

# Study on Converting Glycerol to 2,3-Butanediol by Suspended and Immobilized Bacterial Strains

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## ABSTRACT

Among the promising chemical, 2,3-butanediol (2,3-BDO) is a bio-based diols, which for a wide range of applications in food and cosmetic industry, antifreezes and additives. This study is reported the isolation of two strains *Klebsiella* sp. Wu1 and *K. pneumoniae* Wu2, capable to convert glycerol in to 2,3-BDO. The effects of pH, carbon and nitrogen substrate concentration, aeration and agitation rate on the production of 2,3-BDO were investigated. Peptone was a better nitrogen source when considering the 2,3-BDO production performance. The 2,3-butanediol formation reached a maximum yield were 7.2 and 8.4 g/l for *Klebsiella* sp. Wu1 and *K. pneumoniae* Wu2 in batch culture, respectively, when glycerol concentration were 40 and 60 g/L. Additionally, the study was also to focus on the feasibility of production of 2,3-BDO using PVA-PU immobilized-cell beads in the fluidized bed reactor. The effect of typical process variables such as hydraulic retention (HRT), influent glycerol concentration level and particle number density on 2,3-BDO production were studied. The maximum 2,3-BDO production by immobilized *Klebsiella* sp. Wu1 and *K. pneumoniae* Wu2 beads in fluidized bed reactor were 16.35 and 17.12 g/L at HRT of 24h, respectively.

Keywords : 2,3-butanediol、glycerol、immobilization、fluidized bed reactor、Polyvinyl Alcohol (PVA)、polyurethane (PU)、*K. pneumoniae*、*Klebsiella* sp.

