

利用獸疫鏈球菌生產透明質酸之研究

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摘要

本研究主要是研究Streptococcus zooepidemicus BCRC 15414在批次發酵下，探討不同培養條件對生產hyaluronic acid (HA)之分子量及特性影響。這些不同培養條件包含不同葡萄糖濃度(0-40 g/L)、攪拌速度(50 and 150 rpm)、曝氣速率(0.5 and 2 Lmin⁻¹)、絕對厭氧及添加不同NaCl濃度(0-5%, w/v)。實驗結果顯示出在pH為9.0的條件下，當攪拌速率為150 rpm且葡萄糖濃度為20 g/L時，HA產量可達最大值(1.79 g/L)且分子量為 1.76×10^6 Da；當曝氣速率為2.0 Lmin⁻¹時，HA產量可達2.05 g/L且分子量為 1.97×10^6 Da。因此，攪拌速度和曝氣速率對於細胞生長和HA生產是很重要的影響。另外，在利用固定化S. zooepidemicus PVA (polyvinyl alcohol)顆粒進行批次發酵生產HA方面，實驗結果顯示出利用固定化S. zooepidemicus PVA顆粒進行批次發酵生產，最好的HA產量約為1.0 g/L。除此之外，本實驗將純化後的HA樣品以NMR (nuclear magnetic resonance)、GPC (gel permeation chromatography)及EA (elemental analyzer)進行分析，確定產物之結構及分子量範圍，結果顯示此產物確實為 HA。在HA發酵過程中，由於HA累積而使發酵液黏度增加。因此，氧氣質傳速率會明顯地減少。在培養期間，氧氣質傳係數是扮演重要角色。所以，本研究主要探討攪拌速度、曝氣速率及HA溶液黏度對kLa值(oxygen mass transfer coefficient)的影響。實驗結果顯示出攪拌速度和曝氣速率增加時，kLa值也會隨之增加。當攪拌速度為300 rpm，kLa值為最大，其值約為0.3787 min⁻¹；當曝氣速率為2.0 Lmin⁻¹，kLa值為最大，其值約為0.1328 min⁻¹。此外，本研究亦探討在批次發酵系統下，用S. zooepidemicus生產胞外多醣HA之發酵動力學。本實驗所用simple model是以Logistic equation模擬菌體生長、Luedeking-Piret equation來模擬HA生產、而葡萄糖和氧氣消耗則是利用Luedeking- Piret-like equation模擬得知。

關鍵詞：獸疫鏈球菌；透明質酸；固定化；氧氣質傳係數

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參考文獻

- 李孫榮，張錦松，張錦輝，陳健民和曾如娟編著。1995。環工單元操作。高立圖書有限公司。台北。台灣。
- 高海軍、陳堅、管軼眾、堵國成和傅世儀。1999。獸疫鏈球菌搖瓶發酵法生產透明質酸。無錫輕工業大學學報。18:17-22。
- 高海軍。1999。S. *zooepidemicus*生物合成透明質酸的過程優化與代謝路徑分析。無錫輕工業大學碩士論文。江蘇。
- 張文會。1997。皮膚的乾燥與保濕。日用化學品科學。93:9-11。
- 郭學平，凌沛學，王春喜和張天民。2000。透明質酸的生產。藥物生物技術。7:61-64。
- 陳國誠，1992。微生物酵素工程學。藝軒圖書出版社。台北。台灣。
- 曾義雄。1989。細菌代謝。藝軒圖書出版社。台北。台灣。8:218-224。
- 楊文彬，2000。偶氮染料於PVA固定化菌體顆粒內的擴散特性及生物分解動力學之研究。國立清華大學碩士論文。新竹。
- 謝慧冰。2005。攪拌剪應力是玻尿酸發酵放大設計之關鍵因素。國立成功大學化工工程學系碩士論文。台南。
- Abbe, K., Takahashi, S. and Yamada, T. 1982. Involvement of oxygen-sensitive pyruvate formate-lyase in mixed-acid fermentation by *Streptococcus mutans* under strictly anaerobic conditions. *Journal of Bacteriology* 152:175-182.
- Adams, D. H., Wang, L., Hubscher, S. G. and Neuberger, J. M. 1989. Hepatic endothelial cells. Targets in liver allograft rejection? *Transplantation* 47:479-482.
- Adams, M. E., Lussier, A. J. and Peyron, J. G. 2000. A risk-benefit assessment of injections of hyaluronan and its derivatives in the treatment of osteoarthritis of the knee. *Drug Safety* 23:115-130.
- Akasaka, H., Seto, S., Yanagi, M., Fukushima, S. and Mitsui, T. 1988. Industrial production of hyaluronic acid by *Streptococcus zooepidemicus*. *Journal of the Society Cosmetic of Chemists Japan* 22:35-42.
- Aksu, Z. and Bulbul, G. 1999. Determination of the effective diffusion coefficient of phenol in Ca-alginate-immobilized *P. putida* beads. *Enzyme and Microbial Technology* 25:344-348.
