

影響Bacillus sp. DYU1 菌株所生產之生物絮凝劑環境因子探討

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摘要

生物絮凝劑是一種天然高分子絮凝劑，具有無毒、安全、高效、可生物降解、無二次污染等特點，已被廣泛使用於廢水處理、食品與發酵工業以及自來水處理。生物絮凝劑DYU 300是由Bacillus sp. DYU1的發酵液所製備，具有良好之絮凝活性。本篇研究利用高嶺土懸浮液(kaolin suspension)為模擬對象，以探討絮凝劑DYU 300之絮凝能力。DYU 300之組成成分中，總糖、糖醛酸(uronic acid)、蛋白質及聚醯胺所占百分比分別為13.5%、3.4%、4.7%和48.7%。經傅立葉紅外線光譜(FT-IR)分析顯示，DYU 300具有羧基和胺基官能基團。而經絮凝試驗發現，絮凝活性、絮凝率及黏度會隨Bacillus sp. DYU1生長而增加，由此可知，生物絮凝劑DYU 300為菌株生長期間所生產。此外，實驗結果發現，添加二價金屬離子(Ca²⁺和Mg²⁺)於高嶺土懸浮液中可促進DYU 300的絮凝活性，而金屬離子的協同作用在酸鹼值pH 6-8範圍最為顯著。室溫下，添加40 mg-DYU 300/L及41.5 mM MgSO₄至懸浮液中(pH 8)，可獲得最佳絮凝活性及絮凝率分別為19.5和97.4%。

關鍵詞：絮凝、生物絮凝劑、高嶺土、生物聚合物

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參考文獻

- 1.張天送。2003。環境荷爾蒙 從限用塑膠袋說起，科學發展，370: 54-59.
- 2.Allen, M. M. 1984. Cyanobacterial cell inclusions. Annual Review of Microbiology 38: 1-25.
- 3.Allen, M. M., Hutchison, F. and Weathers, P. J. 1980. Cyanophycin granule polypeptide formation and degradation in the cyanobacterium Aphanocapsa 6308. Journal of Bacteriology 141: 687-693.
- 4.Anderson, A. and Dawes, E. 1992. Occurrence, Metabolism, Metabolic Role and Industrial Uses of Bacterial Polyhydroxyalkanoates, Microbiology Reviews 54: 450-472.
- 5.Baker, S. A. and Young, N. M. 1966. Isolation of hyaluronic acid by gel filtration on agarose. Carbohydrate Research 2: 363-370.
- 6.Balazs, E. A. and Band, P. 1984. Hyaluronic acid: Its structure and use. Cosmetics and Toiletries 99: 65-72.
- 7.Bender, H., Rodriguez-Eatun, S., Ekanemesang, U. and Phillips, P. 1994. Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial mats. Applied and Environmental Microbiology 60: 2311-2315.
- 8.Bender, J., Archibold, E. R., Ibeanusi, V. and Gould, J. P. 1989. Lead removal from contaminated water by a mixed microbial ecosystem. Water Science and Technology 21: 1661-1665.
- 9.Bender, J., Gould, J. P., Vatcharapijarn, Y. and Saha, G. 1991. Uptake, transformation and fixation of Se (VI) by a mixed, selenium tolerant ecosystem. Water, Air and Soil Pollution 59: 359-367.
- 10.Birrer, G. A., Cromwick, A. M. and Gross, R. A. 1994. -Poly(glutamic acid) formation by Bacillus licheniformis 9945a: physiological and biochemical studies. International Journal of Biological Macromolecules 16: 265-275.
- 11.Borzi, A. 1887. Le comunicazioni intracellulari delle Nostochineae. Malpighia 1: 28-203.
- 12.Bovarnick, M. 1942. The formation of extracellular D(-)-glutamic acid polypeptide by Bacillus subtilis. The Journal of Biological Chemistry 145: 415-424.
- 13.Bruus, J. H., Nielsen, P. H. and Keiding, K. 1992 On the stability of activated sludge flocs with implications to dewatering. Water Research 26: 1597-1604.
- 14.Champney, K. J., Levine, D. P., Levy, H. B., and Leaner, A. M. 1979. Modified polyriboinosinic-polyribocytidylic acid complex:sustained interferonemia and its physiological associates in humans. Infection and Immunity 25: 831-837.
- 15.Chang, M. C., Chuang, S. H., and Lin, H. L. 1997. Effects of calcium ion on sludge conditioning. Water Science and Technology 35(8): 217-222.
- 16.Chibnall, A.C., Rees, M. W. and Richards, F. M. 1958. Structure of the polyglutamic acid from Bacillus subtilis. The