## Table of Contents

封面內頁	簽名頁	中文摘要iii	英文摘要iv	誌謝v	目錄vii	圖目錄xiii	表目錄xix	1.緒論1	1.1前言1	1.2研究動機及目的4	2.文獻回顧6	2.1甘油簡介6	2.1.1甘油轉化2,3-BDO代謝途徑8	2.2 2,3-BDO簡介9	2.2.1 2,3-BDO之特性與應用9	2.2.2生物方法生產2,3-BDO10	2.2.3生產2,3-BDO之菌株介紹11	2.3 培養基探討17	2.3.1基質濃度17	2.3.2外加有機酸濃度18	2.4環境因子對微生物生產2,3-BDO之影響19	2.4.1溶氧19	2.4.2 pH之影響20	2.4.3溫度的影響21	2.5 固定化技術簡介23	2.5.1固定化細胞技術23	3. 材料與方法26	3.1實驗材料26	3.1.1實驗藥品26	3.1.2儀器設備29	3.2懸浮污泥馴養30	3.2.1馴養可生產2,3-BDO之培養基30	3.2.2培養條件30	3.2.3菌株篩選31	3.2.4純菌生產2,3-BDO之培養基32	3.3菌株之篩選與DNA鑑定33	3.4實驗方法39	3.4.1 環境因子及培養基組成對2,3-BDO生產菌株生長及生產之影響39	3.4.1.1pH值之影響39	3.4.1.2氮源種類之影響39	3.4.1.3氮源濃度之影響40	3.4.1.4甘油為主要碳源, 額外添加不同碳源之影響40	3.4.1.5甘油濃度之影響41	3.4.1.6振盪速率及曝氣速率之影響41	3.4.1.7溫度之影響42	3.4.2微生物發酵液之分析方法42	3.4.2.1總糖分析42	3.4.2.2以氣相層析儀(GC)分析2,3-BDO及副產物44	3.4.3固定化菌株之製備45	3.4.3.1菌體之大量培養45	3.4.3.2菌體量之量測46	3.4.3.3固定化菌株之製備46	3.4.3.4 PU+PVA顆粒製備方法和物性測定47	3.4.4固定化生化反應器設計與操作50	3.4.5物質結構之分析53	3.4.5.1掃描式電子顯微鏡 (Scanning Electron Microscopy, SEM)53	4. 結果與討論54	4.1生產2,3-BDO之活性污泥馴養54	4.1.1探討額外添加醋酸對9種污泥在不同培養條件下2,3-BDO之產量比較55	4.2經由活性污泥篩選出生產2,3-BDO之菌株並進行鑑定59	4.3 PH對2,3-BDO生產之影響61	4.4探討不同氮源種類對2,3-BDO生產之影響68	4.4.1氮源對 <i>Klebsiella</i> sp. Wu1生產2,3-BDO之影響68	4.4.2氮源對 <i>K. pneumoniae</i> Wu2生產2,3-BDO之影響68	4.5氮源濃度對2,3-BDO生產之影響72	4.5.1 Peptone、urea及(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 對 <i>Klebsiella</i> sp. Wu1菌株生產2,3-BDO之影響72	4.5.2 Peptone、urea及(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 對 <i>K. pneumoniae</i> Wu2菌株生產2,3-BDO之影響72	4.6探討以甘油為主要碳源, 額外添加不同碳源條件對 <i>Klebsiella</i> sp. Wu1和 <i>K. pneumoniae</i> Wu2菌株生產2,3-BDO之影響80	4.6.1額外添加不同碳源對 <i>Klebsiella</i> sp. Wu1菌株生產2,3-BDO之影響80	4.6.2額外添加不同碳源對 <i>K. pneumoniae</i> Wu2菌株生產2,3-BDO之影響81	4.7甘油濃度對篩選菌株生產2,3-BDO之影響87	4.7.1固定乳糖濃度下, 甘油濃度對 <i>Klebsiella</i> sp. Wu1生產2,3-BDO之影響 87	4.7.2 固定乳糖濃度下, 甘油濃度對 <i>K. pneumoniae</i> Wu2生產2,3-BDO之影響88	4.8 振盪速率及曝氣速率對篩選菌株生產2,3-BDO之影響94	4.8.1振盪速率及曝氣速率對 <i>Klebsiella</i> sp. Wu1生產2,3-BDO之影響94	4.8.2振盪速率及曝氣速率對 <i>K. pneumoniae</i> Wu2生產2,3-BDO之影響95	4.9 溫度對篩選菌株生長及生產2,3-BDO之影響98	4.10添加丙酮酸對 <i>Klebsiella</i> sp. Wu1 及 <i>K. pneumoniae</i> Wu2兩菌株有無提升2,3-BDO產量之影響100	4.11選擇最佳固定化製備條件固定化 <i>Klebsiella</i> sp. Wu1與 <i>K. pneumoniae</i> Wu2菌株於生產2,3-BDO之能力影響104	4.11.1探討不同PU濃度與Alginate 對固定化之可行性104	4.11.2不同CaCl <sub>2</sub> 對固定化及懸浮 <i>Klebsiella</i> sp. Wu1與 <i>K. pneumoniae</i> Wu2菌株之影響111	4.11.3 利用固定化 <i>Klebsiella</i> sp. Wu1與 <i>K. pneumoniae</i> Wu2以PU+PVA與Alginate+PVA作為固定化方法可行性之研究117	4.11.4 添加不同丙酮酸濃度對固定化與懸浮 <i>Klebsiella</i> sp. Wu1與 <i>K. pneumoniae</i> Wu2菌株生產2,3-BDO之影響 124	4.11.5振盪速率, 曝氣速率及靜置對固定化 <i>Klebsiella</i> sp. Wu1與固定化 <i>K. pneumoniae</i> Wu2兩菌株生產2,3-BDO之影響130	4.11.6 pH對固定化 <i>Klebsiella</i> sp.
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Wu1和K. pneumonia Wu2菌株生產2,3-BDO之影響134 4.11.7溫度對固定化Klebsiella sp. Wu1菌株與K. pneumonia Wu2菌株之影響144 4.11.8探討不同介面活性劑有無提高Klebsiella sp. Wu1與K. pneumonia Wu2菌株生產2,3-BDO之影響150 4.12以PU+PVA作為固定化單體在單一菌株下進行流體化床反應器之連續生產測試156 4.12.1不同水力滯留時間與顆粒填充比生產2,3-BDO之影響156 4.12.2 初始Glycerol濃度對固定化Klebsiella sp. Wu1與K. pneumoniae Wu2 菌株在反應器生產2,3-BDO之影響163 4.12.3 探討固定化Klebsiella sp. Wu1及K. pneumonia Wu2以流體化床連續饋料並以不同曝氣量對2,3-BDO之影響167 5. 