- Alam, C. A., Seed, M. P. and Willoughby, D. A. 1995. Angiostasis and vascular regression in chronic granulomatous inflammation induced by diclofenac in combination with hyaluronan in mice. *Journal of Pharmacy and Pharmacology* 47:407-412.
- Al-Assaf, S., Meadows, J., Phillips, G. O., Williams, P. A. and Parsons, B. J. 2000. The effect of hydroxyl radicals on the rheological performance of hylan and hyaluronan. *International Journal of Biological Macromolecules* 27:337-348.
- Altman, R. D. and Moskowitz, R. 1998. Intraarticular sodium hyaluronate (HyalganR) in the treatment of patients with osteoarthritis of the knee: a randomized clinical trial. *Journal of Rheumatology* 25:2203-2212.
- Amanullah, A., Tuttiett, B. and Nienow, A. 1998. Agitator speed and dissolved oxygen effects in xanthan fermentations. *Biotechnology and Bioengineering* 57:198-210.
- Armstrong, D. C. and Johns, M. R. 1997. Culture conditions affect the molecular weight properties of hyaluronic acid produced by *Streptococcus zooepidemicus*. *Applied and Environmental Microbiology* 63:2759-2764.
- Armstrong, D. C., Cooney, M. J. and John, M. R. 1997. Growth and amino acids requirements of hyaluronic-acid-producing *Streptococcus zooepidemicus*. *Applied Microbiology and Biotechnology* 47:309-312.
- Arvidson, S. A., Todd Rinehart, B. and Gadala-Maria, F. 2006. Concentration regimes of solutions of levan polysaccharide from *Bacillus* sp. *Carbohydrate Polymers* 65:144-149.
- Ashbaugh, C. D., Alberti, S. and Wessels, M. R. 1998. Molecular analysis of the capsule gene region of group A *Streptococcus*: the hasAB genes are sufficient for capsule expression. *Journal of Bacteriology* 180:4955-4959.
- Asteriou, T., Vincent, J-C., Tranchepain, F. and Deschrevel, B. 2006. Inhibition of hyaluronan hydrolysis catalysed by hyaluronidase at high substrate concentration and low ionic strength. *Matrix Biology* 25:166-174.
- Atkins, E. D. T., Meader, D. and Scott, J. E. 1980. Model for hyaluronic-acid incorporating intramolecular hydrogen-bonds. *International Journal of Biological Macromolecules* 2:318-319.
- Axelsson, A. and Persson, B. 1988. Determination of effective diffusion coefficients in calcium alginate gel plates with varying yeast cell content. *Applied Biochemistry and Biotechnology* 18:231-250.
- Bai, M., Brown, K. A., Fischer, W., Roser, T., Tsoupas, N. and Van Zeijts, J. 2000. Adiabatic excitation of longitudinal bunch shape oscillations. *Physical Review Special Topics-Accelerators and Beams Volume 3*, 064001. Brookhaven National Laboratory, Upton, New York 11973.
- Bailey, J. E. 1977. *Biochemical Engineering Fundamentals*, McGrawHill Publishing Company, New York p.424.
- Baker, C. J. and Kasper, D. L. 1976. Microcapsule of type III strains of group B *Streptococcus*: production and morphology. *Infection and Immunity* 13:189-194.
- Balazs, E. A. 1983. Sodium hyaluronate and viscosurgery. In Miller, D. and Stegmann, R. (eds.), *Healon, a guide to its use in ophthalmic surgery*. John Wiley, New York, USA.
- Balazs, E. A. 1984. Hyaluronic acid: its structure and use. *Polymers in Cosmetics* 99:65-72.
- Balazs, E. A., Briller, S. and Denlinger, J. L. 1981. Na-hyaluronate molecular size variations in equine and arthritic synovial fluid and effect on phagocytic cells. *Seminars in Arthritis and Rheumatism* 11:141-143.
- Balazs, E. A., Cowman, M. K. and Briller, S.

