

Ethanol Production using Thermophilic Cellulose-degrading Anaerobes for Pennisetum Alopecoider

吳秋芬、林啟文

E-mail: 9708328@mail.dyu.edu.tw

ABSTRACT

Pennisetum Alopecoider (Napiergrass Taishigrass No.2) was selected as a main carbon source for cellulose in this study. The microbes were grown under anaerobic and thermophilic conditions, and isolated from *Pennisetum Alopecoider* and sheep dung composts. It was focused on bioconversion of *Pennisetum Alopecoider* to ethanol in a single-step process and determining the best growth conditions by using response surface methodology analysis. In addition, *Clostridium thermocellum* was used to set up the protocol and conditions of culturing. Results indicated that the degradation capability is similar for cellulose-degrading mixed culture and *Clostridium thermocellum*, and the ethanol production increased as the carbon source addition increased. Moreover, *Pennisetum Alopecoider* also provides certain type of sugar and will even increase ethanol production. The results also showed that microbes can use avicel and *Pennisetum Alopecoider* to produce ethanol. The best culturing time was approximately 95-142 hr. It was found that the maximum ethanol production concentrations (the ratio of Ethanol/Acetic acid) were 1066 mg/L (2.06) and 1582 mg/L (1.73) under substrate concentrations at 10 g/L and 40 g/L for avicel and *Pennisetum Alopecoider*, respectively. Furthermore, the microbe can produce butanol, and the maximum production concentration was 876 mg/L using avicel at 10 g/L. The effect of initial pH on microbes showed that the best pH was pH 7 and ethanol concentration was 530 mg/L under 20 g/L *Pennisetum Alopecoider*. The best growth conditions of microbes were pH 7.18 and incubating time of 129.2 hr using response surface methodology analysis, and the best ethanol producing concentration was 754 mg/L. Co-culture of *Clostridium thermocellum* and microbes in this study resulted in enhancement for ethanol production.

Keywords : bioenergy ; cellulose ; ethanol ; *Pennisetum Alopecoider* ; response surface methodology

Table of Contents

博碩士論文暨電子檔案上網授權書.....	iii	中文摘要.....	iv	英文摘要.....	iv
目錄.....	vi	誌謝.....	vii	目錄.....	vii
目錄.....	ix	圖目錄.....	xii	表目.....	xii
第一章 緒論 1.1 前言.....	1	1.2 研究目的與內容.....	1	1.2 研究目的與內容.....	1
2 第二章 文獻回顧 2.1 生質能源.....	2	2.2 生質乙醇之優劣.....	4	2.2 生質乙醇之優劣.....	4
5 2.3 生質乙醇發展狀況.....	5	2.4 生質乙醇之製造.....	6	2.4 生質乙醇之製造.....	6
9 2.5 狼尾草.....	9	2.6 纖維素結構.....	10	2.6 纖維素結構.....	10
17 2.7 纖維素分解酵素.....	17	2.8 纖維素分解酵素之應用.....	18	2.8 纖維素分解酵素之應用.....	18
21 2.9 纖維素分解酵素降解系統.....	21	2.10 嗜熱性厭氧菌株.....	22	2.10 嗜熱性厭氧菌株.....	22
23 2.11 回應曲面法.....	23	第三章 研究方法 3.1 實驗流程.....	27	第三章 研究方法 3.1 實驗流程.....	27
29 3.2 菌種來源與培養基.....	29	3.3 還原糖之測定.....	31	3.3 還原糖之測定.....	31
32 3.4 糖、酸、醇之測定.....	32	3.5 狼尾草之前處理.....	35	3.5 狼尾草之前處理.....	35
39 3.6 回應曲面法.....	39	第四章 結果與討論 4.1 <i>Clostridium thermocellum</i> 之乙醇生成特性探討.....	44	4.2 混合菌群產醇能力之探討.....	44
52 4.3 狼尾草前處理.....	52	69 4.4 <i>Clostridium thermocellum</i> 與混合菌群之比較.....	69	4.4 <i>Clostridium thermocellum</i> 與混合菌群之比較.....	69
73 第五章 結論與建議 5.1 結論.....	73	81 5.2 建議.....	81	5.2 建議.....	81
82 參考文獻.....	82	84	84		

