

Optimizing and Modeling Specific Growth Rate of *Candida tropicalis* by Designed Experiment

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ABSTRACT

Candida tropicalis can produce several economical productions such as xylitol, ethanol, , -dicarboxylic acid etc.; and can treat wastewater to decontaminate phenol and m-cresol. In this study, a effective designed experiment was used to find the conditions of the optimal growth of *Candida tropicalis* in continuous fermentation. The response surface methodology method (RSM), a set of designed experiment including two-level factorial design, path of steepest ascent and central composite design etc., was selected. The physical and chemical factors are changed by reaction time in batch fermentation; hence, the chemostat operation through the continuous fermentation is more adopted in this study. Feedback control must be employed in this examination in order to keep the fermentation conditions in setting values even under the unstable region. The parameters in controllers was tuned by a set of proved tuning method. These controller can change the temperature and sugar concentration to designed values very soon and correct. Base on RSM test, the relation among the specific growth rate (μ), temperature (T) and sugar concentration (S) was formulated as $\mu = -240.31 - 8.15S + 15.88T - 0.296S^2 - 0.245T^2 + 0.317ST$. This surface plane predicts that the maximum growth rate is 0.687 h⁻¹ at 5.5 g sugar/L medium and 35.9 . Base on optimal condition, the real specific growth rate in the continuous fermentation is 0.701 h⁻¹ which very close to the predicted result. Moreover, the yield is 0.27 g biomass/g glucose whish is almost same as in batch fermentation.

Keywords : *Candida tropicalis* ; two-step fermentation ; continuous fermentation

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REFERENCES

- 1.吳俊彥。2003。半纖維素水解液中木糖之分離及其酸酵。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。利用餌料醣酵生產納豆激?，妞膚。大同大學生物工程系研究所碩士論文。台北。4.莊正道。1994。溶氧對木糖酒精醣酵之研究。大葉大學食品工程研究所碩士論文。彰化。5.陳玉青。2004。酵母菌醣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* 進行二階段醣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。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醣酵程序。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. Optimal substrate feeding policy 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 Riehards, 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. Atom, 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 xyli-tol 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 guillermondii*. 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. B13: 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 regard 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 kononekoae* and *Saccharomyces 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 environmental goals. Science 311: 506-508. 29. Furlan, S. A., Boutlouad, P., Strehaino, 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 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. 31. Grindle, 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 recovery from fermentaed sugar cane bagasse hydrolyzate. Bioresource Technology., 52: 219-213. 34. Haldane, J. B. S. 1930. Enzymes, Longmans Green, London. 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 organic chemicals. Vol. I: Large production and priority 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 synthesis tuning method of unstable first order plus time delay processes. J. Process control. 9: 265-269. 42. Keith, L. H. and Tellier, 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* oxidation of long-chain fatty acids to dienoic 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 chemically defined medium. Biotechnol. Lett. 25: 2085-2088. 46. Klinke, H. B., Thomsen, A. B. and Ahring, B. K. 2004. Inhibition of ethanol-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-Sandholm, T. 1998. Oat bran β -gluco- and xylo-oligosaccharides as fermentative substrates for lactic acid bacteria. Food Mi-crob., 45: 163-169. 48. Kretzschmar, 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 Picha stipits, Candida shehatae, Saccharo-myces cerevisiae and Zymomonas mobilis: oxygen requirement as a key 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. Xylitol production from corn fibre hydrolysates by two stage fermentation process. *Process Biochemistry.*, 35: 765-769.