O. 1983. On the limiting viscosity number of hyaluronan in potassium phosphate buffers between pH 6.5 and 8. *Biopolymers* 22:589-591. 33. Bang, W., Nikov, I., Delmas, H. and Bascoul, A. 1998. Gas-liquid mass transfer in a new three-phase stirred airlift reactor. *Journal of Chemical Technology and Biotechnology* 72:137-142. 34. Bauchop, T. and Elsden, S. J. 1960. The growth of micro-organisms in relation to their energy supply. *The Journal of General Microbiology* 23:457-469. 35. Benyhaia, F., Jones, L. and Plantaz, D. 1996a. Mass transfer in a rectangular airlift reactor. *Chemical Engineering and Technology* 32:1128-1137. 36. Benyhaia, F., Jones, L. and Plantaz, D. 1996b. Mass transfer studies in pneumatic reactors. *Chemical Engineering and Technology* 19:425-431. 37. Bertheim, U. and Hellstrom, S. 1994. The distribution of hyaluronan in human skin and mature, hypertrophic and keloid scars. *British Journal of Plastic Surgery* 47:483-491. 38. Bibal, B., Goma, G., Vayssier, Y. and Pareilleux, A. 1988. Influence of pH, lactose and lactic acid on the growth of *Streptococcus cremoris*: a kinetic study. *Applied Microbiology and Biotechnology* 28:340-344. 39. Bibal, B., Vayssier, Y., Tournou, M. and Pareilleux, A. 1989. Enhanced inhibitory effect of lactic acid on growth kinetics of *Streptococcus cremoris* during nutritional medium limitations. *Applied Microbiology and Biotechnology* 30:630-635. 40. Biotechnology General Corporation. 1986. Method of producing high molecular weight sodium hyaluronate by fermentation of *Streptococcus*. World Patent WO 8604355 A. 41. Blumberg, B. S. and Oster, G. 1954. Light-scattering studies on hyaluronic acid. *Science* 120:432-433. 42. Blumberg, B. S., Ogston, A. G., Lowther, D. A. and Rogers, H. J. 1958. Physicochemical properties of hyaluronic acid formed by *Streptococcus haemolyticus*. *Biochemical Journal* 70:1-4. 43. Bother, H. and Wik, O. 1987. Rheology of hyaluronate. *Acta Otolaryngologica Supplement* 442:25-30. 44. Bothner, H., Waaler, T. and Wik, O. 1988. Limiting viscosity number and weight average molecular weight of hyaluronate samples produced by heat degradation *International Journal of Biological Macromolecules* 10:287-291. 45. Bourne, J. R., Zurita, E. P. and Heinze, E. 1992. Bioreactor scale-up for the oxygen-sensitive culture *Bacillus subtilis*: The influence of stirrer shaft geometry. *Biotechnology Progress* 8:580-582. 46. Bracke, J. W. and Thacker, K. 1985. Hyaluronic acid from bacterial culture. U.S. patent 4517295. 47. Bravo, P. and Gonzalez, G. 1991. Continuous ethanol fermentation by immobilized yeast cell in a fluidized-bed reactor. *Journal of Chemical Technology and Biotechnology* 52:127-134. 48. Brown, M., Marriot, C. and Martin, G. P. 1995. The effect of hyaluronan in the in vitro deposition of Diclofenac within the skin. *International Journal Tissue Reactions* 17:33-37. 49. Brown, W. and Chitumbo, K. 1975. Solute diffusion in hydrated polymer networks Part 2. Polyacrylamide, hydroxylethylcellulose and cellulose gels. *Journal of the Chemical Society Faraday Transactions* 71:12-21. 50. Bu?ko, M., Vikartovska, A., Gemeiner, P., Lacik, I., Kollarikova, G. and Marison, I. W. 2006. Nocardia tartaricans cells immobilized in sodium alginate-cellulose sulfate-poly(methylene-co-guanidine) capsules: mechanical resistance and operational stability. *Journal of Chemical Technology and Biotechnology* 81:500-504. 51. Burger, K., Rethay, I., Stefko, B., Gebhardt, I., Kiraly nee Gyongyver Soos, A., Nagy, G. T., Illes, J., Nesmelyi, E., Racz, I. and Varkonyi, V. 1995. U.S. Patent 5472950. 52. Carlsson, J. and Griffith, C. J. 1974. Fermentation products and bacterial yields in glucose-limited and nitrogen-limited cultures of *Streptococci*. *Archives of Oral Biology* 19:1105-1109. 53. Carriere, C., Amis, E., Schrag, J. and Ferry, J. 1993. Dilute-solution dynamic viscoelastic properties of xanthan polysaccharide. *Journal of Rheology* 37:469-478. 54. Casas, J., Santos, V. and Garcia-Ochoa, F. 2000. Xanthan gum production under several operating condition: molecular structure and rheological properties. *Enzyme and Microbial Technology* 26:282-291. 55. Casione, M. 1995. Blends of synthetic and natural polymers as drug delivery systems from growth hormone. *Biomaterials* 16:569-576. 