REFERENCES

- Aristidou, A., and M. Penttila, (2000) " Metabolic engineering applications to renewable resource utilization " Current Opinion in Biotechnology, 11: 187-198. Bayer, E.A., H. Chanzy, R. Lamed, and Y. Shoham, (1998) " Cellulose, cellulases and cellulosomes " Current Opinion in Structural Biology, 8: 548-557. Balusu, R., R.R. Paduru, S.K. Kuravi, G. Seenayya, and G. Reddy, (2005) " Optimization of critical medium components using response surface methodology for ethanol production from cellulosic biomass by *Clostridium thermocellum* SS19 " Process Biochemistry, 40:

3025-3030. Bandaru, V.V.R., S.R. Somalanka, D.R. Mendu, N.R. Madicherla, and A. Chityala, (2006) " Optimization of fermentation conditions for the production of ethanol from sago starch by co-immobilized amyloglucosidase and cells of *Zymomonas mobilis* using response surface methodology " *Enzyme and Microbial Technology*, 38: 209-214. Beguin, P., (1987) " Cloning of cellulose gene " *Critical Reviews in Biotechnology*, 6: 129-162. Bertoldo, C., and G. Antranikian, (2002) " Starch-hydrolyzing enzymes from thermophilic archaea and bacteria " *Current Opinion in Chemical Biology*, 6: 151-160. Bhat, M.K., and S. Bhat, (1997) " Cellulose degrading enzymes and their potential industrial applications " *Biotechnology Advances*, 15: 583-620. Blackburn, B., T. MacDonald, M. McCormack, P. Perez, V. Tiangco, M. Scharff, and S. Unnasch, (1999) " Report to the governor: evaluation of biomass-to-ethanol fuel potential in California " California Energy Commission, California. Bowles, L.K., and W.L. Ellefson, (1985) " Effects of butanol on *Clostridium acetobutylicum* " *Applied and Environmental Microbiology*, 50: 1165-1170. Bowman, L., and E. Geiger, (1984) " Optimization of fermentation conditions for alcohol production " *Biotechnology and Bioengineering*, 26: 1492-1497. Campbell, C.J., and J.H. Laherrere, (1998) " The end of cheap oil " *Scientific American*, 3: 78-83. Cheynier, V., M. Feinberg, C. Chararas, and C. Ducauze, (1983) " Application of response surface methodology to evaluation of bioconversion experimental conditions " *Applied and Environmental Microbiology*, 45: 634-639. Chinn, M.S., S.E. Nokes, and H.J. Strobel, (2007) " Influence of process conditions on end product formation from *Clostridium thermocellum* 27405 in solid substrate cultivation on paper pulp sludge " *Bioresource Technology*, 98: 2184-2193. Davis, L., P. Rogers, J. Pearce, and P. Peiris, (2006) " Evaluation of *Zymomonas*-based ethanol production from a hydrolysed waste starch stream " *Biomass and Bioenergy*, 30: 809-814. Demain, A.L., M. Newcomb, and J.H. David Wu, (2005) " Cellulase, clostridia, and ethanol " *Microbiology and Molecular Biology Reviews*, 69: 124-154. Desvaux, M., E. Guedon and H. Petitdemange, (2000) " Cellulose catabolism by *Clostridium cellulolyticum* growing in batch culture on defined medium " *Applied and Environmental Microbiology*, 66: 2461-2470. George, H.A., J.L. Johnson, W.E.C. Moore, L.V. Holdeman, and J.S. Chen, (1983) " Acetone, isopropanol, and butanol production by *Clostridium beijerinckii* (syn. *Clostridium butylicum*) and *Clostridium aurantibutyricum* " *Applied and Environmental Microbiology*, 45: 1160-1163. Gong, C.S., C.S. Chen, and L.F. Chen, (1993) " Pretreatment of sugar cane bagasse hemicellulose hydrolyzate for ethanol production by yeast " *Applied Biochemistry and Biotechnology*, 39: 83-88. Gregg, D.J., and J.N. Saddler, (1996) " Factors affecting cellulose hydrolysis and the potential of enzyme recycle to enhance the efficiency of an integrated wood to ethanol process " *Biotechnology and Bioengineering*, 51: 375-383. Hamelinck, C.N., G. Hooijdonk, and A.P. Faaij, (2005) " Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle-, and long-term " *Biomass and Bioenergy*, 28: 384-410. Herrero, A.A., R.F. Gomez, B. Snedecor, C.J. Tolman, and M.F. Roberts, (1985) " Growth inhibition of *Clostridium thermocellum* by carboxylic acids: a 48 mechanism