Enhancement of ethanol production using cellulose-degrading and immobilized-ethanolic microbes in co-culturing ststems

趙金賢、葉啟輝、林啟文

E-mail: 321456@mail.dyu.edu.tw

ABSTRACT

It is well known that the expense to purify cellulose for cellulose degradation and ethanol production is relatively high. To reduce the cost, this study has made a effort to construct a co-culturing system. The unique system is a microbial process in which cellulolytic strain severs a function that to hydrolyze cellulose and generate reducing sugar which is being utilized by ethanolic strains. To overcome the inhibition from ethanol of fermentative strains, the study applied immobilized ethanolic strain. The study used batch operation to investigate ethanol production with different strategies. In the phase I study, effects of environmental settings on ethanol production using Zymomonas mobilis were investigated. Moreover, comparison on ethanol production between suspended cultures and cell-immobilized bead cultures is conducted. In the phase II study, a feasibility study was conducted to investigate a co-culturing system. The results showed: (1) Immobilized Z. mobilis can well produce ethanol at incubation temperature range of 30 – 37 ℃ and initial pH of 3-11 during 9 – 27 hours fermentation; (2) Because immobilized Z. mobilis seemed to be protected by cell-immobilized bead matrix, ethanol production was not significantly affected under different initial pH; (3) The best co-culturing combinations are Bacillus thermoamylovorans K2 and immobilized Klebsiella pneumoniae THLB0109 in producing ethanol from various cellulosic source among three co-culturing combinations at 30 ℃ incubation; (4) In the co-culturing system of Bacillus thermoamylovorans K2 and immobilized Klebsiella pneumoniae THLB0109, the produced ethanol concentrations at 37 ℃ are in the descending order of Napiergrass, avicel, and caboxymethyl cellulose (CMC) ; and (5) The incubation temperature affected ethanol production under co-culturing systems.

Keywords : co-culturing system、cell-immobilized technology、bioethanol、cellulose cellulose cellulose

