

# 生物絮凝劑之生產及影響絮凝作用因子之探討

毛禹蓁、吳建一

E-mail: 362535@mail.dyu.edu.tw

## 摘要

本研究第一部分係利用自行篩選菌株Bacillus subtilis DYU1作為生產生物絮凝劑(聚穀胺酸(polyglutamic acid, PGA))生產菌株，於5-L發酵槽中探討不同攪拌速率對於PGA生產之影響。在37℃、攪拌速率300 rpm、曝氣量1 vvm以及初始pH值為8的培養條件下，可獲得最佳的PGA產量為12.6 g/L。B. subtilis DYU1生產之生物聚合物(biopolymer)經核磁共振(nuclear magnetic resonance spectrometry, NMR)與胺基酸分析(amino acid identification)得知生物聚合物主要結構為PGA。第二部分係影響生物絮凝劑(PGA)絮凝影響因子之探討。Bacillus subtilis DYU1生產的PGA對澱粉廢水、SiO<sub>2</sub>廢水、活性污泥、Chlorella sp. Y8-1懸浮液(淡水條件下)具有良好的絮凝能力。在所有的絮凝對象添加三價陽離子Fe<sup>3+</sup>或Al<sup>3+</sup>明顯地促進PGA的絮凝活性。此外，根據實驗觀察到的結果提出並描述絮凝劑在絮凝過程中的機制。

關鍵詞：生物絮凝劑、聚穀胺酸

## 目錄

封面內頁 簽名頁 中文摘要 iii 英文摘要 iv 誌謝 v 目錄 vi 圖目錄 xi 表目錄 xvii 1. 緒論 1 1.1 前言 1 1.2 動機與目的 3 2. 文獻回顧 5 2.1 天然聚合物之絮凝應用 5 2.1.1 絮凝劑之簡介 5 2.1.2 天然聚合物之絮凝機制 9 2.1.2.1 幾丁聚醣 9 2.1.2.1 陽離子澱粉 9 2.1.2.3 PGA 10 2.2 影響絮凝劑效果之影響因子 12 2.2.1 絶凝劑分子量 12 2.2.2 pH值 13 2.2.3 溫度 14 2.2.4 重金屬離子 14 2.2.5 絶凝劑濃度 15 2.3 PGA (polyglutamic acid)之簡介 16 2.3.1 PGA之特性 16 2.3.2 PGA之合成以及相關酵素與機制 17 2.3.2.1 PGA之合成 17 2.3.2.2 相關酵素與機制 19 2.3.3 PGA之降解作用 21 2.4 PGA生產菌株及其特性 26 2.4.1 需L-穀胺酸之菌株 32 2.4.2 不需L-穀胺酸之菌株 38 2.5 PGA之相關應用 40 2.5.1 食品工業上之應用 42 2.5.2 生醫材料/藥物上之應用 43 2.5.3 水處理之應用 45 2.5.4 化妝品之應用 48 2.5.5 其他之應用 49 2.6 目前使用藻類收集之方法 51 2.6.1 離心 52 2.6.2 混凝/絮凝 53 2.6.2.1 聚電解質 54 2.6.2.2 無機絮凝劑 56 2.6.2.3 調控pH值絮凝 57 2.6.3 過濾 58 2.6.4 電解絮凝 59 2.7 先前研究結果 61 3. 材料與方法 62 3.1 實驗材料 62 3.1.1 實驗藥品 62 3.1.2 儀器設備 64 3.2 PGA生產菌株培養 66 3.2.1 菌株來源 66 3.2.2 菌株活化 66 3.2.3 PGA生產培養 66 3.3 分析方法 67 3.3.1 發酵液之分析方法 67 3.3.1.1 菌體濃度分析 67 3.3.1.2 麥芽糖濃度分析 67 3.3.1.3 總醣含量分析 - 酚-硫酸法 68 3.3.1.4 黏度之測量 68 3.3.2 PGA純化方法及特性分析 69 3.3.2.1 PGA之回收與純化 69 3.3.2.2 PGA產量測定 70 3.3.2.3 氨基酸分析 71 3.3.2.4 核磁共振(NMR)分析 71 3.4 絶凝能力分析 71 4. 結果與討論 77 4.1 發酵槽中攪拌速率對PGA生產之影響 77 4.2 PGA產物之分析 82 4.2.1 核磁共振(NMR)分析 82 4.2.2 PGA組成之胺基酸分析 84 4.3 模擬食品與發酵工業排放水之懸浮微粒 85 4.3.1 pH值對於PGA絶凝效果之影響 85 4.3.2 PAC助凝劑濃度對於PGA絶凝效果之影響 89 4.3.3 PGA絶凝劑濃度對於絶凝效果之影響 91 4.3.4 金屬離子價數對於PGA絶凝效果之影響 95 4.4 模擬半導體產業放流水與冷卻水之懸浮微粒 99 4.4.1 沉降時間對於PGA絶凝效果之影響 99 4.4.2 pH值對於PGA絶凝效果之影響 101 4.4.3 PAC助凝劑濃度對於PGA絶凝效果之影響 104 4.4.4 PGA絶凝劑濃度對於絶凝SiO <sub>2</sub> 效果之影響 106 4.4.5 金屬離子價數對於PGA絶凝效果之影響 110 4.5 提高污泥沉降速率 112 4.5.1 pH值對於PGA絶凝活性污泥效果之影響 112 4.5.2 PAC助凝劑濃度對於PGA絶凝活性污泥效果之影響 116 4.5.3 PGA絶凝劑濃度對於絶凝活性污泥效果之影響 118 4.5.4 金屬離子價數及濃度對於PGA絶凝活性污泥效果之影響 122 4.5.5 污泥容積指標(sludge volume index, SVI) 125 4.6 Chlorella sp. Y8-1懸浮液之絶凝實驗 127 4.6.1 沉降時間對於PGA絶凝Chlorella sp. Y8-1懸浮液效果之影響 128 4.6.2 pH值對於PGA絶凝Chlorella sp. Y8-1懸浮液效果之影響 132 4.6.3 PAC助凝劑濃度對於PGA絶凝Chlorella sp. Y8-1懸浮液效果之影響 137 4.6.4 PGA絶凝劑濃度對於絶凝Chlorella sp. Y8-1懸浮液效果之影響 141 4.6.5 鹽類濃度(NaCl)對於PGA絶凝Chlorella sp. Y8-1懸浮液效果之影響 147 4.6.7 金屬離子價數及濃度對於PGA絶凝Chlorella sp. Y8-1懸浮液效果之影響 150 4.7 絶凝機制 156 5. 結論 158 參考文獻 160 圖目錄 Figure 1-1 Schematic of this study procedure. 4 Figure 2-1 Chemical structure of polyglutamic acid. 17 Figure 2-2 Proposed pathway of PGA synthesis in B. subtilis IFO 3335 24 Figure 2-3 The genetic elements required for PGA synthesis in B. anthracis, B. subtilis and B. licheniformis. 25 Figure 2-4 Schematic diagram of electrolytic unit 60 Figure 3-1 Standard curve of total sugar. 68 Figure 3-2 Preparation of pure -PGA 70 Figure 4-1 Time course of PGA production and cell growth of by B. subtilis DYU1 in batch fermentation at various agitation speed. 80 Figure 4-2 The effect of agitation speed on Y <sub>p</sub> /s, Y <sub>x</sub> /s, Y <sub>p</sub> /x, vPGA, vmaltose of B. subtilis DYU1 in batch fermentation. 81 Figure 4-3 1H NMR spectra of PGA product from B. subtilis DYU1 in chemical defined medium. 83 Figure 4-4 13C NMR spectra of PGA product from B. subtilis DYU1 in chemical defined medium. 83 Figure 4-5 Amino acid analysis of purified PGA hydrolysate. 84 Figure 4-6 The effect of pH on flocculating activity and flocculating rate of PGA 200 mg/L against
---