based on uncoupling by weak acids " *Applied Microbiology and Biotechnology*, 22: 53-62. Henrissat, B., (1994) " Cellulases and their interaction with cellulose " *Cellulose*, 1: 169-196. Himmelblau, D.M., (1970) " Process analysis by statistical methods " John Wiley and Sons, New York, 230-292. Hiu, S.F., C.X. Zhu, R.T. Yan, and J.S. Chen, (1987) " Butanol-ethanol dehydrogenase and butanol-ethanol- isopropanol dehydrogenase: different alcohol dehydrogenases in two strains of *Clostridium beijerinckii* (*Clostridium butylicum*). " *Applied and Environmental Microbiology*, 53: 697-703. Huang, C.Y., B.K. Patel, R.A. Mah and L. Baresi, (1998) " *Caldicellulosiruptor owensensis* sp. nov., an anaerobic, extremely thermophilic, xylanolytic bacterium " *International Journal of Systematic Bacteriology*, 48: 91-97. Huang, L., (2007) " Application of microbial resources for bioethanol production " The 2007 Taiwan Bioethanol Development Trend Workshop, January 24, Taoyuan, Taiwan. Ingram, L.O., (1990) " Ethanol tolerance in bacteria " *Critical Reviews in Biotechnology*, 9: 305-319. Jones, R.P., (1989) " Biological principles for the effects of ethanol " *Enzyme and Microbial Technology*, 11: 130-153. Dimitar, K., G. Danka, and S. Ivan, (2003) " A simple and rapid test for differentiation of aerobic from anaerobic bacteria " *World Journal of Microbiology & Biotechnology*, 19: 233-238. Knowles, J., P. Lethovaara, T.T. Reeri, (1987) " Cellulase families and their genes " *Trends Biotechnol*, 5: 255-261. Knutson, B.L., H.J. Strobel, S.E. Nokes, K.A. Dawson, J.A. Berberich, and C.R. Jones, (1999) " Effect of pressurized solvents on ethanol production by the thermophilic bacterium *Clostridium thermocellum* " *The Journal of Supercritical Fluids*, 16: 149-156. Krassig, H.A., (1993) " Cellulose: structure, accessibility, and reactivity " Gordon and Breach Science Publishers, Netherlands. Lamed, R., and J.G. Zeikus, (1980) " Ethanol production by thermophilic bacteria: relationship between fermentation product yields of and catabolic enzyme activities in *Clostridium thermocellum* and *Thermoanaerobium brockii* " *Journal of Bacteriology*, 144: 569-578. Lee, J., (1997) " Biological conversion of lignocellulosic biomass to ethanol " *Journal of Biotechnology*, 56: 1-24. Levina, D.B., R. Islamc, N. Cicekc, and R. Sparlingd, (2006) " Hydrogen production by *Clostridium thermocellum* 27405 from cellulosic biomass substrates " *International Journal of Hydrogen Energy*, 31: 1496-1503. Lin, Y.L., and H.P. Blaschek, (1983) " Butanol production by a butanol-tolerant strain of *Clostridium acetobutylicum* in extruded corn broth " *Applied and Environmental Microbiology*, 45: 966-973. Lovitt, R.W., G. Shen, and J.G. Zeikus, (1988) " Ethanol production by thermophilic bacteria: biochemical basis for ethanol and hydrogen tolerance in *Clostridium thermohydrosulfuricum* " *Journal of Bacteriology*, 170: 2809-2815. Lynd, L.R., P.J. Weimer, W.H. van Zyl, and I.S. Pretorius, (2002). " Microbial cellulose utilization: fundamentals and biotechnology. " *Microbiology and Molecular Biology Reviews*, 66: 506-577. Mohagheghi, A., M. Ruth, and D.J. Schell, (2006) " Conditioning hemicellulose hydrolysates for fermentation: effects of overliming pH on sugar and ethanol yields " *Process Biochemistry*, 41: 1806-1811. Frederic, M., J.R. Martin, H. Petitdemange, and R. Gay, (1982) " Acetone and butanol production by *Clostridium acetobutylicum* in a synthetic medium. " *Applied and Environmental Microbiology*, 44: 1318-1324. Moresi, M., A. Colicchio, and F. Sansovini, (1980) " Optimization of whey fermentation in a jar fermenter. " *Applied and Environmental Microbiology*, 9: 173-183. O'Sullivan, A.C., (1997). " Cellulose: the structure slowly unravels. " *Cellulose*, 4:173-207. Ozkan, M., S.G. Desai, Y. Zhang, D.M. Stevenson, J. Beane, E.A. White, M.L. Gueriot, and L.R. Lynd, (2001) " Characterization of 13 newly isolated strain of anaerobic, cellulolytic thermophilic bacteria "