Biochemical Journal 68: 129-135. 17. Chitikela, S. and Dentel, S. 1998. Dual chemical conditioning and dewatering of anaerobically digested biosolids: laboratory evaluations. *Water Environment Research* 70: 1062-1069. 18. Dembinska, M. E. and Allen, M. M. 1988. Cyanophycin granule size variation in *Aphanocapsa*. *The Journal of General Microbiology* 143: 295-298. 19. 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. 20. Dubios, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric method of determination of sugars and related substances. *Analytical Chemistry* 28: 350-356. 21. Dugan, P. R. 1970. Removal of mine water ions by microbial polymers. *Proceedings of the Third Symposium on Coal Mine Drainage Research*. Pittsburgh (PA): Mellon Institute: 279-283. 22. Dugan, P. R. 1987. The function of microbial polysaccharide in bioflocculation and biosorption of mineral ions. In: Attia YA, editor. *Flocculation in biotechnology and separation systems*. Amsterdam: Elsevier: 337-350. 23. Dugan, R., in: Y.A. Attia (Ed.). 1984. *Flocculation in Biotechnology and Separation System*. Elsevier Science, Amsterdam: 337-351. 24. Endo, T., Nakamura, K. and Takahashi, H. 1976. Pronase susceptible floc forming bacteria: relationship between flocculation and calcium ion. *Agricultural and Biological Chemistry* 40: 2289-2295. 25. Esser, K. and Kues, U. 1983. Flocculation and its implication for biotechnology. *Process Biochemistry* 18: 21-23. 26. Esser, K., Hinrichs, J. and Kues, U. 1987. Genetic control of flocculation of yeast with respect to application in biotechnology. In: Attia YA, editor. *Flocculation in biotechnology and separation systems*. Amsterdam: Elsevier: 383-398. 27. Fattom, A. and SbBo, M. 1984. Phormidium J-1 bioflocculant: production and activity. *Archives of Microbiology* 139: 421-426. 28. Friedman, B. A. and Dugan, P. R. 1968. Concentration and accumulation of metallic ions by the bacterium *Zoogloea*. *Developments in Industrial Microbiology* 9: 381-388. 29. Fujii, H. 1963. On the formation of mucilage by *Bacillus natto*. Part . Chemical constitutions of mucilage in natto (1). *Nippon Nogeikagaku Kaishi* 37: 407-411. 30. 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 Microbiology and Biotechnology* 89: 40-46. 31. Golecki, J. R. and Heinrich, U. R. 1991. Ultrastructural and electron spectroscopic analyses of cyanobacteria and bacteria. *Journal of Microscopy* 162: 147-154. 32. Gonzales, D., Fan, K. and Sevoian, M. 1996. Synthesis and swelling characterizations of a poly(-glutamic acid) hydrogel. *Journal of Polymer Science. Part A, Polymer Chemistry* 34: 2019-2027. 33. Guez-Eatun, S., Ekanemesang, U., and Phillips, P. 1994. Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial mats. *Applied and Environmental Microbiology* 60: 2311-2315. 34. Gutcho, S. 1977. *Waste treatment with Polyelectrolytes and other flocculants*. Noyes Data Corp., Park Ridge, NJ: 1-37. 35. Hai, T., Oppermann-Sanio, F. B. and Steinbuchel, A. 1999. Purification and characterization of cyanophycin and cyanophycin synthetase from the thermophilic *Synechococcus* sp. MA19. *FEMS Microbiology Letters* 181: 229-236. 36. Hanby, W. E. and Rydon, H. N. 1946. The capsule substance of *Bacillus anthracis*. *The Biochemical Journal* 40: 297-309. 37. Hantula, J. and Bamford, D. H. 1991a. The efficiency of the protein dependent flocculation of *Flavobacterium* sp. *Applied Microbiology and Biotechnology* 36: 100 – 104. 38. Hantula, J. and Bamford, D. H. 1991b. Bacterial phage resistance and flocculation of *Flavobacterium* sp. Are phenotypically interrelated. *Applied Microbiology and Biotechnology* 36: 105-108. 