starch wastewater, containing PAC 50 mg/L, in flashwater. 87 Figure 4-7 Zata-potential of starch wastewater at diffeent pH. 88 Figure 4-8 The effect of PAC concentration on flocculating activity and flocculating rate of PGA 200 mg/L against starch wastewater. 90 Figure 4-9 The effect of PGA concentration and molecular weight of PGA on flocculating activity and flocculating rate against starch wastewater . 93 Figure 4-10 The photo and SEM of starch wastewater before and after flocculation by PGA. 94 Figure 4-11 Effect of cation concentrations on flocculating activity of PGA (200 mg/L) against starch wastewater at pH 9.0 97 Figure 4-12 Effect of cation concentrations on flocculating rate of PGA (200 mg/L) against starch wastewater at pH 9.0 98 Figure 4-13 The time course of flocculating rate of PGA (200 mg/L) against SiO<sub>2</sub> wastewater, containing PAC 50 mg/L. 100 Figure 4-14 The effect of pH on flocculating rate of PGA 200 mg/L against SiO<sub>2</sub> wastewater, containing PAC 50 mg/L. 102 Figure 4-15 Zata-potential of SiO<sub>2</sub> wastewater at different pH conditions. 103 Figure 4-16 The effect of PAC concentration on flocculating rate of PGA 200 mg/L against SiO<sub>2</sub> wastewater. 105 Figure 4-17 The effect of PGA concentration on flocculating rate against SiO<sub>2</sub> wastewater. 108 Figure 4-18 The photo and SEM of SiO<sub>2</sub> wastewater before and after flocculation by PGA. 109 Figure 4-19 Effect of cation concentrations on flocculating rate of PGA (200 mg/L) against SiO<sub>2</sub> wastewater at pH 9.0 111 Figure 4-20 The effect of pH on flocculating active and flocculating rate of PGA (200 mg/L) against activated sludge suspension, containing PAC 50 mg/L. 114 Figure 4-21 Zata-potential of activated sludge suspension at different pH condition. 115 Figure 4-22 The effect of PAC concentration on flocculating activity and flocculating rate of PGA (200 mg/L) against activated sludge suspension. 117 Figure 4-23 The effect of PGA concentration on flocculating activity and flocculating rate against activated sludge suspension. 120 Figure 4-24 The photo and SEM of activated sludge suspension before and after flocculation by PGA. 121 Figure 4-25 Effects of cation concentrations on flocculating activity of PGA (100 mg/L) against activated sludge suspension at pH 7.0 123 Figure 4-26 Effects of cation concentrations on flocculating rate of PGA (100 mg/L) against activated sludge suspension at pH 7.0 124 Figure 4-27 The effect of PGA concentration on sludge volume index 126 Figure 4-28 Time course of the flocculating activity and flocculating rate of VADAN Co. PGA (200 mg/L) against suspendedsolution of Chlorella sp. Y8-1 containing PAC (50 mg/L) at pH 7.0. 130 Figure 4-29 Time course of the flocculating activity and flocculating rate of PGA products (200 mg/L) and fermentation broth (containing PGA concentration of 220 mg/L) B. subtilius DYU1 against suspended solution of Chlorella sp. Y8-1 containing PAC 50 mg/L at pH 7.0. 131 Figure 4-30 The effect of pH on flocculating activity and flocculating rate of VADAN Co. PGA (200 mg/L) against suspended solution of Chlorella sp. Y8-1 containing PAC (50 mg/L). 134 Figure 4-31 The effect of pH on flocculating activity and flocculating rate of PGA products (200 mg/L) and fermentation broth (containing PGA concentration of 220 mg/L) B. subtilius DYU1 against suspended solution of Chlorella sp. Y8-1 containing PAC 50 mg/L. 135 Figure 4-32 Zata-potential of Chlorella sp. Y8-1 at different pH condition in freshwater. 136 Figure 4-33 The effect of PAC concentration on flocculating activity and flocculating rate of VADAN Co. PGA (200 mg/L) against suspended solution of Chlorella sp. Y8-1 at pH 8. 139 Figure 4-34 The effect of PAC concentrationon flocculating activity and flocculating rate of PGA products (200 mg/L) and fermentation broth (containing PGA concentration of 220 mg/L) B. subtilius DYU1 against suspended solution of Chlorella sp. Y8-1at pH 8. 140 Figure 4-35 The effect of PGA concentration on flocculating activity and flocculating rate of VADAN Co. PGA against suspended solution of Chlorella sp. Y8-1 at pH 8. 142 Figure 4-36 The effect of PAC concentrationon flocculating activity and flocculating rate of PGA products and fermentation broth B. subtilius DYU1 against suspended solution of Chlorella sp. Y8-1 at pH 8. 143 Figure 4-37 The effect of NaCl concentration on flocculating activity and flocculating rate against Chlorella sp. Y8-1 at pH 8. 146 Figure 4-38 The effect of algal broth concentration on flocculating activity and flocculating rate against Chlorella sp. Y8-1 at pH 8. 149 Figure 4-39 Effects of metal concentrations on flocculating activity of purified PGA (150 mg/L) from B. subtilis DYU1 against Chlorella sp. Y8-1 at pH 8.0 152 Figure 4-40 Effects of metal concentrations on flocculating rate of purified PGA (150 mg/L) from B. subtilis DYU1 against Chlorella sp Y8-1 at pH 8.0: 153 Figure 4-41 Effects of metal concentrations on flocculating activity of PGA fermentation broth (containing PGA concentration of 220 mg/L) of B. subtilis DYU1 against Chlorella sp. Y8-1 at pH 8.0: 154 Figure 4-42 Effects of metal concentrations on flocculating rate of PGA fermentation broth (containing PGA concentration of 220 mg/L) of B. subtilis DYU1 against Chlorella sp. Y8-1 at pH 8.0 155 Figure 4-43 Proposed flocculation mechanism for polyglutamic acid. 157 表目錄 Table 2-1 Production bacterial strain of bioflocculant. 8 Table 2-2 Comparison with different bacteria strains of culture condition and PGA production (GA-dependent strans) 27 Table 2-3 Comparison with different bacteria strains of culture condition and PGA production (GA-independent strans). 30 Table 2-4 Potential applications of PGA and its derivatives. 41

