行政院國家科學委員會專題研究計畫 成果報告

膜過濾結垢機制及預防之研究--薄膜生物顆粒反應器新程序之開發及機制(3/3)
研究成果報告(完整版)

計 畫 類 別 ： 整合型
計 畫 編 號 ： NSC 96-2221-E-002-110-
執 行 期 間 ： 96 年 08 月 01 日至 97 年 07 月 31 日
執 行 單 位 ： 國立臺灣大學化學工程學系暨研究所

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報 告 附 件 ： 國外研究心得報告

處 理 方 式 ： 本計畫可公開查詢

中 華 民 國 97 年 05 月 15 日

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I. Abstract

Aerobic granulation, a novel environmental biotechnological process, was increasingly drawing interest of researchers engaging in work in the area of biological wastewater treatment. Developed about one decade ago, it was exciting research work that explored beyond the limits of aerobic wastewater treatment such as treatment of high strength organic wastewaters, bioremediation of toxic aromatic pollutants including phenol, toluene, pyridine and textile dyes, removal of nitrogen, phosphate, sulphate and nuclear waste and adsorption of heavy metals.. Despite this intensive research the mechanisms responsible for aerobic granulation and the strategy to expedite the formation of granular sludge, and effects of different operational and environmental factors have not yet been clearly described. This paper provides an up-to-date review on recent research development in aerobic biogranulation technology and applications in treating toxic industrial and municipal wastewaters. Factors affecting granulation, granule characterization, granulation hypotheses, effects of different operational parameters on aerobic granulation, response of aerobic granules to different environmental conditions, their applications in bioremediations, and possible future trends were delineated. The review attempts to shed light on the fundamental understanding in aerobic granulation by newly employed confocal laser scanning microscopic techniques and microscopic observations of granules.

Keywords: aerobic granules, mechanisms, extracellular polymeric substances, structure, interactions

II. 計劃緣由與目的

Aerobic granules were considered to be a special case of biofilm composing of self-immobilized cells. During the last 20 years, intensive research in the field of biological wastewater treatment and other applications demonstrated that biofilms were often more efficient for water purification than suspended activated sludge. To date, the application of aerobic granular sludge was regarded as one of the promising biotechnologies in wastewater treatment. The first patent was granted by Heijnen and van Loosdrecht (1998). De Kreuk *et al.* (2007) provided comments on the state of the art for the aerobic granulation process. Liu and Tay (2004) and Maximova and Dahl (2006) provided an up to date summary of the current understanding towards the bioaggregation processes.

Granular sludge was first described for strictly anaerobic systems in 1980 (Lettinga *et al.*, 1980) and only by the late 1990s had the formation and application of aerobic granules been reported (Morgenroth *et al.*, 1997, Beun *et al.*, 1999, Dangcong *et al.*, 1999). The anaerobic granulation technology exhibited several drawbacks that included a long start-up period, a relatively high operating temperature, unsuitability for low strength organic wastewater, and low efficiency in the removal of nutrients (N and P) from wastewater. This resulted in the development of aerobic granular technology which became a popular topic of discussion for environmental engineers.

Compact structured, biologically efficient aerobic sludge granules with wide diverse microbial species and excellent settling capabilities have been developed in sequencing batch reactors (SBR) (Morgenroth *et al.*, 1997; Beun *et al.*, 1999; Tay *et al.*, 2001a; Yang *et al.*, 2003; Liu and Tay, 2004,

Adav *et al.*, 2007a). Formation by self immobilization of bacteria as hypothesized by several researchers (Kim *et al.*, 2004; McSwain *et al.*, 2004a; Qin *et al.*, 2004a, b; Wang *et al.*, 2004; Hu *et al.*, 2005; Liu *et al.*, 2005), the aerobic granules were densely packed microbial aggregates and their densities were much higher than that of conventional activated sludge. In addition, the aerobic granules were known to exhibit attributes of:

(1) Regular, smooth and nearly round in shape
(2) Excellent settleability
(3) Dense and strong microbial structure
(4) High biomass retention
(5) Ability to withstand at high organic loading
(6) Tolerance to toxicity

Because of the unique granule attributes, the aerobic granulation technology was recently developed for treating high-strength wastewaters containing organics, nitrogen, phosphorus, toxic substances and xenobiotics (Jiang *et al.*, 2002; Moy *et al.*, 2002; Tay *et al.*, 2002b; Lin *et al.*, 2003; Adav *et al.*, 2007a-d, Adav and Lee, 2008a).