39. Higgins, M. J. and Novak, J. T. 1997. The effect of cations on the settling and dewatering of activated sludges. *Water Environment Research* 69: 215-224. 40. Hiroaki, T. and Kiyoshi, K. 1985. Purification and chemical properties of a flocculant produced by *Paecilomyces*. *Agricultural and Biological Chemistry* 49: 3159-3164. 41. Houghton, J. I. and Quarby, J. 1991. Biopolymer in wastewater treatment. *Current Opinion in Biotechnology* 10: 259-262. 42. Huang, L. 1990. Studies on culture condition for production of flocculant by bacterial strain S-4K. *Taiwan Tangye Yanjiu Huibao* 129: 11 – 19. 43. Ike, M., Tachibana, S., Kitada, G., Kim, S. M. and Inoue, Z. 2000. Characterization of a bioflocculant produced by *Citrobacter* sp. TKF04 from acetic and propionic acids. *Journal of Fermentation and Bioengineering* 89: 40-46. 44. Ivanovics, G. and Bruckner, V. 1937. Chemische und immunologische Studien über den Mechanismus der Milzbrandinfektion und Immunität; die chemische Struktur der Kapselsubstanz des Milzbrandbazillus und der serologisch identischen spezifischen Substanz des *Bacillus mesentericus*. *Z Immunitätsforsch Exp Ther* 90: 304-318. 45. Kakii, K., Sugahara, E., Shirakashi, T. and Kuriyama, M. 1986. Isolation and characterization of a Ca²⁺ dependent floc-forming bacterium. *Journal of Fermentation Technology* 64: 57 – 62. 46. Kawai, T., Kubota, T., Hiraki, J. and Izumi, Y. 2003. Biosynthesis of -Poly-L-lysine in a cell-free system of *Streptomyces albus*. *Biochemical and Biophysical Research Communications* 311: 635-640. 47. Khalil, M.I. and Aly, A.A. 2001. Preparation and evaluation of some cationic starch derivatives as flocculants. *Starch/Stärke* 53: 84-89. 48. Kunioka, M. 1997. Biosynthesis and chemical reactions of poly(amino acid)s from microorganisms. *Applied Microbiology and Biotechnology* 47: 469-475. 49. Kurane, R. and Matsuyama, H. 1994. Production of a bioflocculant by mixed culture. *Bioscience, Biotechnology and Biochemistry* 58: 1589 – 1594. 50. Kurane, R. and Nohata, Y. 1994. A new water-absorbing polysaccharide from *Alcaligenes latus*. *Bioscience, Biotechnology, and Biochemistry* 58: 235 – 238. 51. Kurane, R. and Nohata, Y. 1991. Microbial flocculation of waste liquids and oil emulsion by a bioflocculant from *Alcaligenes latus*. *Agricultural and Biological Chemistry* 55: 1127 – 1129. 52. Kurane, R., Hatamochi, K., Kakuno, T., Kiyohara, M., Hirano, M. and Taniguchi, Y. 1994a. Production of a bioflocculant by *Rhodococcus erythropolis* S-1 grown on alcohols. *Bioscience Biotechnology and Biochemistry* 58: 428 – 429. 53. Kurane, R., Hatamochi, K., Kakuno, T., Kiyohara, M., Kawaguchi, K., Mizuno, Y., Hirano, M. and Taniguchi, Y. 1994b. Purification and characterization of lipid bioflocculant produced by *Rhodococcus erythropolis*. *Bioscience Biotechnology, and Biochemistry* 58: 1977-1982. 54. Kurane, R., Toeda, K., Takeda, K. and Suzuki, T. 1986. Culture condition for production of microbial flocculant by *Rhodococcus erythropolis*. *Agricultural and Biological Chemistry* 50: 2309-2313. 55. Kwon, G. S., Moon, S. H., Lee, H. M., Kim, H. S., Oh, H. M. and Yoon, B. D., 1996. A novel flocculant biopolymer produced by *Pestalotiopsis* sp. KCTC 8637P. *Biotechnology Letters* 18: 1459-1464. 56. Lang, N. J. 1968. The fine structure of blue-green algae. *Annual Review of Microbiology* 22: 15-46. 57. Lawry, N. H. and Simon, R. D. 1982. The normal and induced occurrence of cyanophycin inclusion bodies in