56. Chabrecek, P., Soltes, L. and Orvisky, E. 1991. Comparative depolymerization of sodium hyaluronate by ultrasonic and enzymatic treatments. *Journal of Applied Polymer Science Applied Polymer Symposium* 48:233-241. 57. Chemical Market Reporter. 2000. Drug delivery market poised for five years of strong growth. Schnell Publishing, New York. 258, 23. 58. Chen, K. C., Suga, K. I. and Taguchi, H. 1980. Effects of pore and film diffusion resistances and deactivation of enzyme on the overall reaction rate of immobilized enzyme. *Journal of Fermentation Technology* 58:439-448. 59. Chisti, M. Y. 1989. Airlift Bioreactors. Elsevier Applied Science, London, UK. 60. Chong, B. F. and Nielsen, L. K. 2003. Aerobic cultivation of *Streptococcus zooepidemicus* and the role of NADH oxidase. *Biochemical Engineering Journal* 16:153-162. 61. Chong, B. F., Blank, L. M., McLaughlin, R. and Nielsen, L. K. 2005. Microbial hyaluronic acid production. *Applied Microbiology and Biotechnology* 66:341-351. 62. Chronakis, I., Doublier, J. and Piculell, L. 2000. Viscoelastic properties of kappa- and iota-carrageenan in aqueous NaI from the liquid-like to the solid-like behaviour. *International Journal of Biological Macromolecules* 28:1-14. 63. Cifonelli, J. A., Rebers, P. A. and Heddleston, K. H. 1970. The isolation and characterization of hyaluronic acid from *Pasteurella multocida*. *Carbohydrate Research* 14:272-276. 64. Clarke, K. G., Williams, P. C., Smit, M. S. and Harrison, S. T. L. 2006. Enhancement and repression of the volumetric oxygen transfer coefficient through hydrocarbon addition and its influence on oxygen transfer rate in stirred tank bioreactors. *Biochemical Engineering Journal* 28:237-242. 65. Cleary, P. and Larkin, A. 1979. Hyaluronic acid capsule: strategy for oxygen resistance in group A *Streptococci*. *Journal of Bacteriology* 140:1090-1097. 66. Cleland, R. L. 1968. Ionic polysaccharides. II. Comparison of polyelectrolyte behaviour of hyaluronate with that of carboxylmethyl cellulose. *Biopolymers* 6:1519-1529. 67. Cleland, R. L. 1970. Ionic polysaccharide. . Free-rotation dimensions for disaccharide polymers. Comparison with experiment for hyaluronic acid. *Biopolymers* 9:811-824. 68. Cleland, R. L. 1971. Ionic polysaccharide. . Conformation studies of hyaluronic acid, cellulose, and laminaran. *Biopolymers* 10:1925-1948. 69. Cleland, R. L. and Wang, J. L. 1970. Ionic polysaccharides. Dilute solution properties of hyaluronic acid fraction. *Biopolymers* 9: 799-810. 70. Coleman, P. J., Scott, D., Mason, R. M. and Levick, J. R. 1999. Characterization of the effect of high molecular weight hyaluronan on trans-synovial flow in rabbit knees. *Journal of Physiology* 514:265-282. 71. Comte, B., Vincent, G., Bouchard, B., Jette, M., Cordeau, S. and Des Rosiers, C. 1997. A ¹³C mass isotopomer study of anaplerotic pyruvate carboxylation in perfused rat hearts. *The Journal of Biological Chemistry* 272:26125-26131. 72. Condon, S. 1987. Responses of lactic acid bacteria to oxygen. *The Federation of European Microbiology Societies Reviews* 46:269-280. 73. Cooney, M. J., Goh, L-T., Lee, P. L. and Johns, M. R. 1999. Structured model-based analysis and control of the hyaluronic acid fermentation by *Streptococcus*

zooepidemicus: Physiological implications of glucose and complex-nitrogen limited growth. *Biotechnology Progress* 15:898-910. 74. Couvert, A., Bastoul, D., Roustan, M. and Chatellier, P. 2004. Hydrodynamic and mass transfer study in a rectangular three-phase air-lift loop reactor. *Chemical Engineering and Processing* 43:1381-1387. 75. Crank, J. 1975. The mathematics of diffusion. Oxford University Press, Ely House, London. 76. Crater, D. L., Dougherty, B. A. and van de Rijn, I. 1995. Molecular characterization of hasC from an operon required for hyaluronic acid synthesis in group A Streptococci. Demonstration of UDP glucose pyrophosphorylase activity. *The Journal of Biological Chemistry* 270:28676-28680. 77. Crescenzi, V. 1995. Microbial polysaccharides of applied interest: ongoing research activities in Europe. *Biotechnology Progress* 11:251-259. 78. Cromwick, A-M., Birrer, G. and Gross, R. 1996. Effect of pH and aeration on -poly(glutamic acid) formation by *Bacillus licheniformis* in controlled batch fermentor cultures. *Biotechnology and Bioengineering* 50:222-227. 