結論171 參考文獻173 圖目錄 圖1-1本研究架構示意圖3 圖2-1生物發酵法甘油代謝途徑8 圖3-1生產2,3-BDO之微生物篩選流程圖32 圖3-2葡萄糖濃度的標準曲線43 圖3-3各種代謝產物之GC標準圖譜44 圖3-4產物及副產物之標準曲線45 圖3-5PU+PVA固定化流程47 圖3-6測量固定化顆粒強度之實驗裝置49 圖3-7反應器運行過程示意圖52 圖4-1以不同來源的工業廢水，農業污水和農業污泥馴養後2,3-BDO、1,3-PDO與甘油消耗量培養基與培養條件56 圖4-2 以不同來源的工業廢水，農業廢水探討添加醋酸對2,3-BDO產量之影響58 圖4-3 Klebsiella sp. Wu1之親緣圖60 圖4-4 Klebsiella pneumoniae Wu2之親緣圖60 圖4-5在不同pH值條件下Klebsiella sp. Wu1之生長曲線64 圖4-6在不同pH值條件下K. pneumoniae Wu2之生長曲線65 圖4-7不同pH對Klebsiella sp. Wu1生產2,3-BDO產量及產力之影響66 圖4-8不同pH對K. pneumoniae Wu2生產2,3-BDO產量及產力之影響67 圖4-9不同氮源對Klebsiella sp. Wu1生產2,3-BDO產量之影響70 圖4-10不同氮源對K. pneumoniae Wu2生產2,3-BDO產量之影響71 圖4-11不同peptone濃度對Klebsiella sp. Wu1生產2,3-BDO產量和產力之影響74 圖4-12不同urea濃度對Klebsiella sp. Wu1生產2,3-BDO產量和產力之影響75 圖4-13不同(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>濃度對Klebsiella sp. Wu1生產2,3-BDO產量之影響76 圖4-14 不同peptone濃度對K. pneumoniae Wu2生產2,3-BDO產量和產力之影響77 圖4-15不同urea濃度對K. pneumoniae Wu2生產2,3-BDO產量和產力之影響78 圖4-16不同(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>濃度對K. pneumoniae Wu2生產2,3-BDO產量之影響79 圖4-17在不同碳源對Klebsiella sp. Wu1之生長曲線與碳源的消耗率83 圖4-18有/無添加甘油，額外添加不同碳源對Klebsiella sp. Wu1 生產2,3-BDO之影響84 圖4-19在不同碳源對K. pneumoniae Wu2之生長曲線與碳源的消耗率85 圖4-20有/無添加甘油，額外添加不同碳源對K. pneumoniae Wu2生產2,3-BDO之影響86 圖4-21在不同甘油濃度下Klebsiella sp. Wu1添加乳糖及未添加乳糖之生長及甘油的消耗比較90 圖4-22有/無添加乳糖在添加不同甘油濃度對Klebsiella sp. Wu1生產2,3-BDO之影響91 圖4-23在不同甘油濃度下K. pneumoniae Wu2添加乳糖及未添加乳糖之生長及甘油的消耗比較92 圖4-24有/無添加乳糖在不同甘油濃度對K. pneumoniae Wu2生產2,3-BDO之影響93 圖4-25在曝氣條件與攪拌速率下Klebsiella sp. Wu1對2,3-BDO產量之影響96 圖4-26在曝氣條件與攪拌速率下K.pneumoniae Wu2對2,3-BDO產量之影響97 圖4-27不同溫度對篩選的菌株生產2,3-BDO之影響99 圖4-28 Klebsiella sp. Wu1和K. pneumoniae Wu2添加丙酮酸之生長曲線102 圖4-29 添加不同丙酮酸對Klebsiella sp. Wu1和K. pneumoniae Wu2培養72小時生產2,3-BDO及副產物之影響103 圖4-30試驗不同Alginate和PU比例濃度形成固定化Klebsiella sp. Wu1和K. pneumoniae Wu2培養五天後之2,3-BDO及副產物之影響106 圖4-31不同氯化鈣濃度對固定化Klebsiella sp. Wu1之生長情形113 圖4-32不同氯化鈣濃度對固定化K. pneumoniae Wu2之生長情形114 圖4-33 不同氯化鈣濃度對Klebsiella sp. Wu1培養四天後 2,3-BDO及副產物之影響115 圖4-34不同氯化鈣濃度對K. pneumoniae Wu2培養四天後2,3-BDO及副產物之影響116 圖4-35不同固定化條件對Klebsiella sp. Wu1培養四天生產2,3-BDO及副產物之影響120 圖4-36不同固定化條件對K. pneumoniae Wu2培養四天生產2,3-BDO及副產物之影響121 圖4-37 不同丙酮酸濃度對固定化與懸浮Klebsiella sp. Wu1生長情形126 圖4-38不同丙酮酸濃度對固定化與懸浮K. pneumoniae Wu2菌株生長情形 127 圖4-39不同丙酮酸濃度對固定化與懸浮之Klebsiella sp. Wu1菌株培養三天後生產2,3-BDO及副產物之影響128 圖4-40不同丙酮酸濃度對固定化與懸浮之K. pneumoniae Wu2 菌株培養三天生產2,3-BDO及副產物之影響129 圖4-41在曝氣條件與攪拌速率下Klebsiella sp. Wu1和K. pneumoniae Wu2生長情形132 圖4-42在曝氣條件與攪拌速率下Klebsiella sp. Wu1和K.pneumoniae Wu2對2,3-BDO與其他副產物之影響133 圖4-43利用酸鹼液調整pH對固定化與懸浮Klebsiella sp. Wu1菌株之生長情形136 圖4-44 利用磷酸緩衝液調整pH對固定化與懸浮Klebsiella sp. Wu1菌株之生長情形137 圖4-45利用酸鹼液調整pH對固定化與懸浮K. pneumoniae Wu2菌株之生長情形138 圖4-46利用磷酸緩衝液調整pH對固定化與懸浮K. pneumoniae Wu2菌株之生長情形139 圖4-47利用酸鹼液調整pH對Klebsiella sp. Wu1生產2,3-BDO產量及產力之影響140 圖4-48 利用磷酸緩衝液調整pH對Klebsiella sp. Wu1生產2,3-BDO產量及產力之影響141 圖4-49 利用酸鹼液調整pH對K. pneumoniae Wu2生產2,3-BDO產量及產力之影響142 圖4-50 利用磷酸緩衝液調整pH對K. pneumoniae Wu2生產2,3-BDO產量及產力之影響143 圖4-51不同溫度對固定化與懸浮Klebsiella sp. Wu1生長情形146 圖4-52 不同溫度對固定化與懸浮K. pneumoniae Wu2之生長情形147 圖4-53不同溫度對固定化與懸浮Klebsiella sp. Wu1生長2,3-BDO及副產物之影響 148 圖4-54不同溫度對固定化與懸浮K. pneumoniae Wu2生長2,3-BDO及副產物之影響149 圖4-55 額外添加不同界面活性劑Klebsiella sp. Wu1菌株之生長情形152 圖4-56 添加不同界面活性劑K.pneumoniae Wu2菌株之生長情形153 圖4-57添加不同界面活性劑Klebsiella sp. Wu1生產2,3-BDO及副產物之影響154 圖4-58添加不同界面活性劑K. pneumoniae Wu2生產2,3-BDO及副產物之影響155 圖4-59不同顆粒填充比與HRT對固定化 Klebsiella sp. Wu1在流體化床連續饋料之生長曲線158 圖4-60不同顆粒填充比與HRT對固定化K. pneumonia Wu2在流體化床連續饋料之生長曲線159 圖4-61不同顆粒填充比與HRT對固定化Klebsiella sp. Wu1在流體化床連續饋料對2,3-BDO產量之影響160 圖4-62不同顆粒填充比與HRT對固定化K. pneumonia Wu2在流體化床連續饋料對2,3-BDO產量之影響161 圖4-63初始甘油濃度對固定化Klebsiella sp. Wu1在流體化床連續饋料及批次饋料對2,3-BDO產量之影響165 圖4-64初始甘油濃度對固定化K. pneumoniae Wu2在流體化床 連續饋料及批次饋料對2,3-BDO產量之影響166 圖4-65固定化Klebsiella sp. Wu1在流體化床連續饋料中不同曝氣量對2,3-BDO產量之影響169 圖4-66固定化K. pneumoniae Wu2在流體化床連續饋料中不同曝氣量對2,3-BDO產量之影響170 表目錄 表2-1各種微生物利用碳源轉換2,3-BDO之研究文

