

# Study on Production of Bacterial Cellulose by *Acetobacter xylinum* WU1 and Antimicrobial Application of Bacterial Cellulo

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## ABSTRACT

Bacterial cellulose (BC), which is synthesized and secreted by the gram negative bacterium, *Acetobacter xylinum* WU1, displays unique physical, chemical, and mechanical properties including a high crystallinity, a high water holding capacity, a well-developed surface area comprised of nanofibers, elasticity, mechanical strength, and biocompatibility. BC displays such special physicochemical characteristics, applications in the paper and food industries as well as in the medical field as an artificial skin and blood vessel substitute are expected.

Static batch fermentations for bacterial cellulose production were studied in carbon sources, nitrogen sources, pH, temperature, organic acid (citric acid, succinic acid and acetic acid), ethanol concentration (0-10%), acetic acid concentration (0-20%), sweet potato concentration (0-20 g/L) and aeration rate (0.5-2 L/min) in flask under 30 °C by *A. xylinum* WU1. Bacterial cellulose production from *A. xylinum* WU1 was affected by various environmental factors, such as pH and temperature; initial pH 5.5 and 30 °C were favorable for bacterial cellulose production. On the other hand, the experimental results showed that BC dried weight was maximum 3.7 g/L, when aeration rate was controlled at 1.5 L/min, glucose concentration was 20 g/L at pH 5.5 in 5L fermentor.

Structural changes in never-dried, disintegrated bacterial cellulose by treatment with various alkali treatment and various drying process were examined. The pretreated/ treated bacterial cellulose were characterized by X-ray diffractometry (XRD), Fourier Transform Infrared spectroscopy (FTIR) and Field-emission scanning electron microscope (FE-SEM). In additionally, Hunter lab colour parameters were determined to assess the effect of different alkali treatments on the colour characteristics of the bacterial cellulose. The overall quality of the freeze dried membranes had higher 'L' values. On the other hand, alkali treated bacterial cellulose using NaOH and Na<sub>2</sub>CO<sub>3</sub> with higher 'L' values 101.8 and 97.1, respective. The XRD patterns of freeze dried membranes obtained the with reduce in the relative intensity of plane diffraction. In contrast, air dried membranes with higher intensity. No crystallize difference directly caused by alkali treatment could be seen with XRD.

Bacterial cellulose was an interesting material for using as a wound dressing since it provides moist environment to a wound resulting in a better wound healing. But bacterial cellulose itself has no antimicrobial activity to prevent wound infection. To achieve antimicrobial activity, nano-silver were impregnated into bacterial cellulose by immersing bacterial cellulose in AgNO<sub>3</sub> solution. The formation of nano-silver was also evidenced by the scanning electron microscope. The dried nano-silver impregnated bacterial cellulose exhibited strong the antimicrobial activity against *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive).

Keywords : *Acetobacter xylinum*、bacterial cellulose、nano-silver wound dressing、antimicrobial activity