Journal of Industrial Microbiology and Biotechnology, 27: 275-280. Paseephol, T., D. Small, and F. Sherkat, (2007) " Process optimisation for fractionating Jerusalem artichoke fructans with ethanol using response surface methodology " Food Chemistry, 104: 73-80. Rani, K.S., M.V. Swamy, and G. Seenayya, (1997) " Increased ethanol production by metabolic modulation of cellulose fermentation in *Clostridium thermocellum* " Biotechnology Letters, 19: 819-823. Rani, K.S., M.V. Swamy, and G. Seenayya, (1998) " Production of ethanol from various pure and nature cellulosic biomass by *Clostridium thermocellum* Strains SS21 and SS22 " Process Biochemistry, 33: 435-440. Ratnam1, B.V.V., M. Narasimha Rao, M. Damodar Rao, S. Subba Rao and C. Ayyanna, (2003) " Optimization of fermentation conditions for the production of ethanol from sago starch using response surface methodology " World Journal of Microbiology & Biotechnology, 19: 523-526. Sato, K., S. Goto, S. Yonemura, K. Sekine, E. Okuma, Y. Takagi, K. Hon-Nami, and T. Saiki, (1992) " Effect of yeast extract and Vitamin B12 on ethanol production from cellulose by *Clostridium thermocellum* I-1-B " Applied and Environmental Microbiology, 58: 734-736. Schwarz, W.H., (2001) " The cellulosome and cellulose degradation by anaerobic bacteria " Applied Microbiology and Biotechnology, 56: 634-649. Slininger, P.J., P.L. Bolen, and C.P. Kurtzman, (1987) " *Pachysolen tannophilus*: properties and process consideration for ethanol product from D-xylose " Enzyme and Microbial Technology, 9: 5-15. Sun, Y., and J. Cheng, (2002) " Hydrolysis of lignocellulosic materials for ethanol production: a review " Bioresource Technology, 83: 1 – 11. Thomas, K.N., A. Ben-Bassat, and J.G. Zeikus, (1981) " Ethanol production by thermophilic bacteria: fermentation of cellulosic substrates by cocultures of *Clostridium thermocellum* and *Clostridium thermohydrosulfuricum* " Applied and Environmental Microbiology, 41: 1337-1343. Tomme, P., D.P. Driver, E.A. Amandoron, R.C. Miller, J. R Antony, J. Warren, and D. Kilburn, (1995) " Comparison of a fungal (family I) and bacterial (family II) cellulose-binding domain " Journal of Bacteriology, 177: 4356-4363. Valjamae, P., V. Sild, A. Nutt, G. Pettersson, and G. Johansson, (1999) " Acid hydrolysis of bacterial cellulose reveals different modes of synergistic action between cellobiohydrolase I and endoglucanase I " European Journal of Biochemistry, 266: 327-334. Wood, T.M., and V. Garica-Campayo, (1990) " Enzymology of cellulose degradation " Biodegradation, 1: 147-161. Wood, B.E., and L.O. Ingram, (1992) " Ethanol production from cellobiose, amorphous cellulose, and crystalline cellulose by recombinant *Klebsiella oxytoca* containing chromosomally integrated *Zymomonas mobilis* genes for ethanol production and plasmids expressing thermostable cellulase genes from *Clostridium thermocellum* " Applied and Environmental Microbiology, 58: 2103-2110. Xiang, Q., Y.Y. Lee, P.O. Pettersson, and R.W. Torget, (2003) " Heterogeneous aspects of acid hydrolysis of -cellulose " Applied Biochemistry and Biotechnology, 107: 105-108. Zeikus, J.G., M.W. Taylor, and T.D. Brock, (1970) " Thermal stability of ribosomes and RNA from *Thermus aquaticus* " Biochimica et Biophysica Acta, 204: 512-520. Zeikus, J.G., A. Ben-Bassat, and P.W. Hegge, (1980) " Microbiology of methanogenesis in thermal, volcanic environments " Journal of Bacteriology, 143: 432-440. Zertuche, L., and R.R. Zall, (1985) " Optimizing alcohol production from whey using computer technology " Biotechnology and Bioengineering, 27: 547-554. Zhang, Y.H.P., and L.R. Lynd, (2004) " Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems " Biotechnology and Bioengineering, 88: 797-824. Zhang, Y. H. P., M. E. Himmel, and J. R. Mielenz, (2006) " Outlook for cellulase improvement: screening and selection strategies " Biotechnology Advances, 24: 452-481. 尤智立, (2003), 嗜熱纖維分解纖維分解酵素的探討, 國立中山大學生命科學研究所, 碩士論文。王惠君, (2003), 以回應曲面法探討溶膠-凝膠法製備奈米二氧化矽之參數影響, 中原大學化學工程研究所, 碩士論文。王馨儀, (2006), 以牛糞堆肥中嗜熱厭氧菌群分解纖維素產乙醇之研究, 東海大學環境科學與工程研究所, 碩士論文。成游貴, (2004), 狼尾草, 行政院農業委員會畜產試驗所專輯, 第89號, 第66-67頁。林成原、陳永和, (2002), 車用替代性燃料發展現況與展望, 能源季刊, 第三十二卷, 第三期, 第118-128頁。林立僑, (2003), 由谷關溫泉中嗜熱厭氧木質素降解細菌之分離、定性及鑑定, 東海大學環境科學研究所, 碩士論文。林啟文, 呂珊茹, 賴吉永, (2005), 生物能酒精燃料之發展歷程及現況, 台灣機電工程社-生質能專輯, 第1期, 第31-39頁。林俊義, (2006), 國內外生質能源發展潛力與方向, 生質能源開發與應用, 第39-55頁。周柏伸, (2006), 利用酸前處理提高纖維素水解蔗渣效率之研究, 台灣大學生物產業機電工程學研究所, 碩士論文。姚向君、田宜水, (2004), 生物質能資源清潔轉化利用技術, 化學工業出版社, 北京。袁振宏、吳創之、馬隆龍, (2004), 生物質能利用原理與技術, 化學工業出版社, 北京。許峻賓, (2007), 能源「無油化」瑞典再生能源推動概況, 能源報導, 七月, 第23-26頁。陳文恆、郭家倫、黃文松、王嘉寶, (2007), 纖維酒精技術之發展, 農業生技產業季刊, 第九期, 第62-69頁。陳志威、吳文騰, (2002), 生生不息的生質能源, 科學發展, 第359期, 第8-11頁。陳俊吉, (2003), 利用回應曲面法探討添加胺基酸對肌酸酵素生產之影響, 國立成功大學化學工程學系, 碩士論文。彭炳戊, 成游貴, (2000), 堆積對狼尾草收穫後品質的影響, 酪農天地雜誌, 第38期, 第19頁。黃美燕, (2006), 嗜熱厭氧生物降解纖維素產甲烷程序最佳化之探討, 東海大學環境科學與工程研究所, 碩士論文。黃雯靖, (2006), 纖維素物質之厭氧醇化, 逢甲大學環境工程與科學學系, 碩士論文。齊倍慶, (2001), 從堆肥中篩選纖維素分解酵素生產菌及其酵素性質研究, 國立清華大學生命科學系, 碩士論文。葉財記, (2003), 谷關溫泉中嗜熱厭氧木聚醣降解螺旋體菌株之分離與鑑定, 東海大學環境科學研究所, 碩士論文。蔣宇, 邵蔚藍, (2005), 嗜熱厭氧產乙醇桿菌乙醇代謝途徑的初步研究, 南京師大學報(自然科學版), 第28卷, 第3期, 第69-73頁。鄭作林、蕭耀基, (2006), 台糖公司在生質酒精的發展策略, 能源報導, 六月, 第11-13頁。穆春菊、鄭顏昆、張耀南, (2002), 利用回應曲面法探討白米複合培養基組成對紅麴膽固醇合成抑制劑產量影響, 第七屆生化工程研討會論文集, 第739-744頁。韓如暘, 陳美慈, 閔航, 趙宇華, 馬曉航, (1999), 嗜熱厭氧細菌 *Clostridium* sp. EVA4 菌株直接轉化纖維素產乙醇的研究, 應用與環境生物學報, 第5卷。戴上凱, 林畢修平, 劉仲康, (2004), 熱穩定性纖維素分解酵素, 生物科學, 第47卷, 第1期, 第35-47頁。戴上凱, (2004), 熱穩定性纖維素分解細菌分離株之特性探討與親緣關係的研究, 國立中山大學生命科學研究所, 博士論文。羅泳勝, (2005), 以反應曲面實驗設計法探討本土厭氧產氫菌 *Clostridium butyricum* CGS2 之最佳醱酵產氫條件, 國立成功大學化學工程學系, 碩士論文。