79. Cuvelier, G. and Lauvay, B. 1986. Concentration regimes in xanthan gum solutions deduced from flow and viscoelastic properties. *Carbohydrate Polymers* 6:321-333. 80. Danishefsky, I. and Siskovic, E. 1971. Conversion of carboxyl groups of mucopolysaccharides into amides of amino acid esters. *Carbohydrate Research* 16:199-205. 81. Dawson, R. M. C., Elliot, D. C., Elliot, W. H and Jones, K. M. 1968. pH, buffers and physiological media. p. 427-438. In: Data for biochemical research 2nd ed. Oxford: Clarendon Press. 82. DeAngelis, P. L., Papaconstantinou, J. and Weigel, P. H. 1993a. Isolation of a *Streptococcus pyogenes* gene locus that directs hyaluronan biosynthesis in acapsular mutants and in heterologous bacteria. *The Journal of Biological Chemistry* 268:14568-14571. 83. DeAngelis, P. L., Papaconstantinou, J. and Weigel, P. H. 1993b. Molecular cloning, identification, and sequence of the hyaluronan synthase gene from group A *Streptococcus pyogenes*. *The Journal of Biological Chemistry* 268:19181-19184. 84. Derek, C., Charles Gervase, T., Geoffrey, M., Richard, G. and Keith, J. 1992. Production of hyaluronic acid. World patent WO 9208799 A1. 85. Dougherty, B. A. and van de Rijn, I. 1993. Molecular characterization of hasB from an operon required for hyaluronic acid synthesis in group A Streptococci. Demonstration of UDP-glucose dehydrogenase activity. *The Journal of Biological Chemistry* 268:7118-7124. 86. Dougherty, B. A. and van de Rijn, I. 1994. Molecular characterization of hasA from a operon required for hyaluronic acid synthesis in group A Streptococci. *The Journal of Biological Chemistry* 269:169-175. 87. Dussap, C. G., Decrops, J. and Gros, J. B. 1985. Transfert d'oxygène en présence de polysaccharides exocellulaires dans un fermenteur agité aérien et dans un fermenteur de type gazosiphon. *Entropie* 123:11-20. 88. Elibol, M. and Maviturna, F. 1999. A kinetic model for actionrhodin production by *Streptomyces coelicolor* A3(2). *Process Biochemistry* 34:625-631. 89. Ellaiah, P., Prabhakar, T., Ramakrishna, B., Thaer Taleb, A. and Adinarayana, K. 2004. Production of lipase by immobilized cells of *Aspergillus niger*. *Process Biochemistry* 39:525-528. 90. Endre, B., Deak, F. and Kiss, I. 1993. Evolution of the hyaluronan-binding module of link protein. *The Biochemical Journal* 292:947-949. 91. Engstrom-Laurent, A. 1989. Changes in hyaluronan concentration in tissues and body fluids in disease states. In *The Biology of Hyaluronan*, Ciba Foundation Symposium. p. 233-224. Wiley, Chichester, England. 92. Engstrom-Laurent, A. and Laurent, T. C. 1989. Hyaluronan as a clinical marker. In Lindh, E. and Thorell, J. I. (eds.), *Clinical Impact of Bone and Connective Tissue Markers*. p. 235-252. Academic, London, England. 93. Fakheha, A. H., Jibril, B. Y. Ibrahim, G. and Abasaeed, A. E. 1999. Medium effects on oxygen mass transfer in a plunging jet loop reactor with a downcomer. *Chemical Engineering and Processing* 38:259-265. 94. Fan, Z. D., Ge, F. W., Zhang, R. S. and Fang, S. Y. 1982. On Chinese species of *Lasiomma* especially those injurious to the coniferous cones. *Journal of Northeast Forestry University. Chinese* 1:1-12. 95. Figueroa, N. and Chakrabarti, B. 1978. Circular dichroism studies of copper (-)-hyaluronic acid complex in relation to conformation of the polymer. *Biopolymers* 17:2415-2426. 96. Figueroa, N., Nagy, B. and Chakrabarti, B. 1977. Cu²⁺-hyaluronic acid complex: Spectrophotometric detection. *Biochemical and Biophysical Research Communications* 74:460-465. 97. Flores Candia, J-L., Deckwer, W-D., Flickinger, M. C. and Drew, S. W. 1999. Xanthan gum. In: *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*, John Wiley and Sons, New York 5:2695-2711. 98. Fouissac, E., Milas, M. and Rinaudo, M. 1993. Shear rate, concentration, molecular weight and temperature viscosity dependences of hyaluronate, a wormlike polyelectrolytes. *Macromolecules* 26:6945-6951. 99. Fouissac, E., Milas, M., Rinaudo, M. and Borsali, R. 1992. Influence of the strength on the dimensions of sodium hyaluronate. *Macromolecules* 25:5613-5617. 100. Fouissac, E., Milas, M. and Rinaudo, M. 1993. Shear-rate, concentration, molecular weight, and temperature viscosity dependences of hyaluronate, a wormlike polyelectrolyte. *Macromolecules* 26:6945-6951. 101. Francesco, D. V. and Aurelio, R. 1987. European Patent Application EP-265:116. 