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## REFERENCES

1.水野卓、川何正允 編著。1992。菇類的化學生化學。第260-263頁。國立編譯館版。台北。2.尤新 編著。2001。機能性發酵食品之研究。第119-1670頁。藝軒出版社。台北。3.沈玟?。2007。細菌纖維素生產菌之篩選及其最適培養條件之研究。國立中興大學食品暨應用生物技術學系碩士論文。台中。4.黃唯欣。2007a。醋酸菌*Acetobacter* sp. X02利用改良式SH培養基生產細菌纖維素之研究。國立中興大學食品生物應用暨技術學系碩士論文。台中。5.黃德為。2007b。細菌纖維素生產培養基配方改良暨規模放大之研究。國立中興大學食品暨應用生物技術學系碩士論文。台中。6.盧天惠主編。2002。X光繞射與應用。滄海書局:22-36。7.蕭淑娟。2004。利用醋酸菌*Acetobacter xylinum*發酵燒酒糟生產細菌纖維素之研究。私立東海大學食品科學系研究所碩士論文。台中。8.  
<http://isch.skhsslmc.edu.hk/~boyscouts/imagdata/burnt.ppt> 2008.9. <http://www.nhi.gov.tw/> 2008.10.Adams, A. P., Santschi, E. M. and Mellencamp, M. A. 1999. Antibacterial properties of a silver chloride-coated nylon wound dressing. *Veterinary Surgery* 28: 219-225.11.Alaban, C. A. 1962. Studies on the optimum conditions for Nata de coco bacterium or Nata formation in coconut water. *The Philippine Agriculturist* 45: 490-515.12.Anderson, J. M. 2004. Inflammation, wound healing, and the foreign body response. In: Ratner, B. D., Hoffman, A. S., Shoen, F. J. and Lemons, J. E. (Eds.), *Biomaterials Science. An Introduction to Materials in Medicine*. San Diego, Elsevier. C.A.13.Aschner, M. and Hestrin, S. 1946. Fibrillar structure of cellulose of bacterial and animal origin. *Nature* 157: 659.14.Atalla, R. H. and VandelHart, D. L. 1986. Native cellulose: a composite of two crystalline forms. *Science* 223: 283-285.15.Bae, S. and Shoda, M. 2004. Bacterial cellulose production by fed-batch fermentation in molasses medium. *Biotechnology Progress* 20(5): 1366-1371.16.Bae, S., Sugano, Y. and Hoda, M. 2004. Improvement of bacterial cellulose production by addition of agar in a jar fermentor. *Journal of Bioscience and bioengineering* 97(1): 33-38.17.Bakos, D. Soldan, M. and Hernandez-Fuentes, I. 1999. Hydroxyapatite-collagen- hyaluronic acid composite. *Biomaterials* 20: 191-195.18.Barbucci, R. Lamponi, S. and Aloisi, A. M. 2002. Platelet adhesion to commercial and modified polymer materials in animals under psychological stress and in a no-stress condition. *Biomaterials* 23: 1967-1973.19.Bergey, D. H., Harrison, F. C., Breed, R. S., Hammer B. W., and Huntoon, F. M. 1925. *Bergey's Manual of Determinative Bacteriology*, 2nd ed. The Williams and Wilkins Co., Baltimore, p. 1-462.20.Blacljwell, J. 1982. The macromolecular organization of cellulose and chitin. In Brown, R. M., Jr.(ed.) *Cellulose and Other Natural Polymer Systems*. Plenum Publishing Corp.