## 參考文獻

- 1.李春明。2006。混凝程序去除二氧化矽之反應特性研究。朝陽科技大學環境工程與管理系，台中。
- 2.李琳，張清敏，楊建華。2006。複合微生物絮凝處理?薯澱粉廢水的研究。環境科學與技術 29 (7):75-77。
- 3.唐受印，戴友芝，劉忠義。2001。食品工工業水處理。北京:化學工業出版社。
- 4.陳右承。2009。利用B. subtilis DYU1生產聚麩胺酸並進行聚麩胺酸絮凝作用之探討。大葉大學生物產業科技研究所碩士論文，彰化。
- 5.黃志雄，游衛強。2008。廣東、廣西薯類澱粉廢水處理技術研究與發展。化學工程與裝備 8:121-123。
- 6.劉榮元。2005。篩選聚麩胺酸生產菌株及其絮凝性質之研究。大葉大學生物產業科技研究所碩士論文，彰化。
- 7.蔡傑寧。2004。以量筒沉降試

驗評估載體混凝技術之可行性。朝陽科技大學環境工程與管理系，台中。8.Antoni, D., Zverlov, V. V. and Schwarz, W. H. 2007. Biofuels from microbes. *Applied Microbiology and Biotechnology*. 77:23 – 35. 9.Ashiuchi, M. and Misono, H. 2002a. Poly- $\gamma$ -glutamic acid. In: *Biopolymers Vol. 7*, S.R. Fahnestock, and A. Steinbuchel, eds.(Weinheim, Germany: Wiley-VCH) pp. 123 – 174. 10.Ashiuchi, M. and Misono, H. 2005. Poly- $\gamma$ -glutamic acid. In: Steinbuchel A, Marchessault R. H., Editors. *Biopolymers for medical and pharmaceutical applications*, Vol. 1. Wiley-VCH, Weinheim, p. 619-634. 11.Ashiuchi, M., Kamei, T., Baek, D. H, Shin, S. Y., Sung, M. H., Soda, K., Yagi T, Misono H. 2001b. Isolation of *Bacillus subtilis* (chungkookjang), a poly- $\gamma$ -glutamate producer with high genetic competence. *Appl Microbiol Biotechnol*; 57: 764 – 769. 12.Ashiuchi, M., Tani, K., Soda, K., Misono, H. 1998. Properties of glutamate racemase from *Bacillus subtilis* IFO 3336 producing poly- $\gamma$ -glutamate. *The Journal of Biochemistry* 123:1156-1163. 13.Avichezer, D., Schechter, B., Arnon, R. 1998. Functional polymers in drug delivery: carrier-supported CDDP (cis-platin) complexes of polycarboxylates-effect on human ovarian carcinoma. *React Functional Polym*; 36: 59 – 69. 14.Bajaj, I. B. and Singhal, R. S. 2009a. Sequential optimization approach for enhanced production of poly(  $\gamma$ -glutamic acid) from *Bacillus subtilis* of marine origin. *Food Technol Biotechnol*; Article in Press. 15.Bajaj, I. B., Lele, S. S., Singhal, R. S. 2009. A statistical approach to optimization of fermentative production of poly(  $\gamma$ -glutamic acid) from *Bacillus licheniformis* NCIM 2324. *Bioresource Technology* 100: 826-832. 16.Belitsky, B. R. and Sonenshein, A. L. 1998. Role and regulation of *Bacillus subtilis* glutamate dehydrogenase genes. *J Bacteriol*; 180: 6298-6305. 17.Bender, J., Rodriguez-Eaton, S., Ekanemesang, U. M. and Phillips, P. 1994. Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial mats. *Applied and Environment Microbiology*. 60: 2311-2315. 18.Benemann, J. R., Kopman, B. L., Weissman, D. E., Eisenberg, D. E. and Goebel, R.P. 1980. Development of microalgae harvesting and high rate pond technologies in California. In: Shelef G, Soeder CJ, editors. *Algal biomass*. Amsterdam: Elsevier. p. 457. 19.Bernhardt, H. and Clasen, J. 1991. Flocculation of Micro-organisms. *J. Water SRT-Aqua*. 40: 76-87. 20.Bernhardt, H. and Clasen, J. 1994. Investigations into the Flocculation Mechanisms of small Algal Cells. *Journal of Water SRT-AQUA*. 43: 222-232. 21.Bhattacharyya, D., Hestekin, J. A., Brushaber, P., Cullen, L., Bachas, L. G., Sikdar, S., K. 1998. Novel polyglutamic acid functionalized microfiltration membranes for sorption of heavy metals at high capacity. *J Membr Sci*; 141: 121 – 135. 22.Bilanovic, D., Shelef, G. and Sukenik, A. 1988. Flocculation of microalgae with cationic polymers : effects of medium salinity. *Biomass*. 17: 65 – 76. 23.Birrer, G. A., Cromwick, A. M., Gross, R. A. 1994. -Poly(glutamic acid) formation by *Bacillus licheniformis* 9945a: physiological and biochemical studies. *Int J Biol Macromol*; 16: 265 – 275. 24.Blanchemain, A. and Grizeau, D. 1999. Increased production of eicosapentaenoic acid by *Skeletonema costatum* cells after decantation at low temperature. *Biotechnology Techniques*. 13: 497 – 501. 25.Borowitzka, M. A. 1997. Microalgae for aquaculture: opportunities and constraints. *Journal of Applied Phycology*. 9:393 – 401. 26.Briley, D.S. and Knappe, D.R.U. 2002. Optimizing ferric sulfate coagulation of algae with streaming current measurements. *Journal American Water Works Association*. 94: 80 – 90. 27.Buescher, J. M. and Margaritis, A. 2007. Microbial biosynthesis of polyglutamic acid biopolymer and applications in the biopharmaceutical, biomedical and food industries Criterion Review *Biotechnology* 27: 1-19. 28.Candela, T. and Fouet, A. 2006. Poly-gamma-glutamate in bacteria-Micro Review. *Mol Microbiol*;60 (5): 1091-1098. 29.Candela, T., Mock, M., Fouet, A. 2005. CapE, a 47-amino-acid peptide, is necessary for *Bacillus anthracis* polyglutamate capsule synthesis. *J Bacteriol*; 187: 7765 – 7772. 30.Chen, Y.M., Liu, J.C. and Ju, Y. 1998. Flotation Removal of Algae from Water. *Colloids and Surfaces B: Biointerfaces*. 12: 49-55. 31.Cheng, C., Asada, Y., Aida, T. 1989. Production of -polyglutamic acid by *Bacillus subtilis* A35 under denitrifying conditions. *Agri Biol Chem*; 53: 2369 – 2375. 32.Chisti, Y. 1999. Shear sensitivity. In: Flickinger MC, Drew SW, editors. *Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation*, vol. 5. New York: Wiley. p. 2379 – 406. 33.Choi, H. J. and Kunioka, M. 1995. Preparation conditions and swelling equilibria of hydrogel prepared by -irradiation from microbial poly- $\gamma$ -glutamic acid. *Radiat Phys Chem*; 46: 175-179. 34.Chung, P., Chol, Y. H., Shin, H. J., Poo, H., Song, J. J., Kim, C. J., Sung, M. H. 2005. Effect of high-molecularweight poly- $\gamma$ -glutamic acid from *Bacillus subtilis* (chungkookjang) on Ca solubility and intestinal absorption. *J Microbiol Biotechnol*; 15: 855-858. 35.Chung, Y., Choi, Y. C., Choi, Y. H. and Kang, H. S. 2000. A Demonstration Scaling-up of the Dissolved Air Flotation. *Water Research*. 34: 817 – 824. 36.Cromwick, A. M. and Gross, R. A. 1995a. Effect of manganese(II) on *Bacillus licheniformis* ATCC 9945A physiology and -poly(glutamic acid) formation. *Int J Biol Macromol*; 16: 265 – 75. 37.Cromwick, A. M. and Gross, R. A. 1995b. Investigation by NMR of metabolic routes to bacterial -poly(glutamic acid) using  $^{13}\text{C}$ -labeled citrate and glutamate as media carbon sources. *Can J Microbiol*; 41:902 – 909. 38.Cromwick, A. M., Birrer, G. A., Gross, R. A. 1996. Effects of pH and aeration on -poly(glutamic acid) formation by *Bacillus licheniformis* in controlled batch fermentor cultures. *Biotechnol Bioeng*; 50: 222 – 227. 39.Daninippon Pharmaceutical Co, Ltd. 1972. Ice cream stabilizer. JP 19735/72. 40.Dearfield, K. L., Abernathy, C. O., Ottley, M. S., Brantner J. H., Hayes, P. F. 1988. Acrylamide: its metabolism, developmental and reproductive effects, genotoxicity and carcinogenicity. *Mutation Research* 195: 45-77. 41.Dearfield, K. L., Abernathy, C. O., Ottley, M. S., Brantner, J. H. and Hayes, P. F. 1988. Acrylamide: its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. *Mutation Research*. 195: 45-77. 42.Deng, S. B., Bai, R. B., Hu, X. M. and Luo, Q. 2003. Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment. *Agricultural and Biological Chemistry*. 60: 588-593. 43.Dodd, J. C. 1979. Algae production and harvesting from animal wastewaters. *Agricultural Wastes*. 1: 23 – 37. 44.Dries Vandamme and Imogen Foubert. 2010. Flocculation of microalgae using cationic starch. *Journal of Applied Phycology*: 10.1007/s10811-009-9488-8. 45.Du, G., Yang, G., Qu, Y., Chen, J., Lun, S. 2005. Effects of glycerol on the production of poly(  $\gamma$ -glutamic acid) by *Bacillus licheniformis*. *Process Biochemistry* 40: 2143-2147. 46.Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350-356. 47.Edzwald, J. K. 1993. Algae, bubbles, coagulants, and dissolved air flotation. *Water Science and Technology*. 27: 67 – 81. 48.Elmaleh, S., Coma, J., Grasmick, A. and Bourgade, L. 1991. Magnesium induced algal flocculation in a fluidized bed.