Table of Contents

REFERENCES

田志偉，(2006)，「利用Bacillus thermoamylovorans K2、Clostridium thermocellum與Clostridium butyricum M1 共培養分解纖維素」，國立高雄第一科技大學環境與安全衛生工程系，碩士論文。 吳秋芬，(2008)，「嗜熱厭氧狼尾草分解菌之產醇特性研究」，大葉大學環境工程學研究所，碩士論文。 陳芃，(2008)，「二代生質技術上路—以纖維素產製生質燃料」，能源報導，12-14。 陳威廷，(2004)，「纖維素水解菌之培養策略與纖維素水解酵素之鑑定」，國立成功大學化學工程學系，碩士論文 陳榮耀、許清森，(1986)，「纖維質廢棄物之生化組成及其微生物分解」，工業技術，142:60-69。 陳錠玄，(2008)，「竹纖維分解菌株之篩選及其分解酵素之探討」，大葉大學環境工程學研究所，碩士論文。 葉丁源，(1997)，「嗜高溫放射線菌纖維素分解酵素之探討」，國立台灣大學農業化學研究所，碩士論文。 Ahamed, A., and P. Vermette, (2008) “ Enhanced enzyme production from mixed cultures of Trichoderma reesei RUT-C30 and Aspergillus niger LMA grown as fed batch in a stirred tank bioreactor ” Biochemical Engineering Journal, 42: 41 – 46. Aurilia, V., J.C. Martin, S.I. McCrae, K.P. Scot, M.T Rincon, and H.J. Flint, (2000) “ Three multidomain esterases from the cellulolytic rumen anaerobe Ruminococcus flavefaciens 17 that carry divergent dockerin sequences ” Microbiology, 146: 1391 – 1397. Avitia,C.I., F.X. Castellanos-Juarez, E. Sanchez, A. T?llez-Valencia, P. Fajardo-Cavazos, W.L. Nicholson, and M. Pedraza-Reyes, (2000) “ Temporal secretion of a multicellulolytic system in Myxobacter sp. AL-1, Molecular cloning and heterologous expression of cel 9 encoding a modular endocellulase clustered in an operon with cel 48, an exocellobiohydrolase gene ” European Journal of Biochemistry, 267(24): 7058 – 7064. Beery, K.E., and M.R. Ladisch, (2001) “ Chemistry and properties of starch based desiccants ” Enzyme and Microbial Technology, 28(7-8): 573 – 581. Coughlan, M.P. and G.P. Hazlewood, (1993) “ Beta-1,4-D-xylan-degrading enzyme systems: biochemistry, molecular biology and application ” Biotechnology and Applied Biochemistry, 17: 259 – 289. Dekker, R.F.H., (1986) “ Kinetic, inhibition, and stability properties of a commercial β-D-glucosidase (cellobiase) preparation from aspergillus niger and its suitability in the hydrolysis of lignocellulose ” Biotechnology and Bioengineering, 28: 1438 – 1442. Elberson, M.A., F. Malekzadeh, M.T. Yazdi, N. Kameranpour, M.R. Noori-Daloii, M.H. Matte, M. Shahamat, R.R. Colwell, and K.R. Sowers, (2000) “ Cellulomonas persica sp. nov. and Cellulomonas iranensis sp. nov., mesophilic cellulose-degrading bacteria isolated from forest soil ” International Journal of Systematic and Evolutionary Microbiology, 50: 993 – 996. Eliasson, A., C. Christensson, C.F. Wahlbom, and B. Hahn-Hagerdal, (2000) “ Anaerobic xylose fermentation by recombinant Saccharomyces cerevisiae carrying XYL1, XYL2, and XKS1 in mineral medium chemostat cultures ” Applied and Environmental Microbiology, 66(8): 3381 – 3386. Fields, M.W., S. Mallik, and J.B. Russell, (2000) “ Fibrobacter succinogenes S85 fermentsball-milled cellulose as fast as cellobiose until cellulose surface area is limiting ” Applied Microbiology and Biotechnology, 54(4): 570 – 574. Gupta, R., K.K. Sharma, and R.C. Kuhad, (2008) “ Separate hydrolysis and fermentation (SHF) of Prosopis juliflora, a woody substrate, for the production of cellulosic ethanol by Saccharomyces cerevisiae and Pichia stipitis-NCIM 3498 ” Bioresource Technology, 100(3): 1214 – 1220. Haruta, S., Z. Cui, Z. Huang, M. Li, M. Ishii, Y. Igarashi, (2002) “ Construction of a stable microbial community with high cellulose-degradation ability ” Jounal of Applied of Microbiology Biotechnology, 59: 529 – 534. Hillmann, F., R. Fischer, F. Saint-Prix, L. Girbal, H. Bahl, (2008) “ PerR acts as a switch for oxygen tolerance in the strict anaerobe Clostridium acetobutylicum ” Molecular. Microbiology, 68(4): 848 – 860. IEA, (2008) “ Energy Technology Perspectives 2008 ” Scenarios and Strategies to 2050 – Fact Sheets. Jeffries, T.W., (1994) “ Biodegradation of lignin and hemicellulose ” Ch.8 ” C. Ratledge (ed.), Biochemistry of Microbial Degradation, Kluwer Academic Publishers, London: 233 – 277. Jin, Y.S., J.M. Laplaza, and T.W. Jeffries, (2004) “ Saccharomyces cerevisiae engineered for xylose metabolism exhibits a respiratory response ” Applied and Environmental Mlcrobiology, 70(11): 6816 – 6825. Lednicka, D., J. Mergaert, M.C. Cnockaert, and J. Swings, (2000) “ Isolation and identification of cellulolytic bacteria involved in the degradation of natural cellulosic fibres ” Systematic and Applied Microbiology, 23(2): 292 – 299. Lin, C.Y., and W.C. Hung, (2008) “ Enhancement of fermentative hydrogen/ethanol production from cellulose using mixed anaerobic aultures ” International Journal of Hydrogen Energy, 33: 3660 – 3667. Liu, Y., P. Yua, X. Songa, and Y. Qua, (2008) “ Hydrogen production from cellulose by co-culture of Clostridium thermocellum JN4 and Thermoanaerobacterium thermosaccharolyticum GD17 ” International Journal of Hydrogen Energy, 33: 2927 – 2933. Mandels, M., E.T. Reese, (1957) “ Induction of cellulase in Trichoderma Viride as influenced by carbon sources and metals ” Journal of Bacteriology, 73: 269 – 278. Miyazaki, K., C. Irbis, J. Takada, and A. Matsuura, (2008) “ An ability of isolated strains to efficiently cooperate in ethanolic fermentation of agricultural plant refuse under initially aerobic thermophilic conditions: Oxygen deletion process appended to consolidated bioprocessing (CBP) ” Bioresource Technology, 99: 1768 – 1775. Ohara, H., S. Karita, T. Kimura, K. Sakka, and K. Ohmiya, (2000) “ Characterization of the cellulolytic complex (cellulosome) from Ruminococcus albus ” Bioscience, Biotechnology, and Biochemistry, 64(2): 254 – 260. Okuda, N., K. Ninomiya, Y. Katakura, and S. Shioya, (2008) “ Strategies for reducing supplemental medium cost in bioethanol production from waste house wood hydrolysate by ethanologenic Escherichia coli: inoculum size increase and coculture with Saccharomyces cerevisiae ” Journal of Bioscience and Bioengineering, 105(2): 90 – 96. Olsson, L., and B. Hahn-Hagerdal, (1996) “ Fermentation of lignocellulosic hydrolysates for ethanol production ” Enzyme and Microbial Technology, 18(5): 312 – 331. Patel, G.B., (2007) “ Ethanol production during D-xylose, L-arabinose, and D-ribose fermentation by Bacteroides polypragmatus ” Applied Microbiology and Biotechnology, 20(2): 111 – 117. Saha, B.C., L.B. Iten , M.A. Cotta, and Y.V. Wu, (2005) “ Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol ” Process Biochemistry, 40(12): 3693 – 3700. Sanchez,