327-339.21.Bowler, P. G. and Davies, B. 1999. The microbiology of infected and noninfected leg ulcers. *International Journal of Dermatology* 38: 573-578.22.Brown, A. J. 1886. On acetic ferment which forms cellulose. *Journal of Chemical Society* 49: 432-439.23.Brown, R. M. Jr. 1989. Cellulose structure and function aspects. *Ellis Howoodide* 144-151.24.Brown, R. M. Jr., Kudlicka, K., Cousins, S. K. and Nagy, R. 1992. Gravity effects on cellulose assembly. *American Journal of Botany* 79: 1247 – 1258.25.Cannon, R. E. and Anderson, S. M. 1991. Biogenesis of bacterial cellulose. *Critical Reviews in Microbiology* 17: 415-447.26.Chambers, C. W., Proctor, C. M. and Kabler, P. W. 1962. Bactericidal effect of low concentrations of silver. *Journal of the American Water Works Association* 54: 208-216.27.Chao, Y., Ishida, T., Sugano, Y. and Shoda, M. 2000. Bacterial cellulose production by *Acetobacter xylinum* in a 50-l internal-loop airlift reactor. *Biotechnology and Bioengineering* 68: 345-352.28.Chao, Y., Mitarai, M., Sugann, Y. and Shoda, M. 2001. Effect of addition of water-soluble polysaccharides on bacterial cellulose production in a 50-L airlift reactor. *Biotechnology progress* 17 (4): 781-785.29.Chao, Y., Sugano, Y. and Shoda, M. 2001. Bacterial cellulose production under oxygen-enriched air at different fructose concentrations in a 50-liter internal-loop airlift reactor. *Applied Microbiology and Biotechnology* 55: 673-679.30.Charpentier, P. A., Maguire, A. and Wan, W. K. 2006. Surface modification of polyester to produce a bacterial cellulose-based vascular prosthetic device. *Applied Surface Science* 252: 6360-6367.31.Cho, K. H., Park, J. E., Osaka, T. and Park, S. G. 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. *Electrochimica Acta* 51: 956-960.32.Coates, D. M. and Kaplan, S. L. 1996. Modification of polymeric surfaces with plasmas. *MRS Bull* 21:1.33.Czaja, W. K., Young, D. J., Kawecki, M. and Brown, R. M. 2007. The Future Prospects of Microbial Cellulose in Biomedical Applications. *Biomacromolecules*. 8: 1-12.34.De Faveri, D., Torre, P., Molinari, F., Perego, P. and Converti, A. 2003. Carbon material balances and bioenergetics of 2,3-butanediol bio-oxidation by *Acetobacter hansenii*. *Enzyme and Microbial Technology* 33: 708-719.35.Delmer, D. P. 1999. Cellulose biosynthesis: exciting times for a difficult field of study. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 245-276.36.Dudman, W. F. 1959. Cellulose production by *Acetobacter acetigenum* in defined medium. *The Journal of General Microbiology* 21: 327-337.37.Embucado, M. E., Marks, J. S. and Bemiller, J. N. 1994. Bacterial cellulose . Optimization of cellulose production by *Acetobacter xylinum* through response surface methodology. *Food hydrocoll* 8: 419-430.38.Embucado, M. E., Marks, J. S. and Bemiller, J. N. 1994a. Bacterial cellulose. I. Factors affecting the production of cellulose by *Acetobacter xylinum*. *Food Hydrocoll* 8: 407-418.39.Eming, S., Smola, H. and Kreig, T. 2002. Treatment of chronic wounds: state of the art and future concepts. *Cells Tissues Organs* 172: 105-117.40.Farina, J. I., Sineriz, F., Molina, O. E. and Perotti, N. I. 2001. Isolation and physico-chemical characterization of soluble scleraoglucan from *Sclerotium rolfsii* Rheological properties, molecular weight and conformational characteristics. *Carbohydrate Research* 44: 41-50.41.Fontana, J. D., de Souza, A. M., Fontana, C. K., Torriani, I. L., Moreschi, J. C., Gallotti, B. J., de Souza, S. J., Narcisco, G. P., Bichara, J. A. and Farah, L. F. X. 1990. *Acetobacter cellulose pellicle as a temporary skin substitute*. *Applied Biochemistry and Biotechnology* 24: 253-264.42.Godbey, W. T. and Atala, A. 2002. In vitro systems for tissue engineering. *The New York Academy of Sciences* 961: 10-26.43.Guhados, G., Wan, W. and Hutter, J. L. 2005. Measurement of the elastic modulus of single bacterial cellulose fibers using atomic force microscopy. *Langmuir* 21: 6642.44.Gupta, B., Hilborn, J., Hollenstein, C., Plummer, C. J., Houriet, R. and Xanthopoulos, N. 2000. Surface modification of polyester films by RF plasma. *Journal of Applied Polymer Science*. 78: 1083-1091.45.Ha, J. H., Shehzad, O., Khan, S., Lee, S. Y., Park, J. W., Khan, T. and Park, J. K. 2008. Production of bacterial cellulose by a static cultivation using the waste from beer culture broth. *Korean Journal of Chemical Engineering* 25(4): 812-815.46.Haigler, C. H. and Weimer, P. J. 1991. Biosynthesis and Biodegradation of Cellulose. *Marcel Dekker, Inc* p.5-23, p.71-98, p.219-243.47.Helenius, G., B?ckdahl, H., Bodin, A., Nannmark, U., Gatenholm, P. and Risberg, G. 2007. In vivo biocompatibility of bacterial cellulose. *Journal of Biomedical Materials Research* 76: 431-438.48.Henneberg, W. 1906. Zur Kenntniss der Schnellessig und Weinessigbakterien. *Deutsche Essigindustrie* 10:106-108.49.Hestrin, S. and Schramm, M. 1954. Synthesis of cellulose by *Acetobacter xylinum*: preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *The Biochemical Journal* 58: 345-352.50.Hestrin, S. Aschner, M. and Mager, J. 1947. Synthesis of cellulose by resting Cells of *Acetobacter xylinum*. *Nature* 159: 64-65.51.Humphries, A. W., Hawk, W. A. and Cuthbertson, A. M. 1961. Arterial prosthesis of collagen-impregnated dacron tulle. *Surgery* 50: 947-954.52.Hwang, J. W., Yang, Y. K., Hwang, J. K., Pyun, Y. R. and Kim, Y. S. 1999. Effects of pH and dissolved oxygen on cellulose production by *Acetobacter xylinum* BRC5 in agitated culture. *Journal of Bioscience and Bioengineering* 88(2): 183-188.53.Hwang, J. W., Yang, Y. K., Hwang, J. K., Pyun, Y. R. and Kim Y. S. 1999. Effects of pH and dissolved oxygen on cellulose production by *Acetobacter xylinum* BRC5 in agitated culture. *Journal of Bioscience and Bioengineering* 88(2): 183-188.54.Iguchi, M., Yamanaka, S. and Budhiono, A. 2000. Bacterial cellulose – a masterpiece of nature 's arts. *Journal of Materials Science* 35: 261-270.55.Ishida, T., Sugano, Y. and Shoda, M. 2002. Novel glycosyltransferase genes involved in the acetan biosynthesis of *Acetobacter xylinum*. *Biochemical and Biophysical Research Communications* 295(2): 230-235.56.Ishida, T., Sugano, Y., Nakai, T., and Shoda, M. 2002. Effects of acetan on production of bacterial cellulose by *Acetobacter xylinum*. *Journal of Bioscience and Bioengineering* 66: 1677-1681.57.Ishihara, M., Matsunaga, M., Hayashi, N. and T?ler, V. 2002. Utilization of D-xylose as carbon source for production of bacterial cellulose. *Enzyme and Microbial Technology* 31(7): 986-991.58.Ishikawa, A., Matsuoka, M., Tsuchida, T. and Yoshinaga, F. 1995. Increasing of bacterial cellulose production by sulfoguanidine-resistant mutants derived from *Acetobacter xylinum* subsp. *Sucrofermentans* BPR2001. *Bioscience Biotechnology and Biochemistry* 59: 2259-2263.59.Ishikawa, A., Tsuchida, T. and Yoshinaga, F. 1996. Production of bacterial cellulose using microbial strain resistant to inhibitor of DHO-dehydrogenase. Japanese patent 08009965A.60.Ishikawa, A., Matsuoka, M., Tsuchida, T. and Yoshinaga, F. 1995. Increase in cellulose production by sulfoguanidine-resistant mutants derived from *Acetobacter xylinum* subsp. *Sucrofermentans*. *Bioscience, Biotechnology, and Biochemistry*. 59(12): 2259-2262.61.Jonas, R. and Farah, L. F. 1998. Production and application of microbial cellulose. *Polymer Degradation and Stability* 59(1): 101-106.62.Joo S. H., Goo, K.