102. Freitas, C. and Teixeira, J. A. 2001. Oxygen mass transfer in a high solids loading three-phase internal-loop airlift reactor. *Chemical Engineering Journal* 84:57-61. 103. Friedman, M. R. and Gaden, E. L. 1970. Growth and acid production by *Lactobacillus delbrueckii* in a dialysis culture system. *Biotechnology and Bioengineering* 12:961-974. 104. Funami, T., Kataoka, Y., Omoto, T., Yasunori, G., Asai, T. and Nishinari, K. 2005. Food hydrocolloids control the gelatinization and retrogradation behavior of starch. Functions of guar gums with difference molecular weights on the gelatinization behavior of corn starch. *Food Hydrocolloids* 19:15-24. 105. Galindo, E. and Herrera, R. 1989. Effects of different impeller combinations and agitation speeds on the culture of a highly oxygen-sensitive bacteria. *The Chemical Engineering Journal* 42:9-12. 106. Garbayo, I., Leon, R., Vigara, J. and Vilchez, C. 2002. Diffusion characteristics of nitrate and glycerol in alginate. *Colloids and Surfaces B: Biointerfaces* 25:1-9. 107. Garcia-Ochoa, F. and Castro, E. G. 2001. Estimation of oxygen mass transfer coefficient in stirred tank reactors using artificial neural networks. *Enzyme and Microbial Technology* 28:560-569. 108. Garcia-Ochoa, F. and Gomez, E. 1998. Mass transfer coefficient in stirrer tank reactors for xanthan solutions. *Biochemical Engineering Journal* 1:1-10. 109. Garcia-Ochoa, F., Gomez, E. and Santos, V. 2000. Oxygen transfer and uptake rates during xanthan gum production. *Enzyme and Microbial Technology* 27:680-690. 110. Garcia-Ochoa, F., Santos, V. and Alcon, A. 1995. Xanthan gum production: an unstructured kinetic model. *Enzyme and Microbial Technology* 17:206-217. 111. Gatej, I., Popa, M. and Rinaudo, M. 2005. Role of the pH on hyaluronan behavior in aqueous solution. *Biomacromolecules* 6:61-67. 112. Ghaly, A. E. and Ben Hassan, R. M. 1994. Kinetics of batch production of single cell protein from

cheese whey. Applied Biochemistry and Biotechnology Journal 50:79-92. 113. Gibbs, D. A., Merrill, E. W., Smith, K. A. and Balazs, E. A. 1968. Rheology of hyaluronic acid. Biopolymers 6:777-791. 114. Gibbs, P. and Seviour, R. 1996. Does the agitation rate and/or oxygen saturation influence exopolysaccharide production by *Aureobasidium pullulans* in batch culture. Applied Microbiology and Biotechnology 46:503-510. 115. Giraud, E., Bertrand, L. and Raimbault, M. 1991. Influence of pH and initial lactate concentration on the growth of *Lactobacillus plantarum*. Applied Microbiology and Biotechnology 36:96-99. 116. Girelli, M. A. and Mattei, E. 2005. Application of immobilized enzyme reactor in on-line high performance liquid chromatography: A review. Journal of Chromatography B 819:3-16. 117. Goa, K. L. and Benfield, P. 1994. Hyaluronic acid: a review of its pharmacology and use as a surgical aid in ophthalmology and its therapeutic potential in joint disease and wound healing. Drugs 47:536-566. 118. Godbole, S., Schumpe, A., Shah, Y. and Carr, N. 1984. Hydrodynamics and mass transfer in non-Newtonian solutions in a bubble column. American Institute of Chemical Engineers Journal 30:213-220. 119. Goh, L-T. 1998. Fermentation studies of hyaluronic acid production by *Streptococcus zooepidemicus*. Department of Chemical Engineering University of Queensland 5:1-38. 120. Goto, A. and Kunioka, M. 1992. Biosynthesis and hydrolysis of poly(γ -glutamic acid) from *Bacillus subtilis* IFO3335. Bioscience, Biotechnology, and Biochemistry 56:1032-1035. 121. Goudar, C. T., Strevett, K. A. and Shah, S. N. 1999. Influence of microbial concentration on the rheology of non-Newtonian fermentation broths. Applied Microbiology and Biotechnology 51:310-315. 122. Gowthaman, M. K., Raghava Rao, K. S. M. S., Ghildyal, N. P. and Karanth, N. G. 1995. Estimation of kLa in solid-state fermentation using a packed-bed bioreactor. Process Biochemistry 30:9-15. 123. Gura, E., Huckel, M. and Muller, J. 1998. Specific degradation of hyaluronic acid and its rheological properties. Polymer Degradation and Stability 59:297-302. 124. Hamilton, R. G. and Raksha, R. A. 1989. PCT International Application. WO 89/02445. 125. Hardie, J. M. 1986. Genus *Streptococcus*. In Sneath, P. H. A., Mair, N. S. and Sharpe, M. E. (eds.), Bergeys manual of determinative bacteriology. Willians and Wilkins, Baltimore. 2:1043-1071. 