several blue-green algae. *Journal of Phycology* 18: 391-399. 58. Lee, H. L., Lee, S. O., Jang, K. L. and Lee, T. H. 1995. Microbial flocculant from *Arcuadendron* sp. TS-49. *Biotechnology Letters* 17: 95-100. 59. Levy, H. B., Baer, G., Baron, S., Buckler, C. E., Gibbs, C. J., Iadarola, M. J., London, W. T. and Rice, J. 1975. A modified polyriboinosinic-polyribocytidylic acid complex that induces interferon in primates. *The Journal of Infectious Diseases* 132: 434-439. 60. Levy, N., Magdasi, S. and Bar-Or, Y. 1992. Physico-chemical aspects in flocculation of bentonite suspensions by a cyanobacterial. *Water Research* 26: 249 – 254. 61. Maeda, S., Kunimoto, K. K., Sasaki, C., Kuwae, A. and Hanai, K. 2003. Characterization of microbial poly (-L-lysine) by FT-IR, Raman and solid state ¹³C NMR spectroscopies. *Journal of molecular structure* 655: 149-155. 62. Michaels, A.S. 1954. Aggregation of suspensions by polyelectrolytes. *Industrial & Engineering Chemistry* 46: 1485-1490. 63. Naganishi, I., Kimura, K., Suzuki, T., Ishikawa, M., Banno, I., Sakene, T., and Harada, T. 1976. Demonstration of curdlan-type polysaccharide and some other -1,3-glucan in microorganisms with aniline blue. *The Journal of General and Applied Microbiology* 22: 1-11. 64. Nakamura, J., Miyashiro, S. and Hirose, Y. 1976a. Conditions for production of microbial cell flocculant by *Aspergillus sojae* AJ7002. *Agricultural and Biological Chemistry* 40: 1341-1347. 65. Nakamura, J., Miyashiro, S. and Hirose, Y. 1976b. Screening, isolation and some properties of microbial cell flocculants. *Agricultural and Biological Chemistry* 40: 377 – 383. 66. Nakata, K. and Kurane, R. 1999. Production of an extracellular polysaccharide bioflocculant by *Klebsiella pneumoniae*. *Bioscience, Biotechnology, and Biochemistry* 63: 2064-2068. 67. Norberg, A. B. and Enfors, S. O. 1982. Production of extracellular polysaccharide by *Zoogloea ramigera*. *Applied and Environmental Microbiology* 44: 1231 – 1237. 68. Novak, J. T. and Haugan, B. E. 1981. Polymer extraction from activated sludge. *Journal of Water Pollution Control Fed* 53: 1420-1425. 69. Obst, M. and Steinbuchel, A. 2004. Microbial degradation of poly(amino acid)s. *Biomacromolecules* 5: 1166-1176. 70. Oh, H. M., Lee, S. J., Park, M. H., Kim, H. S., Kim, H. C., Yoon, J. H., Kwon, G. S. and Yoon, B. D. 2001. Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. AM 49. *Biotechnology Letters* 23: 1229-1234. 71. Oppermann, F. B., Pickartz, S. and Steinbuchel, A. 1998. Biodegradation of polyamides. *Polymer Degradation and Stability* 59: 337-344. 72. Oppermann-Sanio, F. B. and Steinbuchel, A. 2002. Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production. *Naturwissenschaften* 89: 11-22. 73. Perez-Camero, G., Congreado, F., Bou, J. J. and Munoz-Guerra, S. 1999. Biosynthesis and ultrasonic degradation of bacterial poly(-glutamic acid). *Biotechnology and bioengineering* 63: 110-115. 74. Pitman, A. R. 1975. Bioflocculation as means of improving the dewatering characteristics of activated sludges. *Journal of Water Pollution Control Fed* 74: 688-700. 75. Poiriner, Y., Dennis, D., Klomparens, S., Namrach, C. and Sommerville, C. 1992. Perspectives on the production of polyhydroxyalkanoates in plants. *FEMS Microbiology Reviews* 103: 237-246. 76. Riley, F. L., Morin, M. L., Lvovsky, E., Stephens, E. E. and Levy, H.B. 1982. Polyriboinosinic-polyribocytidylic acid-poly-lylysine complex (Poly(ICL)) without carboxymethylcellulose (CMC): a new primate-effective interferon inducer (41329). *Proceedings of the Society for Experimental Biology and Medicine* 169: 183-188. 77. Roger C, Herdman. 