H., Ki, K. K., Soo, K. H., Gyun, K. Y. and Joon, L. S. 2003. Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture conditions. *Bioresource Technology* 86(3): 215-219.

63. Joseph, G., Rowe, G. E., Margaritis, A. and Wan, W. 2003. Effects of polyacrylamide on bacterial cellulose production. *Journal of Chemical Technology and Biotechnology* 78(7): 964-970.

64. Kačurková, M., Smith, A. C., Gidley, M. J. and Wilson, R. H. 2002. Molecular interactions in bacterial cellulose composites studied by 1D FT-IR and dynamic 2D FT-IR spectroscopy. *Carbohydrate Research* 337: 1145-1153.

65. Kamide, K., Matsuda, Y., Iijima, H. and Okajima, K. 1990. Effect of culture condition of acetic acid bacteria on cellulose biosynthesis. *British Polymer Journal* 22: 167-171.

66. Kawano, S., Tajima, K., Kono, H., Erata, T., Munekata, M. and Takai, M. 2002. Effect of endogenous endo-beta-1,4-glucanase on cellulose biosynthesis in *Acetobacter xylinum* ATCC23769. *Journal of Bioscience and Bioengineering* 94: 275-281.

67. Keshk, S. 2002. *Gluconacetobacter xylinus* : a new resource for cellulose. *Egypt Journal of Biotechnology* 11: 305-310.

68. Keshk, S. and Sameshima, K. 2006. Influence of lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture. *Enzyme and Microbial Technology* 40(1): 4-8.

69. Keshk, S. and Sameshima, K. 2006a. The utilization of sugar cane molasses with/without the presence of lignosulfonate for the production of bacterial cellulose. *Applied Microbiology and Biotechnology* 72: 291-296.

70. Kim, S. Y., Kim, J. N., Wee, Y. J., Park, D. H. and Ryu, H. W. 2006. Production of bacterial cellulose by *Gluconacetobacter* sp. RKY5 isolated from persimmon vinegar. *Applied Biochemistry and Biotechnology* 131(1-3):705-715.

71. Kitano, T., Ateshian, G. A. Mow, V. C. Kadoya, Y. and Yamano, Y. 2001. Constituents and pH changes in protein rich hyaluronan solution affect the biotribological properties of artificial articular joints. *Journal of Biomechanics* 34(8): 1031-1037.

72. Klamm, D., Schumann, D., Udhardt, U. and Marsch, S. 2001. Bacterial synthesized cellulose - artificial blood vessels for microsurgery. *Progress in Polymer Science* 26: 1561-1603.

73. Klemm, D., Heublein, B., Fink, H. P. and Bohn, A. 2005. Cellulose: fascinating biopolymer and sustainable raw material. *Angewandte Chemie International Edition* 44: 3358-3393.

74. Kongrang, S. 2008. Bacterial cellulose production by *Acetobacter xylinum* strains from agricultural waste products. *Applied Biochemistry and Biotechnology* 148: 245-256.