獻13 表3-1污泥培養基組成30 表3-2 *Klebsiella sp. Wu1*培養基組成33 表3-3 *K. pneumoniae Wu2*培養基組成 33 表3-4本研究使用的PCR引子36 表3-5 PCR配方的組成36 表3-6 PCR的操作條件36 表3-7 SDS-PAGE組成38 表3-8 5X TBE (Tris-Borate-EDTA)緩衝液38 表3-9 GC-FID 型號及操作條件44 表4-1 *Klebsiella sp. Wu1*以不同PU比例濃度顆粒凝膠測試107 表4-2 *K. pneumoniae Wu2*.以不同PU比例濃度顆粒凝膠測試108 表4-3利用SEM觀察不同PU比例濃度固定化*Klebsiella sp. Wu1*之顆粒變化109 表4-4利用SEM觀察不同PU比例濃度固定化*K. pneumoniae Wu 2*之顆粒變化110 表4-5不同固定化製備方法對固定化*Klebsiella sp. Wu1*與*K. pneumoniae Wu2*之影響122 表4-6利用SEM 觀察固定化 *Klebsiella sp. Wu1*和*K. pneumoniae Wu2*之顆粒變化123 表4-7在不同顆粒填充比及水力滯留時間對固定化菌株在流體化床連續反應器之動力學參數162 表5-1 *Klebsiella sp. Wu1* 最佳培養基組成172 表5-2 *K. pneumoniae Wu2* 最佳培養基組成172