C., (2009) “ Lignocellulosic residues: biodegradation and bioconversion by fungi ” Biotechnology Advances, 27: 185-194. Sprenger, G.A ., (1996) “ Carbohydrate metabolism in Zymomonas mobilis: a catabolic highway with some scenic routes ” FEMS Microbiology Letters, 145(3): 301 – 307. Turner, P., G. Mamo, E.N. Karlsson, (2007) “ Potential and utilization of thermophiles and thermostable enzymes in biorefining ” Microbial Cell Factories, 6: 1 – 23. Viesturs, U., D. Zarina, S. Strikauska, and A. Berzins, (2004) “ Utilisation of food and woodworking production byproducts by composting ” Bioautomation, 1: 83 – 98. Webb, C., H. Fukuda, and B. Alkinson, (1986) “ The production of cellulose in a spouted bed fermentor using cells immobilized in biomass support particles ” Biotechnology and Bioengineering, 28: 41 – 50. Xiao, Z., X. Zhang, D.J. Gregg, and J.N. Saddler, (2004) “ Effects of sugar inhibition on cellulases and β-glucosidase during enzymatic hydrolysis of softwood substrates ” Applied Biochemistry and Biotechnology, 116(1-3): 1115 – 1126. Yoshikawa, K., H. Suzuki, and K. Nisizawa, (1974) “ Biogenesis of Multiple Cellulase Components of Pseudomonas fluorescens var. cellulose ” J. Biochem., 75: 531 – 540. Yu, J., and H. Stahl, (2008) “ Microbial utilization and biopolyester synthesis of bagasse hydrolysates ” Bioresource Technology, 99(17): 8042 – 8048. Zhang, Y.H.P., and L.R. Lynd, (2005) “ Cellulose utilization by Clostridium thermocellum: bioenergetics and hydrolysis product assimilation ” Proceedings of the National Academy of Sciences, 102(20): 7321 – 7325. Zhao, Y., Y. Wang, J.Y. Zhu, A. Ragauskas, and Y. Deng, (2008) “ Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature ” Biotechnology and Bioengineering, 99(6): 1320 – 1328.