Water Science and Technology. 23: 1695 – 1702. 49.Esser, K. and Kues, U. 1983. Flocculation and its implication for biotechnology. Process Biochemistry. 18: 21-23. 50.Fujita, M., Ike, M., Tachibana, S., Kitada, G., Kim, S. M. and Inoue, Z. 2000. Characteristics of a bioflocculant produced by *Citrobacter* sp. YKF04 from acetic and propionic acids. Journal of Bioscience and Bioengineering. 89: 40 – 46. 51.Gardner, J. M. and Troy, F. A. 1979. Chemistry and biosynthesis of the poly( -D-glutamyl)capsule in *Bacillus licheniformis*. Activation, racemization, and polymerization of glutamic acid by a membranous polyglutamyl synthetase complex. The Journal of Biological Chemistry 254:6262 – 6269.

52.Ghosh, S. C., Auzenne, E., Khodadadian, M., Farquhar, D., Klostergaard, J. 2009. N,N-Dimethylsphingosine conjugates of poly-L-glutamic acid: Synthesis, characterization, and initial biological evaluation Bioorg Med Chem Lett; 19 (3): 1012-1017. 53.Goto, A. and Kunioka, M. 1992. Biosynthesis and hydrolysis of poly( -glutamic acid) from *Bacillus subtilis* IFO 3335. Bioscience, Biotechnology, and Biochemistry. 56: 1031-1035.