126. Hardie, J. M. and Wiley, R. A. 1995. The genus *Streptococcus*. In Wood, B. J. B. and Holzapfel, W. H. (eds.), The genera of lactic acid bacteria. p. 55-124. Blackie Academic and Professional, Glasgow. 127. Harding, S. G., Wik, O., Helander, A., Ahnfelt, N. O. and Kenne, L. 2002. NMR velocity imaging of the flow behaviour of hyaluronan solutions. Carbohydrate Polymer 47:109-119. 128. Hasegawa, S., Nagatsuri, M., Shibutani, M., Yamamoto, S. and Hasebe, S. 1999. Productivity of concentrated hyaluronic acid using a MaxblendR fermentor. Journal of Bioscience and Bioengineering 88:68-71. 129. Hassan, I. T. M. and Robinson, C. W. 1977. Oxygen transfer in mechanically agitated aqueous systems containing dispersed hydrocarbon. Biotechnology and Bioengineering 19:661-682. 130. Heatley, F. and Scott, J. E. 1988. A water molecule participates in the secondary structure of hyaluronan. The Biochemical Journal 254:489-493. 131. Hedin, P. and Laurent, T. C. 2000. Biosynthesis of hyaluronan. In Ernst B, Hart G, and Sinay, P. (eds.), Carbohydrates in chemistry and biology. p. 363-372. Wiley/WCH, Weinheim. 132. Hellstrom, S. and Laurent, C. 1987. Hyaluronan and healing of tympanic membrane perforations. An experimental study. Acta Oto-laryngological Supplement 442:54-61. 133. Hiroshi, M. and Masahiro, F. 1991. Process for preparing hyaluronic acid. U.S. patent 5071751. 134. Hiruta, O., Yamamura, K., Takebe, H., Futamura, T., Iimura, K. and Tanaka, H. 1997. Application of MaxblendR fermentor for microbial processes. Journal of Bioscience and Bioengineering 88:68-71. 135. Ho, C. S., Ju, L. K. and Baddour, R. F. 1990. Enhancing penicillin fermentations by increased oxygen solubility through the addition of n-hexadecane. Biotechnology and Bioengineering 36:1110-1118. 136. Hokputsa, S., Jumel, K., Alexander, C. and Harding, S. E. 2003. Hydrodynamic characterization of chemically degraded hyaluronic acid. Carbohydrate Polymers 52:111-117. 137. Holger, H., Udo, P. H., Soon, S. I., Adrian, S. and Dieter, D. W. 1988. Mass transfer in xanthan fermentation. Chemical Engineering and Technology 60:407-410. 138. Holmstrom, B and Ricica, J. 1967. Production of hyaluronic acid by a Streptococcal strain in batch culture. Applied Microbiology and Biotechnology 15:1409-1413. 139. Honda, H., Toyama, Y., Takahashi, H., Nakazeko, T. And Kobayashi, Y. 1995. Effective lactic acid production by two-stage extractive fermentation. Journal of Fermentation Technology 79:589-593. 140. Hortacsu, O. 1965. Gas liquid mass transfer in an agitated vessel: air-water system. MSc. Thesis. The graduate school of the Oklahoma State University. 141. Jajuee, B., Margaritis, A., Karamanov, D. and Bergougnou, M. A. 2006. Mass transfer characteristics of a novel three-phase airlift contactor with a semipermeable membrane. Chemical Engineering Journal 125:119-126. 142. Johns, M. R., Goh, L-T. and Oeggerli, A. 1994. Effect of pH, agitation and aeration on hyaluronic acid produced *Streptococcus zooepidemicus*. Biotechnology Letters 15:507-512. 143. Joly-Vuillemin, C., de Bellefon, C. and Delmas, H. 1996. Solid effects on gas-liquid mass transfer in three-phase slurry. Catalytic hydrogenation of adiponitrile over raney nickel. Chemical Engineering Science 51:2149-2155. 144. Ju, L. K. and Ho, C. S. 1989. Oxygen diffusion coefficient and solubility an n-hexadecane. Biotechnology and Bioengineering 34:1221-1224. 145. Jumel, K., Harding, S., Mitchell, J., To, K., Hayter, I. and O ' Mullane, J. 1996. Molar mass and viscometric characterization of hydroxylpropylmethyl cellulose. Carbohydrate Polymers 29:105-109. 146. Karel, S. F., Libicki, S. B. and Robertson, C. R. 1985. The immobilization of whole cells: engineering principles. Chemical Engineering Science 40:1321-1354. 147. Kasapis, S., Morris, E., Gross, M. and Rudolph, K. 1994. Solution properties of levan polysaccharide from *Pseudomonas syringae* pv. *Phaseolicola* and its possible primary role as a blocker of recognition during pathogenesis. Carbohydrate Polymers 23:55-64. 148. Kass, E. H. and Seastone, C. V. 1944. The role of the mucoid polysaccharide hyaluronic acid in the virulence of group A hemolytic Streptococci. The Journal of Experimental Medicine 70:319-330. 149. Kawase, Y. and Hashimoto, N. 1996. Gas hold-up and oxygen transfer in three-phase external-loop airlift bioreactor: non-Newtonian fermentation broths. Journal of Chemical Technology and Biotechnology 65:325-334. 150. Kaya, A. and Schumpe, A. 2005. Surfactant adsorption rather than " shuttle effect ". Chemical Engineering Science 60:6504-6510. 151. Kemblowski, Z. and Kristiansen, B. 1986. Rheometry of fermentation liquids. Biotechnology and Bioengineering 28:1474-1483. 152. Kendall, F. E., Heidelberger, M. and Dawson, M. H. 1937. A serologically inactive polysaccharide elaborated by mucoid strains of group a hemolytic Streptococcus. The Journal of Biological Chemistry 118:61-69. 153. Kilonzo, P. M. and Margaritis, A. 2004. The effects of non-Newtonian