## REFERENCES

- 1.吳坤哲。2009。以固定化厭氧污泥與純菌株進行生物燃料之醱酵生產。國立成功大學博士論文。
- 2.Afschar, A. A., Vaz Rossel C. E., Jonas R., Chanto A. Q. and Schallar K. 1993. Microbial production and downstream processing of 2,3-butanediol. *Journal of Biotechnology*. 27: 317-329.
- 3.Afschar, A. S., Bellgardt K. H., Rossel C. E. V., Czok A. and Schaller K. 1991. The production of 2,3-butanediol by fermentation of high test molasses. *Applied Microbiology and Biotechnology*. 34: 582-585.
- 4.Alam, S., Capit, F., Weigand, W. A. and Hong, J. 1990. Kinetics of 2,3-butanediol fermentation by *Bacillus amyloliquefaciens* effect of initial substrate concentration and aeration. *J. Chem Technol Biotechnol*. 47: 71 – 84.
- 5.Asano, H., Myoga, H., Asano, M. and Toyao, M. 1992. Nitrification treatability of whole microorganisms immobilized by the PVA-freezing method. *Water Science & Technology* 26: 1037-1046.
- 6.Barret, E. L., Collins, E. B., Hall, B. J. and Mato, S. H. 1983. Production of 2,3-butylene glycol from whey by *Klebsiella pneumoniae* and *Enterobacter aerogenes*. *Journal of Dairy Science*. 66: 2507-2514.
- 7.Beronio Jr, P. B. and Tsao, G. T. 1993a. Optimization of 2,3-butanediol production by *Klebsiella oxytoca* through oxygen transfer rate control. *Biotechnol Bioeng*. 42: 1263-1269.
- 8.Beronio Jr, P. B. and Tsao G.T. 1993b. An energetic model for oxygen-limited metabolism. *Biotechnol Bioeng*. 42: 1270-1276.
- 9.Biebl, H., Menzel, K., Zeng, A. P. and Deckwer, W. D. 1999. Microbial production of 1,3-PDO. *Appl Microbiol Biotechnol*. 52: 289-297.
- 10.Biebl, H., Zeng, A. P., Menzel, K. and Deckwer, W. D. 1998. Fermentation of glycerol to 1,3-propanediol and 2,3-butanediol by *Klebsiella pneumoniae*. *Appl. Microbiol. Biotechnol*. 50, 24 – 29.
- 11.Cameron, D. C., Altaras, N. E. and Hoffman, M. L. 1998. Metabolic engineering of propanediol pathways *Biotechnol Prog*. 14:116-125.
- 12.Cao, G. M., Zhao, Q. X. and Sun, X. B. 2002. Characterization of nitrifying and denitrifying bacteria coimmobilized in PVA and kinetics model of biological nitrogen removal by coimmobilized cells[J]. *Enzyme and Microbial Technology*. 30: 49-55.
- 13.Cao, N., Xia, Y., Gong, C. S. and Tsao, G. T. 1977. Production of 2,3-butanediol from pretreated corn cob by *Klebsiella oxytoca* in the presence of fungal cellulose. *Appl Biochem Biotechnol*. 63: 129 – 39.
- 14.Champluvier, B., Francart, B. and Rouxhet, P. G. 1989. Co-Immobilized by adhesion of  $\beta$ -galactosidase in nonviable cells of *Kluyveromyces lactis* with *Klebsiella oxytoca* conversion of lactose into 2,3-BDL. *Biotechnol. Bioeng*. 34: 844 – 853.
- 15.Chang, I. S., Kim, C. I. and Nam, B. U. 2005. The influence of poly-vinylalcohol (PVA) characteristics on the physical stability of encapsulated Immobilized media for advanced wastewater treatment[J]. *Process Biochemistry*. 40: 3050 – 3054.
- 16.Chen, K. C. and Huang, C. T. 1988. Effect of the growth of *Trichosporon cutaneum* in calcium alginate gel beads upon bead structure and oxygen transfer characteristics. *Enzyme Microbial Technology*. 23: 311-320.
- 17.Cheng, K. K., Liu, H. J. and Liu, D. H. 2005. Multiple growth inhibition of *Klebsiella pneumoniae* in 1,3-propanediol fermentation. *Biotechnol Lett*. 27: 19-22.
- 18.Cheng, K. K., Liu, Q., Zhang, J. A., Li, J. P., Xu, J. M. and Wang, G. H. 2010. Improved 2,3-butanediol production from corncob acid hydrolysate by fed-batch fermentation using *Klebsiella oxytoca*. *Process Biochemistry*. 45: 613-616.
- 19.Cheng, K. K., Zhang, J. A., Liu, D. H., Sun, Y., Liu, H. J., Yang, M. D and Xu, J. M. 2007. Pilot-scale production of 1,3-PDO using *Klebsiella pneumoniae*. *Process Biochemistry*. 42: 740 – 744.
- 20.Chum, H. L. and Overend, R. P. 2001. Biomass and renewable fuels. *Fuel Processing Technology*. 71: 187-195.
- 21.Dharmadi, Y., Murarka, A. and Gonzalez, R. 2006. Anaerobic Fermentation of Glycerol by *Escherichia coli*: A New Platform for Metabolic Engineering. *Biotechnology and Bioengineering*. Vol. 94. No. 5: p821-829.
- 22.Doran, P. M and Bailey, J. E. 1986. Effects of Immobilized on growth, fermentation properties, and macromolecular composition of *Saccharomyces cerevisiae* attached to gelatin. *Biotechnol. Bioeng*. 28: 73-87.
- 23.Eiteman, M. A. and Miller, J. H. 1995. Effect of succinic acid on 2,3-bitanediol production by *Klebsiella oxytoca*. *Biotechnology Letters*. 17: 1057-1062.
- 24.Flickinger, M. C. 1980. Current biological research in conversion of cellulosic carbohydrates into liquid fuels: how far have we come. *Biotechnol Bioeng* 22: 27-48.
- 25.Frazer, F. R. and McCaskey, T. A. 1991. Effect of selected components of acid-hydrolysed hardwood on conversion of D-xylose to 2,3-butanediol by *Klebsiella pneumoniae*. *Enzyme and Microbial Technology*. 13: 110-115.
- 26.Gao, J., Xu, H., Li, Q. J., Feng, X. H. and Li, S. 2010. Optimization of medium for one-step fermentation of inulin extract from Jerusalem artichoke tubers using *Paenibacillus polymyxa* ZJ-9 to produce R,R-2,3-butanediol. *Bioresour Technol* 101: 7087 – 93.
- 27.Garg, S. K. and Jain, A. 1995. Fermentative production of 2,3-butanediol: a review. *Bioresource Technol*. 51: 103-109.
- 28.Giordano, R. L., Hirano, P. C., Goncalves, L. R. and Netto, W. S. 2000. Study of biocatalyst to produce ethanol from starch Coimmobilization of glucoamylase and yeast in gel. *Appl. Biochem Biotechnol*. 84-86: 643-654.
- 29.Grizeau, D., and Navarro, J. M. 1986. Glycerol production by *Dunaliella tertiolecta* immobilized within Ca-alginate beads. *Biotechnol Lett*. 8: 261 – 264.
- 30.Grover, B. S., Garg, S. K. and Verma, J. 1990. Production of 2,3-butanediol from wood hydrolysate by *Klebsiella pneumoniae*. *World Journal of Microbiology and Biotechnology*. 6: 328-332.
- 31.Hai, T., Syed, Q. 1995. Poly vinyl alcohol hydrogels: 2. Effects of processing parameters on structure and properties[J]. *Polymer*. 36: 2531 – 2539.
- 32.Hao, J., Lin, R., Zheng, Z., Liu, H. and Liu, D. 2008. Isolation and characterization of microorganisms