Recent development in aerobic biogranulation technology is now reviewed and presented in this paper. Materials covered by existing reviews are not duplicated herein. Factors affecting granulation, granule characterization, granulation hypotheses, effects of different operational parameters on aerobic granulation, response of aerobic granules to different environmental conditions, their applications in bioremediations, and possible future trends are delineated. Applications in treating municipal and toxic industrial wastewaters as well as useful information on exploring the underlined mechanisms are also highlighted.

結果與討論

EPS and granule stability

One of the most serious barriers to practical applications of aerobic granules was the loss of stability of aerobic granules over long-term operation. Two different patterns were noted: granule break-up and filament overgrowth (Liu and Liu, 2006; Adav *et al.*, 2007b, Zhu *et al.*, 2008). For the former, the granules deteriorated into small pieces to flow out with the upflow liquid stream. For the latter, the outgrown filaments produced light and bulky granules for easy washout. Also, the filaments tended to block the pipelines that led to failed reactors. Long term operation was not possible without stable granules.

An example of the EPS and cell distributions in phenol-fed granule is shown (Fig 1). The β-D-glucopyranose polysaccharides formed the core, while the cells and α-D-glucopyranose polysaccharides accumulated in the granule outer layers along with lipids. Wang *et al.* (2005b) applied only one dye, calcofluor white, to their granules and determined that non-soluble β-polysaccharide formed the outer shell of aerobic granules to provide its strength. Conversely, McSwain *et al.* (2005) stained their granules using fluorescein-isothiocyanate (FITC), concanavalin A (Con A) lectin conjugates and SYTO 63, to probe the content distribution of proteins, α-polysaccharides and cells in the granules. These authors and Zhang *et al.* (2007) argued that a non-cellular protein core in aerobic granule provided its stability. Based on the results by Chen *et al.* (2007a), the Wang's and McSwain's groups in fact highlighted only part of the whole story (Fig. 1). Staining technique should be used with caution.

Adav *et al.* (2007d) selectively hydrolyzed proteins, α-, β-polysaccharides, and lipids using enzymes and determined the stability change following hydrolysis. These authors noted that, although protein was redundant at the core regime, the selective removal of proteins had minimal impacts on the structural stability of granules. Conversely, hydrolysis of β-polysaccharides caused granules' disintegration. The updated view was: the granule structure was stabilized by a network principally composed of β-polysaccharides as the backbone for embedded proteins, lipids, α-polysaccharides, and cells. Hence, enrichment of certain (not all) EPS assisted granulation, and enhanced granule stability. Such a conclusion was significant to enhance granule stability during operation and to reduce granule loss in storage.

Storage reduced granule stability (Tay *et al.*, 2002c; Zhu and Wilderer, 2003). Tay *et al.* (2002c) and Ng (2002) noted that the granules stored for eight weeks became more irregular and smaller compared to fresh granules and released soluble organic material due to cell hydrolysis. The glucose-fed granules cultivated by Zhu and Wilderer (2003) did not significantly change in size, color, or settleability after storage for seven weeks at room temperatures. Meanwhile, Zhu (2004) claimed that their granules remained stable even after storage for two years in tap water at an ambient temperature (16–26° C). This result was too good to be realized in practice. Adav *et al.* (2007f) concluded that phenol-fed aerobic granules could be preserved