fermentation broth viscosity and small bubble segregation on oxygen mass transfer in gas-lift bioreactors: a critical review. Biochemical Engineering Journal 17:27-40. 154. Kim, J. H., Yoo, S. J., Oh, D. K., Kweon, Y. G., Park, D. W., Lee, C. H. and Gil, G. H. 1996. Selection of a *Streptococcus equi* mutant and optimization of culture conditions for the production of high molecular weight hyaluronic acid. Enzyme and Microbial Technology 19:440-445. 155. Kim, S-J., Park, S-Y. and Kim, C-W. 2006. A novel approach to the production of hyaluronic acid by *Streptococcus zooepidemicus*. Journal of Microbiology and Biotechnology 16:1849-1855. 156. Knudson, C. B. and Knudson, W. 1993. Hyaluronan-binding proteins in development, tissue homeostasis, and disease. The Journal of the Federation American Societies for Experimental Biology 7:1233-1241. 157. Kochbeck, B., Lindert, M. and Hempel, D. C. 1992. Hydrodynamics and local parameters in three-phase-flow in airlift-loop reactors of different scale. Chemical Engineering Science 47:3443-3450. 158. Komaromy, P. and Sisak, C. 1994. Investigation of gas-liquid oxygen transport in three-phase bioreactor. Hungarian Journal of Industrial Chemistry 22:147-151. 159. Korda?, M. and Linek, V. 2006. Mechanism of enhanced gas absorption in presence of fine solid particles. Effect of molecular diffusivity on mass transfer coefficient in stirred cell. Chemical Engineering Science 61:7125-7132. 160. Koshiishi, I., Takenouchi, M., Hasegawa, T. and Imanari, T. I. 1998. Enzymatic Method for the Simultaneous Determination of Hyaluronan and Chondroitin Sulfate Using High-Performace Liquid Chromatography. Analytical Biochemistry 265:49-54. 161. Kovarova-Kovar, K. and Egli, T. 1998. Growth kinetics of suspended microbial cells: from single substrate-controlled growth to mixed-substrate kinetics. Microbiology and Molecular Biology Reviews 62:646-666. 162. Krahulec, J. and Krahulcova, J. 2006. Increase in hyaluronic acid production by *Streptococcus equi* subsp. *zooepidemicus* strain deficient in -glucuronidase in laboratory conditions. Biotechnological Products and Process Engineering 71:415-422. 163. Lap?ik, Jr, L., Dammer, Ch. and Valko, M. 1992. Hyaluronic acid-copper () complexes: Spectroscopic characterization. Colloid and Polymer Science 270:1049-1052. 164. Laurent, C. T. 1987. Biochemistry of hyaluronan. Acta Otolaryngological 442:7-24. 165. Laurent, T. C. 1970. Structure of hyaluronic acid. In: Balazs, E. A. (ed.), Chemistry and Molecular Biology of the intercellular Matrix. p. 703-732. New York: Academic Press. 166. Laurent, T. C. 1998. The chemistry, biology and medical applications of hyaluronan and its derivative. Portland Press: London. 167. Laurent, T. C. and Fraser, J. R. E. 1992. Hyaluronan. The Journal of the Federation American Societies for Experimental Biology 4:2397-2404. 168. Laurent, T. C., Ryan, M. and Pietruszkiewicz, A. 1960. Fractionation of hyaluronic acid. The polydispersity of hyaluronic acid from the bovine vitreous body. Biochimical et Biophysical Acta 42:476-485. 169. Lee, 1992. Biochemical engineering. Englewood Cliffs, NJ: Prentice-Hall p. 240. 170. Lee, H. G. and Cowman, M. K. 1994. An agarose gel electrophoretic method for analysis of hyaluronan molecular weight distribution. Analytical Biochemistry 219:278-287. 171. Lee, I., Kim, M., Lee, J., Jung, J., Lee, H., Pary, Y. and Seo, W. 1999. Influence of agitation speed on production of curdlan by Agrobacterium species. Bioprocess Engineering 20:283-287. 172. Leetvaar, J. and Ywenna, T. S. J. 1980. Some dimensionless parameters of impeller power in coagulation-flocculation processes. Water Research 14:135-140. 173. Lerner, M. 1996. Hyaluronic acid market benefits from n