able to produce 1,3-propanediol under aerobic conditions. *World J. Microbiol. Biotechnol.* 24: 1731-1740. 33.Harden, A. and Walpole, G. S. 1906. 2,3-Butylene glycol fermentation by *Aerobacter aerogenes*. *Proc. Royal. Soc. B77*: 399-405. 34.Hardt, H. and Lamport, D. T. A. 1982. Hydrogen fluoride saccharification of wood. *Biotechnology and bioengineering.* 24: 903-918. 35.Hespell, R. B. 1996. Fermentation of xylan, corn fiber, or sugars to acetoin and butanediol by *Bacillus polymyxa* strains. *Current Microbiology.* 32: 291-296. 36.Hilaly, A. K. and Binder, T. P. 2002. Method of recovering 1,3-propanediol from fermentation broth. United States Patent. 6: 479-716. 37.Hilge-Rotmann, B. and Rehm, H. J. 1990. Comparison of fermentation properties and specific enzyme activities of free and calcium-alginate-entrapped *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 33: 54-58. 38.Huang, Y. L., Mann, K., Novak, J. M., and Yang, S. T. 1998. Acetic acid production from fructose by *Clostridium formicoaceticum* immobilized in a fibrous-bed bioreactor. *Biotechnol. Prog.* 14(5), 800-806. 39.Jamuna, R., Saswathi, N., Sheela, R. and Ramakrishna S. V. 1993. Synthesis of cyclodextrin glucosyl transferase by *Bacillus cereus* for the production of cyclodextrins. *Appl. Biochem. Biotechnol.* 43(3): 163-176. 40.Jansen, N. B., Flickinger, M. C. and Tsao, G. T. 1984. Application of bioenergetics to modeling the microbial conversion of D-xylose to 2,3-butanediol. *Biotechnology and Bioengineering.* 27: 573-582. 41.Jansen, N. B., Flickinger, M. C. and Tsao, G. T. 1984. Application of bioenergetics to modeling the microbial conversion of D-xylose to 2,3-butanediol. *Biotechnology and Bioengineering.* 27: 573-582. 42.Jansen, N. B., Flickinger, M.C. and Tsao, G. T. 1984. Production of 2,3-butanediol from d-xylose by *Klebsiella oxytoca* ATCC 8724. *Biotechnol Bioeng.* 26: 362-369. 43.Jansen, N. B. and Tsao, G. T. 1983. Bioconversion of pentoses to 2,3-butanediol by *Klebsiella pneumoniae*. *Adv Biochem. Engng Biotechnol.* 27: 85-100. 44.Jansen, N. B., Flickinger, M. C. and Tsao, G. T. 1984. Production of 2,3-butanediol from Dxylose by *Klebsiella oxytoca* ATCC 8724. *Biotechnol. Bioeng.* 26: 362-369. 45.Jarvis, G. N, Moore E. R. B. and Thiele, J. H. 1997. Formate and ethanol are the major products of glycerol fermentation produced by a *Klebsiella planticola* strain isolated from red deer *J. Appl Microbiol.* 83: 166-174. 46.Ji, X. J., Huang, H., Li, S., Du, J. and Lian, M. 2008. Enhanced 2,3-butanediol production by altering the mixed acid fermentation pathway in *Klebsiella oxytoca*. *Biotechnol Lett.* 30: 731-734. 47.Ji, X. J., Huang, H., Zhu, J. G., Ren, L. J., Nie, Z. K. and Du, J. 2010. Engineering *Klebsiella oxytoca* for efficient 2,3-butanediol production through insertional inactivation of acetaldehyde dehydrogenase gene. *Appl Microbiol Biotechnol.* 85: 1751 – 1758. 48.Ji, X. J., Huang, H., Du, J., Zhu, J. G., Ren, L. J., Hu, N. and Li, S. 2009. Enhanced 2, 3-butanediol production by *Klebsiella oxytoca* using a two-stage agitation speed control strategy. *Bioresour. Technol.* 100: 3410 – 3414. 49.Jimezez-Perez, M. V., Sanchez-Castullo, P. and Romera, O. 2004. Fernandez-Moreno, D. and Perez-Martinez, C. Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure. *Enzyme and Microbial Technology.* 34: 392-398. 50.Junter, G. A., Coquet, L., Vilain, S., and Jouenne, T. 2002. Immobilized-cell physiology: current data and the potentialities of proteomics. *Enzyme Microb. Technol.* 31: 201-212. 51.Karel, S. F., Libicki, S. B., and Robertson, C. R. 1985. Review article number 17. The Immobilizedof whole cells: engineering principles. *Chem. Eng. Sci.* 40(8): 1321-1354. 52.Kargi, F. and Eyiisleyen, S. 1995. Batch biological treatment of synthetic wastewater in a fluidized bed containing wire mesh sponge particle. *Enzyme and Microbial Technology.* 17: 119-123. 53.Laube, V. M., Groleau, D. and Martin, S. M. 1984. 2,3-Butanediol production from xylose and other hemicellulosic components by *Bacillus polymyxa*. *Biotechnology Letters.* 6: 257-262. 54.Laube, V. M., Groleau, D. and Martin, S. M. 1984. The effect of yeast extract on the fermentation of 1221 glucose to 2,3-butanediol by *Bacillus polymyxa*. *Biotechnol. Lett.* 6: 535-40.1222. 55.Ledingham, G. A. and Neish, A. C.1954. Fermentative production of 2,3-butanediol. In *Industrial Fermentations*, Vol. 2, ed. L. A. Underkofler, R. J. Hickey. Chemical Publishing Company. New York. USA. pp: 27-94. 56.Lee, S. Y., Park, S. H. M. Eng., Hong, S.H., M, Eng., Young, L., M, Eng. and Lee, S. H. 2005. Biopolymers Polyesters. Fermentative Production of Building Blocks for Chemical Synthesis of Polyesters. p265-274. 57.Li, D., Dai, J. Y. and Xiu, Z. L. 2010a. A novel strategy for integrated utilization of Jerusalem artichoke stalk and tuber for production of 2,3-butanediol by *Klebsiella pneumoniae*. *Bioresour Technol.* 101:8342 – 8347. 58.Li, H. Z., Wang, J. L. and Wen, X. H. 2002. Improvement of PVAH3BO3 Immobilizedmethod [J]. *Environmental Sciences Research.* 15(5): 25 – 28. 59.Li, Z., Teng, H. and Xiu, Z. 2010. Aqueous two-phase extraction of 2,3-butanediol from fermentation broths using an ammonium sulfate system. *Process Biochem.* 45 (5): 731-737. 60.Liua, Z., Qina, J., Gao, C., Huaa, D., Maa, C., Li, L., Wanga, Y. and Xu, P. 2011. Production of (2S,3S)-2,3-butanediol and (3S)-acetoin from glucose using resting cells of *Klebsiella pneumonia* and *Bacillus subtilis*. *Bioresource Technology* 102 : 10741 – 10744. 61.Long, S. K. and Patrick, R. 1961. Production of 2,3-butylene glycol from citrus wastes I. The *Aerobacter aerogenes* fermentation. *Advances in Applied Microbiology.* 9: 244-248. 62.Long, S. K. and Patrick, R. 1963. The present status of the 2,3-butylene glycol fermentation. *Advances in Applied Microbiology.* 5: 135-155. 63.Long, Z., Huang, Y. H. and Cai, Z. L. 2004. Immobilizedof *Acidithiobacillus ferrooxidans* by a PVA-boric acid method for ferrous sulphate oxidation[J]. *Process Biochemistry.* 39: 2129 – 2133. 64.Ma, C. Q., Wang, A. L., Qin, J. Y., Li, L. X., Ai, X. L. and Jiang, T.Y. 2009. Enhanced 2,3-butanediol production by *Klebsiella pneumoniae* SDM. *Appl Microbiol Biotechnol.* 82:49 – 57. 65.Ma, C., Wang, A., Qin, J., Li, L., Ai, X., Jiang, T., Tang, H. and Xu, P. 2009. Enhanced 2,3-butanediol production by *Klebsiella pneumoniae* SDM. *Appl Microbiol. Biotechnol.* 82: 49-57. 66.Magee, R. J. and Kosaric, N. 1987. The microbial production of 2,3-butanediol. *Advances in Applied Microbiology.* 32: 89-161. 67.Magee, R. J. and Kosaric, N. 1987. The microbial production of 2,3-butanediol. Microbial production and downstream processing of 2,3-butanediol. *Journal of Biotechnology.* 27: 317-329. 68.Mickelson, M. N. and Werkman, C. H. 1940. Formation of trimethylene glycol from glycerol by *Aerobacter*. *Enzymologia.* 8: 252-256. 69.Moes, J., Griot, M., Keller, J., Heinzle, E., Dunn, I. J. and Bourne, J. R. 1985. A microbial culture with oxygen supply on optical purity of 2,3-butanediol produced by *Paenibacillus polymyxa* in chemostat culture. *Biotechnol Lett.* 20: 1133-1138. 70.Moes, J., Griot, M., Keller, J., Heinzle, E., Dunn, I. J. and Bourne, J. R. 1985. A microbial culture with oxygensensitive product distribution as a potential tool for characterizing bioreactor oxygen transport. *Biotechnol Bioeng.* 27:482 – 489. 71.Mu, Y., Ying, Mu., Hu, Teng., Dai-Jia, Zhang., Wei Wang. and Zhi-Long.2006. Microbial Production of 1,3-propanediol by *Klebsiella pneumoniae* using crude glycerol from biodiesel. *Biotechnology*