better than acetate-fed granules at reduced temperatures. Particularly, when stored at sub-freezing temperature (-20°C), the granules could retain 80-99% of the initial activity after 48h reactivation. Furthermore the addition of phenol in the storing solution significantly preserved the bioactivity of granules at all storage temperatures. Chiu *et al.* (2007a, b) revealed that the cell core was free of oxygen since the active cell layer, accumulated at the outer rim regime, consumed most intake oxygen. No oxygen was available during long term storage. Adav *et al.* (2007f) probed obligate anaerobic *Bacteroides* sp. over the entire interior of the granules stored at -20 ^{o}C for 180 d. High storage temperature accompanied with the absence of external substrate yielded endogenous respiration inside the granule. Restated, the EPS core could be "digested" inside out by the anaerobes. Following storage, the protein core presented large "vacuoles" compared with the compact and solid protein core noted for fresh granules.

Low or even freezing temperatures and the presence of toxic substance (phenol) inhibited intra-granular bioactivity, hence assisting in preserving granule stability and cell viability for recovery. Furthermore the phenol-fed granule exhibited a dense β-polysaccharide network in the granule interior, which explained why the latter presented better stability than the former during storage.

Intra-granular transport

Chiu *et al.* (2006, 2007a, b) probed the DO profiles around and inside a single aerobic granules. With the help of two DO microelectrodes these authors established a DO profiles under transient and steady-state conditions. The external mass transfer coefficient around spherical floc or granule was estimated by the Frossling equation. Chiu *et al.* (2006) estimated the apparent oxygen diffusivity (D_{app}) of acetate-fed and phenol-fed aerobic granules in the range between 1.24–2.28 $\times 10^{-9}$ $m^2s^{-1}$of 1.28–2.50 mm acetate-fed granules, and 2.50–7.65 $\times 10^{-10}$ m^2s^{-1} of 0.42–0.78 mm phenol-fed granules by probing the DO level at the granule center. It was noted that the EPS content significantly affected the intra-granular oxygen diffusivity. The combined multiple staining, CLSM and DO by microelectrode test, demonstrated an active layer of 125 μm in thickness that consumed most oxygen in the aerobic granules (Chiu *et al.*, 2007a). The DO tests correlated with the findings by CLSM imaging results. The optimal granular size for treating different wastewaters could be quite different based on the intra-granular transport studies.

Strain interactions

Granules cultivated with inorganic carbon had a dominance of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) (Holben *et al.*, 1998; Hao *et al.*, 2002; Jang *et al.*, 2003; Tsuneda *et al.*, 2003; Yang *et al.*, 2004c). When granules were cultivated with acetate or glucose as a carbon source and nitrate as a nitrogen source, the identified bacterial strains were the members of the genus *Epistylis, Poterioochromonas, Geotrichum, Geotrichum klebahnii* (Williams and Reyes, 2006). Yang *et al.* (2004a, b, c) cultivated aerobic granules with acetate as the sole carbon source and noted that successful granulation could be achieved only when free ammonia concentration was less than 23.5 mg L^{-1}. In addition, an increase in ammonia concentration significantly decreased cell hydrophobicity and affected the EPS production, hence causing failure in granulation. Detailed research work on the mechanisms for ammonium inhibition and on the possible inhibition by other metabolites or chemicals was still not fully understood.

