程系研究所碩士論文。台北。
- 4.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。
- 5.陳玉青。2004。酵母菌醱酵木糖醇生產木糖醇-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。
- 6.曾昭維。2005。以Candida subtropicalis進

行二階段醱酵產製木糖醇之研究。生物產業科技學系碩士班碩士論文。彰化。7.詹孟訓。2003。以分散式物件建構監控系統之研究:以生化反應槽為例。大葉大學食品工程研究所碩士論文。彰化。8.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。9.鄭錫霖。1976。木糖醇的生理作用。科學月刊 7(4): 60-62。2005。簡化比生長速率模型之建立及其應用於醱酵程序。台灣科技大學化學工程系碩士學位論文。台北。10.Altintas, M. M., Ulgen, K. O., Kirdar, B., Onsan, Z.I. and Oliver, S. G. 2003. Op-timal substrate feeding pollicy for fed-batch cultures of *S. Cerevisiae* 11.expressing bifunctional fusion protein displaying amylolytic activities. *Enz. Mi-crobial. Technol.* 33: 262-269. 12.Antal, M. J. J., Leesomboon, T., Mok, W. S. and Riehard, G. N. 1991 Mechanism of formation of 2-furaldehyde from D-xylose. *Carbohydr. Res.*, 217, 71-85. 13.Arvin, E. and Flyvbjerg, J. 1992. Groundwater pollution arising from the disposal of creosote waste. *J. Int. Waste Eng. Manage.* 6: 646-651. 14.Atomi, H., Yu, C. and Hara, A. 1994. Characterization of a dicarboxylic acid-producing mutant of the yeast *Candida tropicalis*. *J. Ferment. Bioent.* 77: 205-207. 15.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.* 17: 129-135. 16.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. 17.Box, G. E. P. and Wilson, K. B. 1951. On the experimental attainment optimum conditions. *J. Roy. Statist. Soc. B*13: 1-45. 18.Bux, F., Akkinson, B. and Kasan, K. 1999. Zinc biosorption by waste activated and digested sludges. *Water Sci. Technol.* 39: 127-130. 19.Cao, N. J., Krishnan, M. S., Du, J. X.,Jong, C. S., Ho, N. W. Y., Chen, Z. D. and Tsao, G. T. 1996. Ethanol production from corn cobs pretreated by the ammonia steeping process using genetically engineered yeast. *Biotech. Lett.* 18: 1013-1018. 20.Chien, I. L. and Fruehauf, P. S. 1990. Consider IMC tuning to improve controller performance. *Chem. Eng. Prog.* 86: 33-41. 21.De Silva, S. S. and Afschar, A. S. 1994. Microbial production of xylitol from xylose using *Candida tropicalis*, *Bioprocess Eng.* 11: 129-134. 22.Delgenes, J. P., Moletta, R. and Navarro, J. M. 1998. Fermentation of D-xylose, D-glucose, L-arabinose mixture by *Pichia stipitis* Y-7124. *Applied Microbiol. Biotechnol.* 29:155-161. 23.Dourado, A. and Calvet, J. L. 1983. The design of controllers for batch bioreactors. *Biotechnol. Bioeng.* 32: 519-526. 24.Du Preez, J. C., Van Driessel, B. and Prior, B. A. 1989. Effect of Aerobiosis on fermentation and key enzyme levels during growth of *Pichia stipitis*, *Candida shehatae* and *Candida tenuis* on D-xylose, *Arch. Microbiol.*, 152: 143-147. 25.Du Toit, P. J., Olivier, S. P. and Van Biljon, P.L. 1984.Sugar cane bagasse with re-gard to monosaccharide, hemicellulose and amino acid composition. *Biotechnol. Bioeng.*, 26: 1071-1078. 26.Edwards, V. H. and Jackson, J. V. 1975. Kinetics of substrate inhibition if exponential yeast growth process models. *Biotechnol. Bioeng.* 17: 943-964. 27.Eksteen, J. M., van Rensburg, P., Cordero otero, R. R. and Pretorius, I. S. 2003. Starch fermentation by recombinant *Saccharomyces cerevisiae* strains expressing the α -amylase and glucoamylase genes from *Lipomyces kononekoe* and *Saccharomycopsis fibuligera*. *Biotechnol. Bioeng.* 84: 639-646. 28.Farrel, A. E., Plevin, R. J., Turner, B. T., Jones, A. D., O'Hare, M. and Kammen, D. M. 2006. Ethanol can contribute to energy and enviromental gaols. *Science* 311: 506-508. 29.Furlan, S. A., Boutloud, P. Strehaiano, P. and Riba, J. P. 1991. Study on xylitol formation from xylose under oxygen limiting conditions, *Biotechnol. Lett.