75. Kouda, T., Naritomi, T., Yano, H. and Yoshinaga, F. 1997. Effects of oxygen and carbon dioxide pressures on bacterial cellulose production by *Acetobacter* in aerated and agitated culture. *Journal of Fermentation and Bioengineering* 84: 124-127.

76. Krystynowicz, A., Galas, E. and Paelak, E. 1997. Method of bacterial cellulose production. Polish patent P-299907.

77. Laboureur, P. 1998. Process for production bacterial cellulose from material plant origin. WO 88/09381.

78. Lala, N. P., Ramaseshan, R., Bojin, L., Sundarajan, S., Barhate, R. S., Ying-Jun, L. and Ramakrishna, S. 2007. Fabrication of nanofibers with antimicrobial functionality used as filters: protection against bacterial contaminants. *Biotechnology and Bioengineering* 79(6): 1357-1365.

79. Lapuz, M. M., Gallardo, E. G. and Dalo, M. A. 1967. The Nata organism - cultural requirement, characteristics and identify. *The Philippine Journal of Science* 96: 91-96.

80. Lee, I. Y., Seo, W. T., Kim, G. J., Kim, M. K., Park, C. S. and Park, Y. H. 1997. Production of curdlan using sucrose and sugar cane molasses by two-step fed-batch cultivation of *Agrobacterium* species. *Journal of Industrial Microbiology Biotechnology* 18: 255-259.

81. Lin, F. C., Brown, R. M. Jr., Drake, R. R. Jr. and Haley, B. E. 1990. Identification of the uridine 5' -diphosphoglucose (UDP-glc) binding subunit of cellulose synthase in *Acetobacter xylinum* using the photoaffinity probe 5-azido-UDP-glc. *Journal of Biological Chemistry*. 265: 4782-4784.

82. Linton, J. D., Ash, S. G. and Huybrechts, L. 1991. Microbial polysaccharides. In: Byron, D. (ed.). *Biomaterials: Novel Materials from Biological Sources*. Stockton Press. New York.

83. Maneerung, T., Tokura, S. and Rujiravanit, R. 2008. Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. *Carbohydrate Polymers* 72: 43-51.

84. Masahiro, K., Mikitomo, A., Akitomo, A., Tomonori, Y. 1998. Feed composition for fish kind cultivation. Japanese patent 10313794.

85. Masaoka, S., Ohe, T. and Ameyama, M. 1993. Production of cellulose from glucose by *Acetobacter xylinum*. *Journal of Fermentation and Bioengineering* 75: 18-22.

86. Masayuki, T. and Yukihiro, N. 1990. Noodle made of rice powder and producing method thereof. Japanese patent 02249466.

87. Matsuoka, M., Tsuchida, T., Matsushita, K., Adachi, O. and Yoshinaga, F. 1996. A synthetic medium for bacterial cellulose production by *Acetobacter xylinum* ssp. *sucrofermentans*. *Bioscience, Biotechnology, and Biochemistry* 60(4): 575-579.

88. Miyamoto, T., Takahashi, S., Ito, H., Inagaki, H. and Noishiki, Y. 1989. Tissue biocompatibility of cellulose and its derivatives. *Journal of Biomedical Materials Research* 23: 125-133.

89. Nakai, T., Moriya, A., Tonouchi, N., Tsuchida, T., Yoshinaga, F., Horinouchi, S., Sone, Y., Mor, H., Sakai, F. and Hayashi, T. 1998. Control of expression by the cellulose synthase (*bcsA*) promoter region from *Acetobacter xylinum* BPR2001. *Gene* 213: 93-100.

90. Naritomi, T., Kouda, T., Yano, H. and Yoshinaga, F. 1998a. Effect of lactate on bacterial cellulose production from fructose in continuous culture. *Journal of Fermentation and Bioengineering* 85(1): 89-95.

91. Naritomi, T., Kouda, T., Yano, H. and Yoshinaga, F. 1998b. Effect of ethanol on bacterial cellulose production from fructose in continuous culture. *Journal of Fermentation and Bioengineering* 85(6): 598-603.

92. Nasser, B. A. and Vacanti, J. P. 2002. Tissue engineering in the 21st century. *Surgical Technology International* 10: 25-37.

93. Nieduszynski, I. A. and Preston, R. D. 1970. Crystallite size in natural cellulose. *Nature* 225: 273-274.

94. Nishi, Y., Uryu, M., Yamanaka, S., Watanabe, K., Kitamura, N., Iguchi, M. and Mitsuhashi, S. 1990. The structure and mechanical properties of sheets prepared from bacterial cellulose. Part 2: improvement of the mechanical properties of sheets and their applicability to diaphragms of electroacoustic transducers. *Journal of Materials Science* 25: 2997-3001.