Jiang *et al.* (2004b, 2006a, b, 2007) isolated ten bacterial strains from aerobic phenol-degrading granules and identified their potential for degrading phenol. The PG-01 strain, a member of α-*proteobacteria*, was common in granules and was the predominant strain in phenol degradation. Another strain affiliated with *β-proteobacteria*, PG-08, had minimal phenol degradation capability, and a high propensity for self-aggregation. Hence, different strains on aerobic granules may have specific roles in granule structural integrity and phenol degradation. Yeast genera, such as *Rodotorula*, *Trichosporon*, and *Candida* could degrade high levels of phenol or phenolic compounds (Neujahr, 1990; Kurtz and Crow, 1997; Chang *et al.*, 1998; Ruiz-Ordaz *et al.*, 2000; Chen *et al.*, 2002; Alexievaa *et al.*, 2004; Margesin *et al.*, 2005). Jiang *et al.* (2005) isolated a *Candida tropicalis* strain from activated sludge, and identified the growth kinetics and phenol-specific degradation rates for this strain with phenol concentrations of 100–2000 mg L^{-1}. Adav *et al.* (2007a) isolated the yeast strain, *Candida tropicalis* from their phenol degrading granules and reported it as a functionally dominant strain in the phenol degrading granules. Heinaru *et al.* (2000) isolated 39 bacterial strains from polluted river water (38 *Pseudomonas* spp. and

1 *Acinetobacter* sp.) and found from the taxonomic analysis of all the strains reported for phenol biodegradation, more than 70% strains belong to *Proteobacterium* phyla.

Adav and Lee (2008b) cultivated granules with a single strain, *Acinetobacter calcoaceticus*, which exhibited a high autoaggregation potential with interconnecting fibrils (Fig. 2). Seed sludge with enriched *Acinetobacter* strains could form the granules faster than the sludge dominated by non-flocculating microbial strain. The auto-aggregating bacterial strains enhanced the granulation process (Adav and Lee, 2008b, Jiang *et al.*, 2006). The single-culture granules cultivated with *Acinetobacter calcoaceticus* degraded phenol at a rate of 9930 mg g^{-1}VSS d^{-1}, significantly higher than that of activated sludge and of other aerobic granular sludge (Tay *et al.*, 2005a; Watanabe *et al.*, 1996). The different process performances noted with the mixed-culture granules revealed the difficulty in precise control of the dominant strains in the granules, partly because of the presence of inhibiting strains. The single-culture granules could be biologically precise for engineered systems. The strains *Acinetobacter calcoaceticus, Bacillus thuringiensis* and *Acinetobacter* sp. were both phenol degraders and autoaggregators, and were capable of forming granules individually when inoculated separately in a SBR (Adav *et al.*, 2008b). These findings contradicted the previous view that autoaggregation and phenol degradation were mutually exclusive in aerobic granules and proposed "trade off" functional model (Jiang *et al.*, 2004b; Jiang *et al.*, 2006). In single culture granule, only one strain existed and the specific phenol degradation rate could be higher than those from multi-strains due to a lack of inhibiting strains, antibacterial substance and growth inhibiting metabolites. The interactions of *Candida tropicalis* with *Acinetobacter calcoaceticus, Bacillus thuringiensis* suggested that the strains coaggregated through the cell surface polymers by lectin-saccharide interactions with the adhesin protein on *Acinetobacter calcoaceticus* and complementary sugar receptors on *B. thuringiensis* and *C. tropicalis* (Adav *et al.*, 2008b). The presence of flagellum on the *B. thuringiensis* and *Bacillus sphaericus* helped the nutrient current towards the granules as both these strain were found to locate on the surface of the granules.

V. 結論

Formation of granules in aerobic conditions has been possible and appears as a promising technique for high strength or highly toxic wastewater treatment. These granular systems allowed, in many cases, a more stable operation, and the treatment of larger loads, removal of multiple toxic pollutants, inferior volumes for the settling systems and production of better quality effluents than any conventional systems. The formation mechanisms and applications and certain recent efforts to explore this technology in depth has been presented. We also propose the following perspectives to the potential development of the aerobic granular sludge technology in the future.