*, 13: 203-206. 30.Girio, F. M., Roseriro, J. C., Sa-Machado, P., Durate-Reis, A. R. and Amar-al-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. 31.Grimble, M. J. 1988. Real-time computer control. p.99-127. Prentice Hall, UK. 32.Gupta, R., Gigras, P., Mohapatra, H., Goswami, V. K. and Chauha, B. 2003. Mi-crobial α -amylases: a biotechnological perspective. *Process Biochem.* 38: 1599-1616. 33. Gurgel, P. V., Mancilha, I. M. Pecanha, R. P. and Siqueira, J. F. 1995. Xylitol re-recovery from fermentaed sugar cane bagasse hydrolyzate. *Bioresource Technology.*, 52: 219-213. 34. Haldance, J. B. S. 1930. *Enzymes*, Longmans Green, Lodon. 35. Hill, F. F., Venn, I. and Lukas K. L. 1986. Studies on the formation of long-chain dicarboxylic acids from pure n-alkanes by a mutant of *Candida tropicalis*. *Appl. Microbiol. Biotechnol.* 24: 168-174. 36. 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 . *Biotechnol. Bioeng.*, 40: 1085-1090. 37. Howard P. H. 1989. *Handbook of environmental fate and exposure data for or-ganic chemicals*. Vol. I: Large production and prority pollutants, Lewis publishers, MI, USA. 38. Jackson, J. V., Edwards, V. H. 1975. Kinetics of substrate inhibition if exponential yeast growth process models. *Biotech. Bioeng.* 17: 943-964. 39. Jaffe, G. M. 1978. Xylitol a Specialty Sweetener. *Sugar Azucar. Biotechnol. Bio-eng.*, 73(4): 36-42. 40. Jiang, Y. Wen, J. P., Li, H. M. and Yang, S. L. 2005. The biodegradation of phenol at high initial concentration by the yeast *Candida tropicalis*. *Biochem. Eng. J.* 24: 49-54. 41. Jung, C. S., Song, H. K. and Hyun, J. C. 1999. A direct synthesisi tuning method of unstable first order plus time delay processes. *J. Prosess control.* 9: 265-269. 42. Keith, L. H. and Telliand, W. A. 1979. Priority pollutants. *Environ. Sci. Technol.* 13: 416-423. 43. Kenneth G. D., Turner, M. K. and Woodley, J. M. 2000. *Candida cloacae* oxida-tion of long-chain fatty acids to dioic acids. *Enzyme Microbial Technol.* 27: 205-211. 44. Kim, J. H., Han, K. C., Koh, Y. H. Ryu, Y. W. and Seo J. H. 2002. Optimization of fed-batch fermentation for xylitol production by *Candida tropicalis*. *J. I. Microbiol. Biotech.* 29: 16-19. 45. Kim, T. B. and Oh, D. K. 2003. Xylitol production by *Candida tropicalis* in chem-ically defined medium. *Biotechnol. Lett.* 25: 2085-2088. 46. Klinke, H. B. Thomsen, A. B. and Ahring, B. K. 2004. Inhibition of etha-nol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Appl. Microbiol. Biotechnol.* 66: 10-26. 47. Kontula, P., Wright. A. and Mattila-Sanholm, T. 1998. Oat bran α -gluco- and xy-lo-oligosaccharides as fermentative substrates for lactic acid bacteria. *Food Mi-crob.*, 45: 163-169. 48. Kretzl, K., Silbernael, H. and Assler, k. 1963. *Naturwize.*, 50:154. 49. Laplace, J. M., Delgenes, J. P., Moletta, R. and Navarro, J. M. 1991. Alcoholic fermentation of glucose and xylose by *Pichia stipitis*, *Candida shehatae*, *Saccharo-mycetes cerevisiae* and *Zymomonas mobilis*: oxygen requirement as akey factor. *Appl. Microbiol. Biotechnol.*, 36: 158-162. 50. Latif, F., Rajoka M. I. and Malik, K. A. 1994. Saccharification of *Leptochloa fusca* (kallar grass) straw by thermostable cellulases. *Biores. Technol.* 50: 107-111. 51. Latifa, J., Khalil, B. Jamal, E. Y. and Mohamed, E. 2006. Production of ethanol from

starch by free and immobilized *Candida tropicalis* in the presence of α -amylase. *Bioresource Technol.* 98: 2765-2770. 52. Leathers Timothy D. and Dien Bruce S. 2000. Xylito production from corn fibre hydrolysates by two stage fermentation process. *Process Biochemistry.*, 35: 765-769.