1993. *Biopolymers: Making Materials Nature's way*. U.S. Congress, Office of Technology Assessment. Washington, DC: U.S. Government Printing Office. 78. Salehizadeh, H., Vossoughi, M. and Alemzadeh, I. 2000. Some investigations on bioflocculant producing bacteria. *Biochemical Engineering Journal* 5: 39-44. 79. Senthilnathan, P. R. and Sigler, R. G. 1993. Improved sludge dewatering by dual polymer conditioning. *Water Science and Technology* 28(1): 53-57. 80. 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. 81. Shih, I. L., Van, Y. T., Yeh, L. C., Lin, H. G. and Chang, Y. N. 2001. Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. *Bioresource Technology* 78: 267-272. 82. Shima, S. and Sakai, H. 1977. Polylysine Produced by *Streptomyces*. *Agricultural and Biological Chemistry* 41: 1807-1809. 83. Shima, S. and Sakai, H. 1981. Poly-L-lysine Produced by *Streptomyces*. Part . Taxonomy and Fermentation Studies. *Agricultural and Biological Chemistry* 45: 2497-2502. 84. Shimofuruya, H., Koide, A., Shirota, K., Tsuji, T., Nakamura, M. and Suzuki, J. 1996. The production of flocculating substances by *Streptomyces griseus*. *Bioscience, Biotechnology, and Biochemistry* 60(3): 498-500. 85. Simon, R. D. 1987. Inclusion bodies in the cyanobacteria: cyanophycin, polyphosphate, polyhedral bodies. In: Fay P, Baalen C van (eds) *The cyanobacteria*. Elsevier, Amsterdam: 199-225. 86. Simon, R. D. and Weathers, P. J. 1976. Determination of the structure of the novel polypeptide containing aspartic acid and arginine which is found in cyanobacteria. *Biochimica et Biophysica Acta. International Journal of Biochemistry and Biophysics* 420: 165-176. 87. Sousa, M., Teixeira, J. and Mota, M. 1992. Difference in flocculation mechanism of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. *Biotechnology Letters* 14: 213 – 218. 88. Stahl, U., Kues, U. and Esser, K. 1983. Flocculation in yeast, an assay on the inhibition of cell aggregation. *Applied Microbiology and Biotechnology* 17: 199 – 202. 89. Suh, H. H., Kwon, G. S., Lee, C. H., Kim, H. S., Oh, H. M. and Yoon, B. D. 1997. Characteristics of bioflocculant produced by *Bacillus* sp. DP-152. *Journal of Fermentation and Bioengineering* 84: 108-112. 90. Takagi, H. and Kadowaki, K. 1985. Poly galactosamine produced by a microorganism. *Chitin Nat Technol* 3: 121 – 128. 91. Takashi, A., Hayashi, S., Higashi, N., Niwa, M. and Kurihara, K. 2000. Charge regulation in polyelectrolyte brushes studied by FT-IR spectroscopy. *Colloids and Surfaces A : Physicochemical and Engineering Aspects* 169: 351 – 356. 92. Takeda, M., Koizhi, J., Matsuoka, H. and Hikuma, M. 1992. Factors affecting the activity of a protein bioflocculant produced by *Nocardia amarae*. *Journal of Fermentation and Bioengineering* 74: 408-409. 93. Takeda, M., Kurane, R., Koizumi, J. and Nakamura, I. 1991. A protein bioflocculant produced by *Rhodococcus erythropolis*. *Agricultural and Biological Chemistry* 55: 2663-2664. 94. Tezuka, Y. 1969. Cation-Dependent flocculation in a *Flavobacterium* species predominant in activated sludge. *Applied Microbiology* 17: 222-226. 95. Toeda, K. and Kurane, R. 1991. Microbial flocculant from *Alcaligenes cupidas* KT201. *Agricultural and Biological Chemistry* 55: 2793-2799. 96. Unz, R. F. and Farrah, S. R. 1976. Exopolymer production and flocculation by *Zoogloea* MP6. *Applied and Environmental Microbiology*: 623-626. 97. Watson, D. W., Cromartie, W. J., Bloom, W. L., Heckly, R. J., McGhee, W. J. and Weissman, N. 1947. Studies on infection with *Bacillus anthracis*. V. The isolation of an inflammatory factory factor from crude extracts of lesions of *B. anthracis* infection and its