Letters. Vol 28. No. 21. p 1755-1759. 72.Nakashimada, Y., Marwoto, B., Kashiwamura, T., Kakizono, T. and Nishio N. 2000. Enhanced 2,3-butanediol production by addition of acetic acid in *Paenibacillus polymyxa*. *Journal of Bioscience and Bioengineering*. 90: 661-664. 73.Neish, A. C., Blackwood, A. C. and Ledingham, G. A. 1945. Dissimilation of glucose by *Bacillus subtilis* Ford-strain. *Can J Res*. 23B:290 – 296. 74.Nilegaonkar, S. S., Bhosale, S. B., Kshirsagar, D. C. and Kapadi, A. H. 1992.Production of 2,3-butanediol from glucose by *Bacillus licheniformis*. *World J Microbiol Biotechnol*. 8:378 – 381. 75.Norton, S. and D'Amore, T. 1994. Physiological effects of yeast cell immobilization: applications for brewing. *Enzyme Microb. Technol*. 16: 365-375. 76.Papanikolaou, S., Muniglia, L., Chevalot, I., Aggelis, G. and Marc, I. 2002. *Journal of Applied Microbiology*. 92: 737-744. 77.Perego, P., Converti, A., Del Borghi, A. and Canepa, P. 2000. 2,3-Butanediol production by *Enterobacter aerogenes*: selection of the optimal conditions and application to food industry residues. *Bioprocess Engineering*. 23: 613-620. 78.Perlman, D. 1944. Production of 2,3-butylene glycol from wood hydrolyzates. *Industrial and Engineering Chemistry*. 36: 803-804. 79.Petrov, K. and Petrova, P. 2010. Enhanced production of 2,3-butanediol from glycerol by forced pH fluctuations. *Appl Microbiol Biotechnol*. 87:943 – 949. 80.Petrov, K. and Petrova, P. 2009. High production of 2,3-butanediol from glycerol by *Klebsiella pneumoniae* G31. *Appl Microbiol Biotechnol*. 84:659 – 665. 81.Preininger, C. and Chiarelli, P. 2001. Immobilized oligonucleotides on crosslinked poly vinyl alcohol for application in DNA chips[J]. *Talanta*. 55: 973 – 980. 82.Qin, J. Y., Xiao, Z. J., Ma, C. Q., Xie, N. Z., Liu, P. H. and Xu, P. 2006. Production of 2,3-butanediol by *Klebsiella pneumoniae* using glucose and ammonium phosphate. *Chin J. Chem Eng*. 14: 132-136. 83.Qureshi, N. and Cheryan, M. 1989. Production of 2,3-butanediol by *Klebsiella oxytoca*. *Appl Microbiol Biotechnol*. 30: 440 – 443. 84.Ramachandran, K.B. and Goma, G. 1988. 2,3-butanediol production from glucose by *Klebsiella pneumoniae* in a cell recycle system. *J. Biotechnol*. 9: 39-46. 85.Ramakrishna, S. V. and Prakasham, R. S. 1999. Microbial fermentations with immobilized cells. *Curr. Sci*. 77: 87-100. 86.Raspoet, D., Pot, B., De Deyn, D., De Vos P., Kessters, K. and De Ley, J. 1991. Differentiation between 2,3-butanediol producing *Bacillus licheniformis* and *Bacillus polymyxa* strains by fermentation product profiles and whole-cell protein electrophoretic patterns. *Syst. Applied Microbiology*. 14: 1-7. *Res. Comm*. 74: 898-902. 87.Rosevear, A. 1984. Immobilised biocatalysts – a critical review. *J. Chem. Technol. Biotechnol*. 34B: 127-150. 88.Sabalayrolles, J. M. and Goma, G. 1984. Butanediol production by *Aerobacter aerogenes* NRRB199: effect of initial substrate concentration and aeration agitation. *Biotechnol. Bioeng*. 26: 148-155. 89.Seo, J. K., Jung, I. H., Kim, M. R., Kim, B. J., Nam, S. W. and Kim, S. K. 2001. Nitrification performance of nitrifiers immobilized in PVA for a marine recirculating aquarium system. *Aquacultural Engineering*. 24: 191-94. 90.Spencer, J. F. T. and Shu, P. 1957. Polyhydric alcohol production by osmophilic yeasts: effect of oxygen tension and inorganic phosphate concentration. *Can J Microbiol*. 3:559. 91.Stahly, G. L. and Werkman, C. H. 1942. Origin and relationship of acetylmethylcarbinol to 2,3-butylene glycol in bacterial fermentations. *Biochem. J*. 36: 575-581. 92.Stanier, R. Y. and Adams. G. A. 1944. The nature of the *Aeromonas* fermentation. *Biochem. J*. 38: 168-171. 93.Stormer, F. C. 1997. Evidence for regulation of *Aerobacter aerogenes* pH 6 acetolactate-forming enzyme by acetate ion. *Biochemical and Biophysical Research Communications*. 74: 898-902. 94.Sun, L. H., Wang, X. D., Dai, J. Y. and Xiu, Z. L. 2009. Microbial production of 2,3-butanediol from Jerusalem artichoke tubers by *Klebsiella pneumoniae*. *Appl Microbiol Biotechnol*. 82: 847 – 852. 95.Syu, M. J. 2001. Biological production of 2,3-butanediol. *Appl. Microbiol. Biotechnol*. 55: 10 – 18. 96.Szczesna, M. and Galas, E. 2001. *Bacillus subtilis* cells immobilised in PVA-cryogels[J]. *Biomolecular Engineering*. 17: 55 – 63. 97.Szczesna, M., Galas, E. and Bielecki, S. 2001. PVA-biocatalyst with entrapped viable *Bacillus subtilis* cells[J]. *Journal of Molecular Catalysis B: Enzymatic*. 11: 671 – 676. 98.Train, A.V. and Chambers, R.P. 1987. The dehydration of fermentative 2,3-butanediol into methyl ethyl ketone. *Biotechnol. Bioeng*. 29: 343-351. 99.Tsao, G. T. 1978. Conversion of biomass from agriculture into useful products. Final Report. USDDE Contact No. EG-77-S-02-4298. 100.Tsao, J. L., Guffanti, A. A. and Montville, T. J. 1992. Conversion of pyruvate to acetoin helps to maintain pH homeostasis in *Lactobacillus plantarum*. *Appl Environ Microbiol*. 58:891 – 894. 101.van Houdt, R., Aertsen ,A. and Michiels, C. W. 2007.Quorum-sensing-dependent switch to butanediol fermentation prevents lethal medium acidification in *Aeromonas hydrophila*. AH-1N. *Res Microbiol*. 158: 379 – 385. 102.Vega, J. L., Clausen, E. C., and Gaddy, J. L. 1988. Biofilm reactors for ethanol production. *Enzyme Microb. Technol*. 10: 390-402. 103.Vogelsang, C., Golembiewski, K. and Ostgaard, K. 1999. Effect of preservation of gel entrapped nitrifying sludge. *Water Research*. 33: 164-168. 104.Voloch, M., Ladisch, M. R., Rodwell, V. and Tsao, G. T. 1983. Reduction of acetoin to 2,3-butanediol in *Klebsiella pneumoniae*: a new model. *Biotechnol. Bioeng*. 25, 173-183. 105.Wang, A. L., Wang, Y., Jiang, T. Y., Li, L. X., Ma, C. Q. and Xu, P. 2010. Production of 2,3-butanediol from corncob molasses, a waste by-product in xylitol production. *Appl Microbiol Biotechnol*. 87: 965 – 970. 106.Wang, J. L., Horan, N. and Qian, Y. 2000. The radial distribution and bioactivity of *Pseudomonas* sp. immobilized in calcium alginate beads[J]. *Process Biochemistry*. 35: 465 – 469. 107.Wang, J. L. and Liu, P. 1996. Comparison of citric acid production by *Aspergillus niger* immobilized in gels and cryogels of polyacrylamide[J]. *Journal of Industrial Microbiology*. 16: 351 – 353. 108.Wang, J. L. and Shi, H. C. 1998. The research and development of microbial Immobilized using polyvinyl alcohol (PVA) gel[J]. *Journal of Industrial Microbiology*. 28(2): 35 – 39. 109.Wang, Z. X., Zhuge, J., Fang, H. and Prior, B. A. 2001. Glycerol production by microbial fermentation: a review. *Biotechnology Advances*. 19: 201 – 223. 110.Willke, T. and Vorlop, K. 2008. Biotransformation of glycerol into 1,3-PDO. *Eur. J. Lipid Sci. Technol*. 110:831 – 840. 111.Wu, K. J., Ganesh, Saratale, D., Lo, Y. C., Chen, W. M., Tseng, Z. J., Chang, M. C., Tsai, B. C., Su, A. and Chang J. S. 2008. Simultaneous production of 2,3-butanediol, ethanol and hydrogen with a *Klebsiella* sp. strain isolated from sewage sludge. *Bioresource Technology*. 99: 7966-7970. 112.Wu, K. J., Saratale., G. D., Lo, Y. C., Chen, W. M., Tseng, Z. J., Chang, M. C., Tsai, B. C., Sud, A. and Chang, J. S. 2008. Simultaneous production of 2,3-butanediol, ethanol and hydrogen with a *Klebsiella* sp. strain isolated from sewage sludge. *Bioresource Technology*. 99: 7966 – 7970 113.Yan, J. and Hu, Y. Y. 2009. Partial nitrification to nitrite for treating ammonium-rich organic wastewater by immobilized biomass system.. *Bioresource Technol*. 100: 2341-2347. 114.Yan, J., Jettem, M.,