54.Gregory, J. 1989. Fundamentals of flocculation. Critical Reviews in Environmental Control. 19: 185 – 194. 55.Grima, E. M., Belarbi, E. H., Fernandez, F. G. A., Medina, A. R., Chisti, Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnology Advances. 20: 491 – 515. 56.Gross, A. 1998. Bacterial -poly(glutamic acid). In Biopolymers from renewable resources, D.L. Kaplan, ed. (New York: Springer Berlin Heidelberg) 195 – 219. 57.Gudin, C. and Therpenier, C. 1986. Bioconversion of solar energy into organic chemicals by microalgae. Advances in Biotechnological Processes. 6: 73 – 110. 58.Gutcho, S. 1977. Waste treatment with Polyelectrolytes and other flocculants. Noyes Data Corp., Park Ridge, NJ, pp. 1-37. 59.Hantula, J. and Bamford, D. H. 1991b. Bacterial phage resistance and flocculation deficiency of *Flavobacterium* sp. Are phenotypically interrelated. Applied Microbiology and Biotechnology 36:105 – 8. 60.Hantula, J. and Bamford, D. H. 1991b. Bacterial phage resistance and flocculation deficiency of *Flavobacterium* sp. are phenotypically interrelated. Applied Microbiology and Biotechnology. 36: 105-108. 61.Hara, T., Aumayr, A., Fujio, Y., Ueda, S. 1982b. Elimination of plasmid-linked polyglutamate production by *Bacillus subtilis* (natto) with acridine orange. Appl Environ Microbiol; 44:1456 – 1458. 62.Hara, T., Fujio, Y., Ueda, S. 1982a. Polyglutamate production by *Bacillus subtilis* (natto). J Appl Biochem; 4: 112-120. 63.Heasman M, Diemar J, O ' Connor W, Sushames T, Foulkes L, Nell J. A. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs—a summary. Aquacult Res 2000;31(8 – 9):637 – 59. 64.Heasman, M., Diemar, J., O ' Connor, W., Sushames, T., Foulkes, L., Nell, J. A. 2000. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs—a summary. Aquaculture Research. 31: 637 – 659. 65.Holzer, H. 1969. Regulation of enzymes by enzyme-catalyzed chemical modification. Adv Enzymol; 32: 297-326. 66.Hsu, S. H. and Lin, C. H. 2007. The properties of gelatin – poly ( -glutamic acid) hydrogels as biological glues. Biorheology; 44: 17 – 28. 67.Hunter, R.J. 1993. Introduction to Modern Colloid Science 33-38, 4th edition, Oxford University Press, New York. 289-290. 68.Ito, Y., Tanaka, T., Ohmachi, T., Asada, Y. 1996. Glutamic acid independent production of poly(-glutamic acid) by *Bacillus subtilis* TAM-4. Biosci Biotechnol Biochem; 60: 1239 – 1242. 69.Ivanovics, G. and Bruckner, V. 1937. Chemische und immunologische Studien über den Mechanismus der Milzbrandinfektion und Immunität; die chemische Struktur der Kapdelsubstanz des Milzbrandbasillus und der serologisch identischen spezifischen Substanz des *Bacillus mesentericus*. Z. Immunitsforsch Exp Ther 90: 304-318. 70.Jung, D. K., Jung, S., Sun, J. S., Kim, J. N., Wee, Y. J., Jang, H. G., Ryu, H. W. 2006. Influences of cultural medium components on the production of poly (gamma-glutamic acid) by *Bacillus* sp. RKY3. Biotechnol Bioprocess Eng; 10: 289 – 295. 71.Kambourova, M., Tangney, M., Priest, F. G. 2001. Regulation of polyglutamic acid synthesis by glutamate in *Bacillus licheniformis* and *Bacillus subtilis*. Appl Environ Microbiol; 67 (2): 1004-1007 72.Kandler, O., Konig, H., Wiegel, J., Claus, D. 1983. Occurrence of poly--D-glutamic acid and poly- -L-glutamine in the genus *Xanthobacter*, *Flexithrix*, *Sporosarcina* and *Planococcus*. Syst Appl Microbiol 1 4(1): 34-41. 73.Kaya VM, Picard G. Stability of chitosan gel as entrapment matrix of viable *Scenedesmus bicellularis* cells immobilized on screens for tertiary treatment of wastewater. Bioresour Technol 1996;56:147 – 55. 74.Kimura, K., Tran, L. S. P., Uchida, I., Itoh, Y. 2004. Characterization of *Bacillus subtilis* gammaglutamyltransferase and its involvement in the degradation of capsule poly-gammaglutamate. Microbiol; 150: 4115 – 4123. 75.King, E. C., Blacker, A. J., Bugg, T. D. H. 2000. Enzymatic breakdown of poly--D glutamic acid in *Bacillus licheniformis*: identification of a polyglutamyl -hydrolase enzyme. Biomacromolecules; 1: 75 – 83. 76.Klute, R. and Neis, U. 1976. Stability of colloidal kaolinite suspensions in the presence of soluble organic compounds. In: Ker M, editor. Proceedings of the international conference, colloid interface science, 50th ed 4: 113. 77.Knuckey, R., Brown, M., Robert, R. and Frampton, D. 2006. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacultural Engineering. 35: 300 – 313. 78.Konno, A., Taguchi, T., Yamaguchi, T. 1989. Bakery products and noodles containing polyglutamic acid. USP 4,888,193. 79.Koopman, B. and Lincoln, E. P. 1983. Autoflotation harvesting of algae from high rate pond effluents. Agricultural Wastes. 5: 231 – 246. 80.Kubota, H., Matsunobu, T., Uotani, K., Takebe, H., Satoh, A., Tanaka, T., Taguchi, M. 1993a. Production of Poly( -glutamic acid)by *Bacillus subtilis* F-2-01. Bioscience, biotechnology, and biochemistry 57, 1212-1213. 81.Kubota, H., Nambu, Y., Endo, T. 1996. Alkaline hydrolysis of poly( -glutamic acid) produced by microorganism. Journal of Polymer Science Part A: Polymer Chemistry 34: 1347-1351. 82.Kunioka, M. 1995. Biosynthesis of poly (-glutamic acid) from L-glutamine, citric acid and ammonium sulfate in *Bacillus subtilis* IFO3335. Appl Microbiol Biotechnol; 44: 501-506 83.Kunioka, M. 1997. Biosynthesis and chemical reactions of poly(amino acid)s from microorganisms. Applied Microbiology and Biotechnology 47: 469-475. 84.Kunioka, M. and Goto, A. 1994. Biosynthesis of poly(-glutamic acid) from L-glutamine, citric acid and ammonium sulfate in *Bacillus subtilis* IFO3335. Appl Microbiol Biotechnol; 40: 867-872. 85.Kurane, R. and Nohata, Y. 1991. Microbial flocculation of waste liquids and oil emulsions by a bioflocculant from *Alcaligenes latus*. Agricultural Biology and Chemistry. 55: 1127 – 1129. 86.Kurane, R., Takeda, K., Suzuki, T. 1986. Screening for and characteristics of microbial flocculant. Agricultural and Biological Chemistry 50: 2301-2307. 87.Kwon, G. S., Moon, S. H., Lee, H. M., Kim, H. S., Oh, H. M., Yoon, B. D. 1996. A novel flocculant biopolymer produced by *Pestalotiopsis* sp. KCTC 8637P. Biotechnology Letters. 18: 1459 – 1464. 88.Lavoie, A. and de la Noue, J. 1983.