biological and chemical relationship to glutamyl polypeptide. *The Journal of Infectious Diseases* 80: 121-136.

98. Yokoi, H., Arima, T., Hirose, J., Hayashi, S. and Takasaki, Y. 1996a. Flocculation Properties of Poly(γ-Glutamic acid) Produced by *Bacillus subtilis*. *Journal of Fermentation and Bioengineering* 82: 84-87.

99. Yokoi, H., Natsuda, O., Hirose, J., Hayashi, S. and Takasaki, Y. 1995. Characteristics of a biopolymer flocculant produced by *Bacillus* sp. PY-90. *Journal of Fermentation Technology* 79: 378-380.

100. Yokoi, H., Shiraki, M., Hirose, J., Hayashi, S. and Takasaki, Y. 1996b. Flocculation properties of xanthan produced by *Xanthomonas campestris*. *Biotechnology Techniques* 10: 789-792.

101. Yokoi, H., Yoshida, T., Mori, S., Hirose, J., Hayashi, S. and Takasaki, Y. 1996c. Biopolymer flocculant produced by an *Enterobacter* sp. *Biotechnology Letters* 19: 569-573.

102. Yu, X. and Somasundaran, P. 1993. Enhanced flocculation with double flocculants. *Colloids Surfaces A* 81: 17-23.

103. Zajic, J. E. and Knetting, E. 1971. Flocculants from paraffinic hydrocarbons. *Development in industrial microbiology*. Washington (DC): American Institute of Biological Science: 87-98.

104. Zhu, X., Reed, B. E., Lin, W., Carriere, P. E. and Roark, G. 1997. Investigation of emulsified oil wastewater treatment with polymer. *Separation Science and Technology* 32: 2173-2187.