Rang, J. and Hu, Y. 2010. Comparison of the effects of different salts on aerobic ammonia oxidizers for treating ammonium-rich organic wastewater by free and sodium alginate immobilized biomass system. *Chemosphere*. 81: 669-673. 115.

Yu, E. K., Deschatelets, L., Louis-Seize, G. and Saddler, J. N. 1985. Butanediol production from cellulose and hemicellulose by *Klebsiella pneumoniae* grown in sequential coculture with *Trichoderma harzianum*. *Applied and Environmental microbiology*. 50: 924-929. 116.

Yu, E. K., Levitin, N. and Saddler, J. N. 1982. Production of 2,3-butanediol by *Klebsiella pneumoniae* grown on acid hydrolysed wood hemicellulose. *Biotechnology Letters*. 4: 741-746. 117.

Yu, E. K. C. and Saddler, J. N. 1982a. Enhanced production of 2,3-butanediol by *Klebsiella pneumoniae* grown on high sugar concentrations in the presence of acetic acid. *Applied and Environmental microbiology*. 44 : 777-784. 118.

Yu, E. K. C. and Saddler, J. N. 1982b. Power solvent production by *Klebsiella pneumoniae* grown on sugars present in wood hemicellulose. *Biotechnology Letters*. 4: 121-126. 119.

Yu, E. K. C. and Saddler, J. N. 1983. Fed batch approach to production of 2,3-butanediol by *Klebsiella pneumoniae* grown on high substrate concentrations. *Applied and Environmental microbiology*. 46: 630-635. 120.

Yu, E. K. C., Deschatelets, L., Levitin, N. and Saddler, J. N. 1984. Production of 2,3-butanediol from HF-hydrolyzed aspen wood. *Biotechnology Letters*. 6: 611-614. 121.

Yu, E. K. and Saddler, J. N. 1982. Enhanced production of 2,3-butanediol by *Klebsiella pneumoniae* grown on high sugar concentrations in the presence of acetic acid. *Appl. Environ. Microbiol.* 44:777-784. 122.

Yutaka, N., Bambang M., Takashi K., Toshihide K. and Naomichi N. 2000. Enhanced 2,3-Butanediol Production by Addition of Acetic Acid in *Paenibacillus polymyxa*. *Journal of Bioscience and Bioengineering*. Vol. 90. No. 6: 661-666 123.

Zeng, A.P., Biebl, H., Schlieker, H. and Deckwer, W.D. 1993. Pathway analysis of glycerol fermentation by *Klebsiella Pneumoniae*: Regulation of reducing equivalent balance and product formation. *Enzyme and Microbial Technology* 15: 770-779. 124.

Zeng, A. P., Byun, T. G., Posten, C. and Deckwer, W. D. 1994. Use of respiratory quotient as a control parameter for optimum oxygen supply and scale-up of 2,3-butanediol production under microaerobic conditions. *Biotechnology and Bioengineering*. 44: 1107-1114. 125.

Zeng, A. P., Biebl, H. and Deckwer, W. D. 1991. Production of 2,3-butanediol in a membrane bioreactor with cell recycle. *Appl Microbiol Biotechnol.* 34: 463 – 8. 126.

Zeng, A. P., Biebl, H. and Deckwer, W. D. 1990. Effect of pH and acetic acid on growth and 2,3-butanediol production of *Enterobacter aerogenes* in continuous culture. *Appl. Microbiol. Biotechnol.* 33: 485-489. 127.

Zhang, L. Y, Sun, J. A., Hao, Y. L., Zhu, J. W., Chu, J. and Wei, D. Z. 2010a. Microbial production of 2,3- butanediol by a surfactant serrawettin-deficient mutant of *Serratia marcescens* H30. *J Ind Microbiol Biotechnol.* 37:857 – 862. 128.

Zhang, L. Y., Yang, Y. L., Sun, J. A., Shen, Y. L., Wei, D. Z. and Zhu, J. W. 2010. Microbial production of 2,3- butanediol by a mutagenized strain of *Serratia marcescens* H30. *Bioresour Technol.* 101:1961 – 1967. 129.

Zhao, Y. N., Chen, G. and Yao, S. J. 2006. Microbial prouction of 1,3-PDO from glycerol by encapsulated *Klebsiella pneumoniae*. *Biochemical Engineering Journal.* 32: 9399.