95. Nishinari, K. and Takahashi, R. 2003. Interaction in polysaccharide solutions and gels. *Current Opinion in Colloid and Interface Science* 8: 396-400.

96. Noro, Y., Sugano, Y. and Shoda, M. 2004. Utilization of the buffering capacity of corn steep liquor in bacterial cellulose production by *Acetobacter xylinum*. *Applied Microbiology and Biotechnology* 64: 199-205.

97. Oikawa, T., Morino, T. and Ameyama, M. 1995. Production of cellulose from D-cellulose from D-arabitol by *Acetobacter xylinum* KU-1. *Bioscience, Biotechnology, and Biochemistry* 59(8): 1564-1565.

98. Okiyama, A., Motoki, M. and Yamanaka, S. 1993. Bacterial cellulose . Application to processed foods. *Food Hydrocoll* 6: 503-511.

99. Okiyama, A., Motoki, M. and Yamanaka, S. 1992a. Bacterial cellulose II. Processing of the gelatinous cellulose for food materials. *Food Hydrocoll*. 6: 479-487.

100. Okiyama, A., Shirae, H., Kano, H. and Yamanaka, K. 1992b. Two-stage

fermentation process for cellulose production by *Acetobacter acetii*. *Food Hydrocoll.* 6: 471-477.101.Park, J. K., Jung, J. Y. and Park Y. H. 2003. Cellulose production by *Gluconacetobacter hansenii* in a medium containing ethanol. *Biotechnology Letters* 25: 2055-2059.102.Pollock, T. J. 1993. Gellan-related polysaccharide and the genus *Sphingomonas*. *Journal of General Microbiology* 13: 1939-1945.103.Qian, X. F., Yin, J., Huang, J. C., Yang, Y. F., Guo, X. X. and Zhu, Z. K. 2001. The preparation and characterization of PVA/Ag<sub>2</sub>S nanocomposite. *Materials Chemistry and Physics* 68: 95-97.104.Ramana, K. V., Tomar, A. and Singh, L. 2000. Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter xylinum*. *World Journal of Microbiology & Biotechnology* 16: 245-248.105.Rambo, C. R., Recouvreux, D. O. S., Carminatti, C. A., Pitlovanciv, A. K., Ant?nio, A. V. and Porto, L. M. 2008. Template assisted synthesis of porous nanofibrous cellulose membranes for tissue engineering. *Materials Science and Engineering C* 28: 549-554.106.Ronen, M., Guterman, H. and Shabtai, Y. 2002. Monitoring and control of pullan peoduction using vision sensor. *Journal of Biochemical and Biophysical Methods* 51: 243-249.107.Rong, M. Z., Zhang, M. Q., Zheng, Y. X., Zeng, H. M. and Friedrich, K. 2001. Improvement of tensile properties of nano-SiO<sub>2</sub>/PP composites in relation to percolation mechanism. *Polymer* 42: 3301-3304.108.Ross, P., Mayer, R. and Benziman, M. 1991. Cellulose biosynthesis and function in bacteria. *Microbiological Reviews* 55: 35-58.109.Ross, P., Weinhouse, H., Aloni, Y., Michaeli, D., Weinbergerohana, P., Mayer, R., Braun, S., Devroom, E., Vandermarel, Ga., Vanboom, J. H. and Benziman, M, 1987. Regulation of cellulose synthesis in *Acetobacter-xylinum* by cyclic diguanylic acid. *Nature* 325 (6101): 279-281.110.Sangkok, B. and Makoto, S. 2005. Production of bacterial cellulose by *Acetobacter xylinum* BPR2001 using molasses medium in a jar fermentor. *Applied Microbiology and Biotechnology*. 67: 45-51.111.Saxena, I. M., Kudlicka, K., Okuda, K. and Brown, R. M. Jr. 1994. Characterization of genes in the cellulose-synthesizing operon (acs Operon) of *Acetobacter xylinum*: implicaton for cellulose crystallization. *The Journal of Bacteriology* 176: 5735-5752.112.Saxena, I. M., Lin, I. F. C. and Brown, R. M. Jr. 1990. Cloning and sequencing of the cellulose synthase catalytic subunit gene of *Acetobacter xylinum*. *Plant Molecular Biology* 15: 673-683.113.Schramm, M. and Hestrin, S. 1954. Factors affecting production of cellulose at the air/liquid interface of a culture of *Acetobacter xylinum*. *The Journal of General Microbiology* 11: 123-129.114.Schramm, M., Gromet, Z. and Hestrin, S. 1957. Role of hexose phosphate in synthesis of cellulose by *Acetobacter xylinum*. *Nature* 179(5): 28-29.115.Serafica, G. C. 1997. Production of Bacterial Cellulose Using a Rotating Disk Film Bioreactor by *Acetobacter xylinum*. PhD Thesis, Rensselaer Polytechnic Institute.116.Serafica, G. Mormino, R. and Bungay, H. 2002. Inclusion of solid particles in bacterial cellulose. *Applied Microbiology and Biotechnology* 58(6): 756-760.117.Shamolina, I. I. 1997. Prospects for use of microbial raw material for fabrication of fibre and film materials. *Review. Fibre Chemistry* 29(1): 1-8.118.Siqueira, J. J. P. e Moreschi, J. C. 2000. Membranas de celulose porosas desidratadas para curativos em ?lceras, escoria??es e queimaduras. *Cir Vasc Angiol* 16: 179-180.119.Son, H. J., Kim, H. G., Kim, K. K., Kim, H. S., Kim, Y. G. and Lee, S.J. 2003. Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture conditions. *Bioresource Technology.*, 86: 215-219.120.Standal, R., Iversen, T. G., Coucheron, D. H., Fjaervik, E., Blatny, J. M. and Valla, S. 1994. A new gene required for cellulose production and a gene encoding cellulolyt IC activity in *Acetobacter xylinum* are co-localized with The Bcs Operon. *The Journal of Bacteriology* 176: 665-672.121.Stredansky, M., Conti, E., Bertocchi, C., Matulova, M., and Zanetti, F. 1998. Succinoglycan production by *Agrobacterium tumefaciens*. *Journal of Fermentation and Bioengineering* 85: 398-403.122.Sugiyama, J., Vuong, R. and Chanzy, H. 1991. Electron diffraction study of two crystalline phases occurring in native cellulose from an algal cell wall. *Macromolecules* 24: 4168-4175.123.Sutherland, I. W. 1998. Novel and established application of microbial polysaccharides. *Trends Biotechnol* 16: 41-46.124.Sutherland, I. W. 2001. Microbial polysaccharides from Gram-negative bacteria. *International Dairy Journal* 11: 663-674.125.Tahara, N., Tabuchi, M., Watanabe, K., Yano, H., Morinaga, Y. and Yoshinaga, F. 1997. Degree of polymerization of cellulose from *Acetobacter xylinum* BPR2001 decreased by cellulase produced by the strain. *Bioscience Biotechnology & Biochemistry* 61: 1862-1865.126.Takayasu, T. and Fumihiko, Y. 1997. Production of bacterial cellulose by agitation culture systems. *Pure and Applied Chemistry* 69(11): 2453-2458.127.Takeda-Hirokawa, N., Neoh, L. P., Akimoto, H., Kaneko, H., Hishikawa, T., Sekigawa, I., Hashimoto, H., Hirose, S. I., Murakami, T., Yamamoto, N., Mimura, T. and Kaneko, K. 1997. Role of curdlen sulfate in the binding of HIV-1 gp120 to CD4 molecules and the production of gp120-mediated THF-a. *Microbiology and Immuneology* 41: 741-745.128.Tanaka, M., Murakami, S., Shinke, R. and Aoki, K. 2000. Genetic characteristics of cellulose-forming acetic acid bacteria identified phenotypically as *Gluconacetobacter xylinus*. *Bioscience, Biotechnology and Biochemistry* 64: 757-760.129.Tantratian. S., Tammarate, P., Krusong, W., Bhattarakosol, P. and Phunsri, A. 2005. Effect of dissolved oxygen on cellulose production by *Acetobacter* sp. *Journal of Scientific Research, Chulalongkorn University* 30(2): 179-186.130.Tarr, H. J. and Hibbert, H. 1931. Polysaccharide synthesis by the action of *Acetobacter xylinum* on carbohydrates and related compounds. *The Canadian Journal of Research* 4: 372-388.131.Tendero, C., Tixier, C., Tristant, P., Desmaison, J. and Leprince. P. 2006. Atmosperic pressure plasmas: A review. *Spectrochimica Acta Part B.* 61: 2-30.132.Timell, T. E. 1967. Recent progress in the chemistry of wood hemicelluloses. *Wood Science and Technology* 1: 45-70.133.Toda, K., Asakura, T., Fukaya, M., Entani, E. and Kawamura, Y. 1997. Cellulose production by acetic acid-resistant *Acetobacter xylinum*. *Journal of Fermentation and Bioengineering* 84(3): 228-231.134.Tokoh, C., Takabe, K., Fujita, M. and Saiki, H. 1998. Cellulose synthesized by *Acetobacter xylinum* in the presence of acetyl glucomannan. *Cellulose.* 5: 249-261.135.Toyosaki, H., Naritomi, T., Seto, A., Matsuoka, M., Tsuchida, T. and Yoshinaga, F. 1995. Screening of bacterial cellulose- producing *Acetobacter* strains suitable for agitated culture. *Bioscience Biotechnology & Biochemistry* 59: 1498-1502.136.Tripodo, M. M., Lanuzza, F., Micali, G., Coppolino, R. and Nucita, F. 2004. Citrus waste recovery: a new environmentally friendly procedure to obtain animal feed. *Bioresource Technology.* 91: 111-115.137.Umeda, Y., Hirano, A., Ishibashi, M., Akiyama, H., Onizuka, T., Ikeuchi, M. And Inoue, Y. 1999. Cloning of cellulose synthase genes from *Acetobacter xylinum* JCM 7664: implication of a novel set of cellulose synthase genes. *DNA Reviews* 6: 109-115.138.Vacanti, J. P. and Langer, R. 1999. Tissue engineering:



The design and fabrication of living replacement devices for surgical reconstruction and transplantation. *Lancet*;354 (Suppl 1): S132-S134.139.Vandamme, E. De-Bates, S., Vanbaelen, A., Joris, k. and De-wulf, P. 1998. Improved production of bacterial cellulose-production Acetobacter strains suitable for agitated culture. *Biosecience Biotechnilogy and Biochemistry* 59: 1498-1502.140.VanderHart, D. L., and Atalla, R. H. 1987. Further carbon-13 NMR evidence for the coexistence of two crystalline forms in native celluloses. In R. H. Atalla, *The structures of celluloses*. ACS Symposium Series (340) p.88 – 118. American Chemical Society. Washington, DC, USA.141.White, D. G. and Brown, Jr. R. M. 1989. Prospects for the commercialization of the biosynthesis of microbial cellulose. In: Schuerch C, editor. *Cellulose and Wood – Chemistry and Technology*. 573-590.142.Wong, H. C., Fear, A. L., Calhoon, R. D., Eichinger G. H, Mayer R, Amikam, D. Benziman, M., Gelfand, D. H., Meade, J. H. and Emerick, A. W. 1990. Genetic organization of the cellulose synthase operon in *Acetobacter xylinum*. *Proceedings of the National Academy of Sciences* 87: 8130-8134.143.Wright, J. B., Lam, K., Hansen, D. and Burrell, R. E. 1999. Efficacy of topical silver against fungal burn wound pathogens. *American Journal of Infection Control* 27(4): 344-350.144.Xiong, J., Ye, J., Liang, W. Z. and Fan, P. M. 2000. Influence of microwave on the ultrastructure of cellulose. *Journal of South China University of Technology* 28(3): 84-89.145.Yamamoto, H., Horii, F. and Hirai, A. 1996. In situ crystallization of bacterial cellulose. II. Influences of different polymeric additives on the formation of cellulose I and I at the early stage of incubation. *Cellulose* 3: 229-242.146.Yamanaka, K., Hasegawa, A., Sawamura, R. and Okada, S. 1989. Dimethylatedarsenics induce DNA strand breaks in lung via the production of active oxygen in mice. *Biochemistry and Biophysical Research Communications* 165: 43-50.147.Yoshida, M. 1998. Cosmetic material. Japanese patent 10203953A.148.Yoshida, M. 1999. Composition for acid hair dye. Japanese patent 11240824A.149.Yoshinaga, F., Tonouchi, N. and Watanabe, K. 1997. Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material. *Bioscience, Biotechnology and Biochemistry* 61(2): 219-24.150.Young, K. Y., Sang, H. P., Jung, W. H., Yu, R. P. and Yu, S. K. 1998. Cellulose production by *Acetobacter xylinum* BRC5 under agitated condition. *Journal of Fermentation and Bioengineering* 85: 312-317.151.Zevenhuizen, L. P. T. M. 1997. Succinoglycan and galactoglycan. *Carbohydrate Polymers* 33: 139-144.152.Zhou, L. L., Sun, D. P., Hu L. Y., Li, Y. W. and Yang, J. Z. 2007. Effect of addition of sodium alginate on bacterial cellulose production by *Acetobacter xylinum*. *Journal of Industrial Microbiology & Biotechnology* 34(7): 483-487.