Harvesting microalgae with chitosan. Journal of the World Mariculture Society. 14: 685 – 694. 89.Lee, D. J. and Mueller, J. A. 2001. Preliminary treatments, in Sludge into Biosolids — Processing Disposal, Utilization. Editors: L. Spinosa and A. Vesilind, IWA Publishing, London, UK. 90.Lee, H. L., Lee, S. O., Jang, K. L., Lee, T. H. 1995. Microbial flocculant from Arcuadendron sp. TS-49. Biotechnology Letters. 17: 95 – 100. 91.Lee, S. H., Lee, S. O., Jang, K. L., Lee, T. H. 1995. Microbial flocculant from Arcuadendron sp. TS-49. Biotechnology Letters 17: 95-100. 92.Lee, S., Kim, S., Kim, J., Kwon, G., Yoon, B. and Oh, H. 1998. Effects of harvesting method and growth stage on the flocculation of the green alga *Botryococcus braunii*. Letters in Applied Microbiology. 27: 14 – 28. 93.Lee, S. H., Lee, S. O., Jang, K. L. and Lee, T. H. 1995. Microbial flocculant from Arcuadendron sp. TS-49. Biotechnology Letters. 17: 95-100. 94.Leonard, C. G., Housewright, R. D., Thorne, C. B. 1958a. Effects of some metallic ions on glutamyl polypeptide synthesis by *Bacillus subtilis*. J Bacteriol; 76: 499 – 503. 95.Levin S. and Friesen, W. T. 1987. Flocculation of colloid particles by water soluble polymers. In: Attia YA, editor. Flocculation in biotechnology and separation system. Amsterdam:Elsevier 3 – 20. 96.Levy, N., Magdasi, S., Bar-Or, Y. 1992. Physico-chemical aspects in flocculation of bentonite suspensions by a cyanobacterial. Water Research. 26: 249-254. 97.Levy, N., Magdasi, S., Bar-Or, Y. 1992. Physico-chemical aspects in flocculation of bentonite suspensions by a cyanobacterial. Water Research 26:249 – 54. 98.Li, C., Yu, D. F., Newman, A., Cabral, F., Stephens, C., Hunter, N., Milas, L., Wallace, S. 1998. Complete regression of well-established tumors using novel water-soluble poly (L-glutamic acid)-paclitaxel conjugate. Cancer Res; 58: 2404 – 2409. 99.Li, Y., He, N., Guan, H., Du, G., Chen, J. 1999b. A novel polygalacturonic acid bioflocculant REA-11 produced by *Corynebacterium glutamicum*: a proposed biosynthetic pathway and experimental confirmation. Appl Microbiol Biotechnol; 52: 698 – 703. 100.Lincoln, E. P. 1985. Resource recovery with microalgae. Arch Hydrobiol. 20: 25 – 34. 101.Lu, W. K., Chiu, T. Y., Hung, S. H., Shih, I. L., Chang, Y. N. 2004. Use of response surface methodology to optimize culture medium for production of poly-gamma-glutamic acid by *Bacillus licheniformis*. Int J Appl Sci Eng; 2: 49 – 58. 102.Lubian L M. 1989. Concentrating cultured marine microalgae with chitosan. Aquacult Eng;8:257 – 65. 103.MacKay, D. and Salusbury, T. 1988. Choosing between centrifugation and crossflow microfiltration. Chemical Enneering. 477: 45 – 50. 104.Madihally, S. V. and Matthew, H. W. T. 1999. Porous chitosan scaffolds for tissue engineering. Biomaterials; 20:1133 – 1142. 105.Mata, T. M., Martins, A. A. and Ceatano, N.S. 2010. Microalgae for biodiesel production and Rather applications: A review. Renewable and Sustainable Energy Reviews. 14: 217-232. 106.Matis, K. A., Gallios, G. P. and Kydros, K. A. 1993. Separation of fines by flotation techniques. Separations Technology. 3: 76-90. 107.McCausland, M. A., Brown, M. R., Barrett, S. M., Diemar J. A. and Heasman, M.P. 1999. Evaluation of live microalgae and microbial pastes as supplementary food for juvenile Pacific oyster (*Crassostrea gigas*). Aquaculture. 174: 323-342. 108.McGarry, M. G. 1970. Algal flocculation with aluminum sulfate and polyelectrolytes. Research Journal of the Water Pollution Control Federation. 42: 191 – 201. 109.McNeil, B. and Harvey, L. 1993. Viscous fermentation products. Crit Rev Biotechnol;13: 275 – 304. 110.Miao, X. and Wu, Q. 2006. Biodiesel production from heterotrophic microalgal oil. Bioresource Technology. 97: 841-846. 111.Michaels, A.S. 1954. Aggregation of suspensions by polyelectrolytes. Ind. Eng. Chem. 46: 1485. 112.Misra, M. 1993. Selective flocculation of fine coal with hydrophobic *Mycobacterium phlei*. Minerals Metall Process. 10: 20 – 23. 113.Mohn, F. H. 1980. Experiences and strategies in the recovery of biomass from mass cultures of microalgae. In: Shelef G, Soeder CJ, editors. Algae biomass. Amsterdam: Elsevier; p. 547 – 571. 114.Molina Grima, E., Belarbi, E. H., Acién Fernandez, F. G. Robles Medina, A., Yusuf Chisti. 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnology Advances. 20: 491 – 515. 115.Moraine, R., Shelef, G., Sandbank, F., Bar-Moshe, Z. and Shvartzbard, I. 1980. Recovery of sewage borne algae: flocculation and centrifugation technique. In: Shelef G, Soeder CJ, editors. Algae biomass. Amsterdam: Elsevier. p. 531 – 546. 116.Morales J, de la Noue J, Picard G. 1985. Harvesting marine microalgae species by chitosan flocculation. Aquaculture Eng;4:257 – 70. 117.Morales, J., de la Noue, J., Picard, G. 1985. Harvesting marine microalgae species by chitosan flocculation. Aquacultural Engineering. 4: 257 – 270. 118.Mpofu, P., Addai-Mensah, J., Ralston, J. 2004. Temperature influence of nonionic polyethylene oxide and anionic polyacrylamide on flocculation and dewatering behavior of kaolinite dispersions. Journal of colloid and interface science 271: 145 – 156. 119.Mueller, J. A. 2000. Pretreatment processes for the recycling and reuse of sewage sludge. Water Science and Technology, Vol. 42, No. 9, p. 167. 120.Nakamura, J., Miyashiro, S., Hirose, Y. 1976a. Modes of flocculation of yeast cell with flocculant produced by *Aspergillus sojae* AJ-7002. Agricultural and Biological Chemistry. 40: 1565-1571. 121.Nakamura, J., Miyashiro, S., Hirose, Y. 1976. Conditions for production of microbial cell flocculant by *Aspergillus sojae* AJ7002. Agricultural and Biological Chemistry 40: 1341-1347. 122.Nigam B. P, Ramanathan P. K, Venkataraman L. V. 1980. Application of chitosan as a flocculant for the cultures of the green alga:*Scenedesmus acutus*. Arch Hydrobiol;88:378 – 87. 123.Nigam, B. P., Ramanathan, P. K., Venkataraman, L. V. 1980. Application of chitosan as a flocculant for the cultures of the green alga: *Scenedesmus acutus*. Archiv fur Hydrobiologie. 88: 378 – 387. 124.Oh, H. M., Lee, S., Park, M. H., Kim, H. S., Kim, H. C., Yoon, J. H., Kwon, G. S., Yoon, B. D. 2001. Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. AM49. Biotechnology Letters. 23: 1229 – 1234. 125.Oppermann-sanio, F. B. and Steinbuchel, A. 2002. Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production. Naturwissenschaften 89: 11-22. 126.Otani, Y., Tabata, Y., Ikada, Y. 1996a. A new biological glue from gelatin and poly (L-glutamic acid). J Biomed Mater Res; 31: 158 – 166. 127.Otani, Y., Tabata, Y., Ikada, Y. 1996b. Rapidly curable biological glue composed of gelatin and poly (Lglutamic acid). Biomaterials; 17: 1387 – 1391. 128.Otani, Y., Tabata, Y., Ikada, Y. 1998. Effect of additives on gelation and tissue adhesion of gelatin-poly (L-glutamic acid) mixture. Biomaterials; 19: 2167 – 2173. 129.Pal S., Mal D., Singh R. 2005. Cationic starch: an effective flocculating agent. Carbohydr Polym 59:417 – 423. 130.Perez-Camero, G., Congregado, F., Bou, J. J., Munoz-Guerra, S. 1999. Biosynthesis and ultrasonic degradation of bacterial poly( -glutamic acid). Biotechnology and Bioengineering 63: 110-115. 131.Petrusevski, B., Bolier, G., Van Breemen, A. N. and Alaerts, G. J. 1995. Tangential flow filtration: a method to concentrate freshwater algae. Water Research. 29:

1419 – 1424. 132.Pieterse, A. J. H. and Cloot, A. 1997. Algal Cells and Coagulation, Flocculation and sedimentation processes. Water Science and Technology. 36: 111-118. 133.Pinotti, A. and Zaritzky, N. 2001. Effect of aluminum sulfate and cationic polyelectrolytes on the destabilization of emulsified wastes. Waste Management 21(6): 535 – 542. 134.Poelman, E., de Pauw, N., Jeurissen, B. 1997. Potential of electrolytic flocculation for recovery of microalgae. Resources, Conservation and Recycling. 19: 1 – 10. 135.Pushparaj, B., Pelosi, E., Torzillo G., Materassi, R. 1993. Microbial biomass recover using a synthetic cationic polymer. Bioresource Technology. 43: 59 – 62. 136.Richard, A. 2001. Margaritis A. Poly (glutamic acid) for biomedical applications. Crit Rev Biotechnol; 21: 219 – 232. 137.Richard, A. and Margaritis, A. 2003. Optimization of cell growth and poly(glutamic acid) production in batch fermentation by *Bacillus subtilis*. Biotechnology Letters 25: 456-468. 138.Richard, A. and Margaritis, A. 2003. Rheology, oxygen transfer, and molecular weight characteristics of poly (glutamic acid) fermentation by *Bacillus subtilis*. Biotechnol Bioeng; 82: 299 – 305. 139.Richmond A, Becker E. W. Technological aspects of mass cultivation, a general outline. In: Richmond A, editor. CRC handbook of microalgal mass culture. Boca Raton: CRC Press; 1986. p. 245 – 63. 140.Richmond, A. 1986. Outdoor mass cultures of microalgae. In: Richmond, A. (Ed.), Handbook of Algal Mass Culture. CRC Press, Boca Raton, FL, USA, pp. 285 – 330. 141.Richmond, A. and Becker, E. W. 1986. Technological aspects of mass cultivation, a general outline. In: Richmond A, editor. CRC handbook of microalgal mass culture. Boca Raton: CRC Press. p.245 – 263. 142.Rossignol, N., Vandanon, L., Jaouen, P. and Quemeneur, F. 1999. Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultra-filtration. Aquacultural Engineering. 20: 191 – 208. 143.Saito, K., Sato, S., Shimoi, H., Iefuji, H. and Tadenuma, M. 1990. Flocculation mechanism of *Hansenula anomala* J224. Agricultural and Biological Chemistry. 54: 1425-1432. 144.Sakai, K., Sonoda, C., Murase, K., Santos, D. P., Bergamini, M. F., Fogg, A. G., Zanoni, M. V. B. 2000. Application of a glassy carbon electrode modified with poly(glutamic acid) in caffeic acid determination. Microchimica Acta 2005; 1-2: 127-134. 145.Salehzadeh, H. 2000. Vossoughi M, Alemzadeh I. Some investigations on bioflocculant producing bacteria. Biochemical Engineering. 5: 39 – 44. 146.Salehzadeh, H. and Shokaosadati, S.A. 2001. Extracellular biopolymeric flocculants recent trends and biotechnological importance. Biotechnology Advances 19: 371-385. 147.Salehzadeh, H., Vossoughi, M., Alemzadeh, H. 2000. Some investigations on bioflocculant producing bacteria. Biochemical Engineering Journal 5: 39-44. 148.Sekine, T., Nakamura, T., Shimizu, Y., Ueda, H., Matsumoto, K., Takimoto, Y., Kiyotani, T. 2000. A new type of surgical adhesive made from porcine collagen and polyglutamic acid. J Biomed Mater Res; 35: 305 – 310. 149.Seo, H. 1993. Bioflocculant production from *Bacillus* sp. A56. Sanop Misaengmul Hakhoechi. 21: 486 – 493. 150.Shelef, G. 1978. Photosynthetic biomass production from sewage. Arch Hydrobiol Beih. 11: 3-14. 151.Shelef, G., Sukenik,A. and Green,M. 1984. Microalgae Harvesting and Processing: A Literature Review. Subcontract Report, No.XK-3-03031-01. U.S. Department of Energy. 152.Shi, F., Xu, Z., Cen, P. 2006. Efficient production of poly--glutamic acid by *bacillus subtilis* ZJU-7. Appl Biochem Biotechnol; 133: 271-281. 153.Shih, I. L. and Van, I. T. 2001. The production of poly(-glutamic acid) from microorganisms and its various applications. Bioresource Technology 79: 207-225. 154.Shih, I. L., Van, Y. T., Chang, Y. N. 2002. Application of statistical experimental methods to optimize production of poly(-glutamic acid) by *Bacillus licheniformis* CCRC 12826. Enzyme Microbial Technol; 31: 213-220. 155.Shih, I. L., Van, Y. T., Shen, M. H. 2004. Biomedical applications of chemically and microbiologically synthesized poly(glutamic acid) and poly(lysine). Mini-Review in Medicinal Chemistry 4: 179 – 188. 156.Shih, I. L., Van, Y. T., Yeh, L. C., Lin, H. G., Chang, Y. N. 2001. Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. Bioresour Technol; 78: 267 – 272. 157.Shimofuruuya, H., Koide, A., Shirota, K., Tsuji, T., Nakamura, M., Suzuki, J. 1996. The production of flocculating substance(s) by *Streptomyces griseus*. Bioscience, Biotechnology and Biochemistry. 60: 498-500. 158.Shimofuruuya, H., Koide, A., Shirota, K., Tsuji, T., Nakamura, M., Suzuki, J. 1996. The production of flocculating substance(s) by *Streptomyces griseus*. Bioscience, Biotechnology and Biochemistry 60: 498-500. 159.Stumm, W. and Morgan, J. J. 1981. Aquation Chemistry, 2nd ed. New York, John Wiley & Sons p.780. 160.Suh, H. H., Kwon, G. S., Lee, C. H., Kim, H. S., Oh, H. M., Yoon, B. D. 1997. Characterization of bioflocculant produced by *Bacillus* sp. DP-152. Journal of Fermentation and Bioengineering 84: 108-112. 161.Suh, J. K. F. and Matthew, H. W. T. 2000. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials; 21: 2589 – 2598. 162.Sukenik, A. and Shelef, G. 1984. Algal autoflocculation—verification and proposed mechanism. Biotechnology and Bioengineering. 26: 142 – 147. 163.Sukenik, A. and Wahnon, R. 1991. Biochemical quality of marine unicellular algae with special emphasis on lipid composition I. Isochrysis galbana. Aquaculture. 97: 61-72. 164.Sung, M. H., Park, C., Kim, C. J., Poo, H., Soda, K., Ashiuchi, M. 2005. Natural and edible biopolymer polygamma-glutamic acid: synthesis, production, and applications. Chem Rec; 5: 352 – 366. 165.Suo, C., Mei, L. H., Huang, J., Sheng, Q. 2007. Selection of -poly glutamic acid high yield strain by 60Co -irradiation and the optimization of its culture medium. J Chem Eng Chinese Univ; 21 (5): 820-825. 166.Suzuki, T. and Tahara, Y. 2003. Characterization of the *Bacillus subtilis* ywtD gene, whose product is involved in -polyglutamic acid degradation. J Bacteriol; 185: 2379 – 2382. 167.Takagi, M., Karseno and Yoshida, T. 2006. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunalidilla* cells. Journal of Bioscience and Bioengineering. 101: 223 – 226. 168.Takeda, M., Koizumi, J., Matsuoka, H., Hikuma, M. 1992. Factors affecting the activity of a protein bioflocculant produced by *Nocardia amarae*. Journal of Fermentation Technology. 74: 408-409. 169.Takeda, M., Kurane, R., Koizumi, J., Nakamura, I. 1991. A protein bioflocculant produced by *Rhodococcus erythropolis*. Agricultural and Biological Chemistry. 55: 2663 – 2664. 170.Taniguchi, M., Kato, K., Matsui, O., Ping, X., Nakayama, H., Usuki, Y., Ichimura, A., Fujita, K. I., Tanaka, T. 2005b. Flocculating activity of cross-linked poly-gamma-glutamic acid against bentonite and *Escherichia coli* suspension pretreated with FeCl3 and its interaction with Fe3+. Journal of Bioscience and Bioengineering 100: 207 – 211. 171.Taniguchi, M., Kato, K., Shimauchi, A., Pingm X., I., Fujita, K. I., Tanaka A. T., Tarui, Y., Hirasawa, E. 2005a. Physicochemical Properties of Cross-linked Poly- -Glutamic Acid and Its Flocculating Activity against Kaolin

Suspension. Journal of Bioscience and Bioengineering 99: 130 – 135. 172.Taniguchi, M., Kato, K., Shimauchi, A., Xu, P., Nakayama, H., Fujita, K., Tanaka, T., Tarui, Y., Hirasawa, E. 2005c. Proposals for wastewater treatment by applying flocculating activity of cross-linked poly-glutamic acid. Journal of Bioscience and Bioengineering 99: 245 – 251 173.Taniguchi, M., Kayo, K., Shimauchi, A., Ping, X., Nakayama, H., Fujita, K. I., Tanaka, T., Tarui, Y., Hirasawa, E. 2004. Proposals for wastewater treatment by applying flocculating activity of cross-linked poly-glutamic acid. Journal of Bioscience and Bioengineering 99: 245 – 251. 174.Tanimoto, H., Fox, T., Eagles, J., Satoh, H., Nozawa, H., Okiyama, A., Morinaga, Y., Susan, J., Fairweather-Tait, S. J. 2007. Acute effect of poly--glutamic acid on calcium absorption in postmenopausal women. J Am College Nutr; 26(6): 645-649. 175.Tanimoto, H., Nozawa, H., Okada, K., Miyano, R., Hidesaki, M., Tsunematsu, M., Ito, K. A. 2003. Calcium supplement containing poly- -glutamic acid increases human calcium absorption. Nippon Nogeikagaku Kaishi; 77 (5): 504-507. 176.Tanimoto, H., Sato, H., Kuraishi, C., Kido, K., Seguto, K. 1995. High absorption mineral-containing composition and foods; USP 447,732. 177.Tarui, Y., Iida, H., Ono, E., Miki, W., Hirasawa, E., Fujita, K., Tanaka, T., Taniguchi, M. 2005. Biosynthesis of poly-gamma-glutamic acid in plants: transient expression of poly-gam