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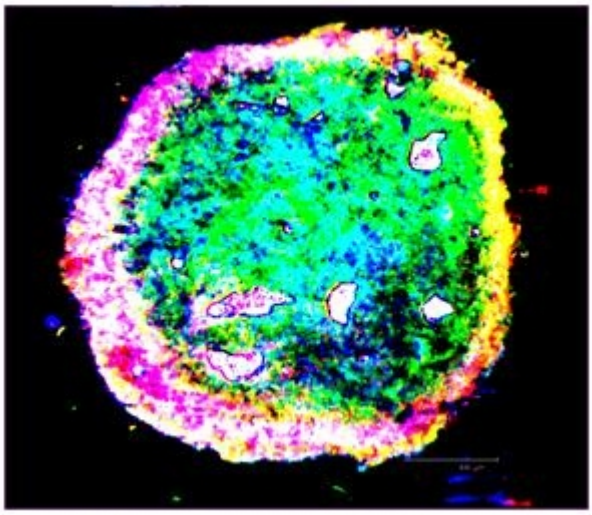

Figure 1. Phenol granule cultivated in sequential batch reactor with synthetic wastewater containing 250 mgL^{-1} phenol, stained for all EPS components [proteins (green): FITC; B - lipids (yellow): Nile red; C-total cells (red): SYTO 63; D – dead cells (violet): Sytox blue; E -α-polysaccharide(Light blue): Con A rhodamine; F-β-polysaccharide (Blue): calcofluor white] and individual images were merged together.

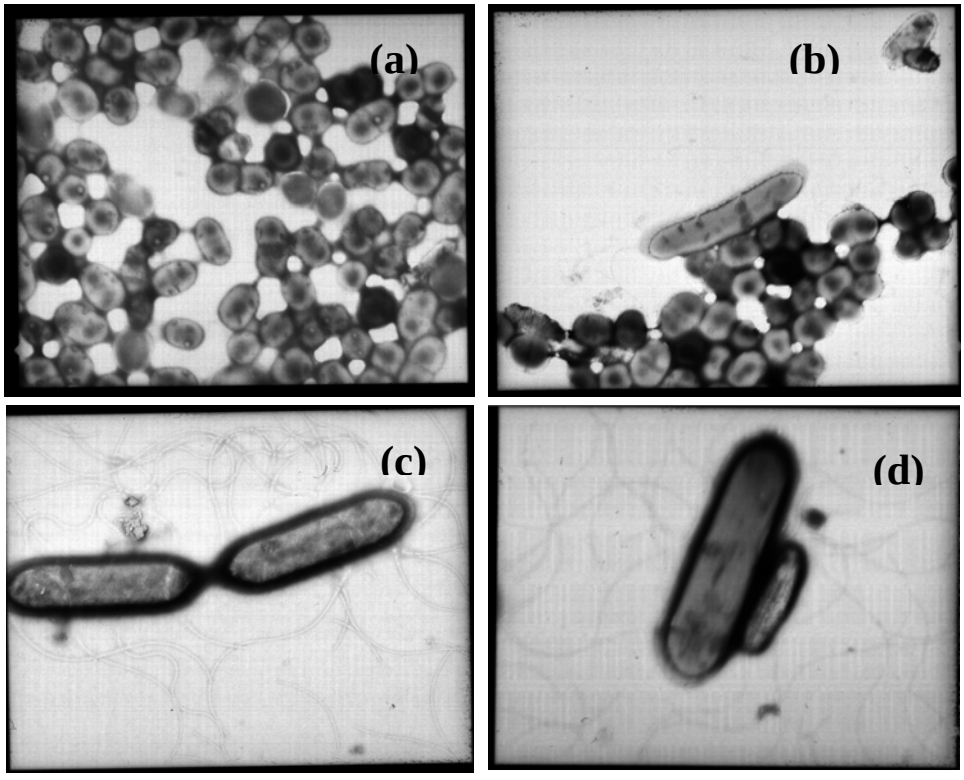

Figure 2. TEM images showing the interactions of the strains (a) *Acinetobacter calcoaceticus* showing interconnecting fibrils; (b) *Bacillus thuringiensis* and *Acinetobacter calcoaceticus* aggregation*;* (b) *Bacillus thuringiensis* with flagellum; (d) *Bacillus sphaericus* with flagella

赴國外研究心得報告

計畫編號	NSC 96-2221-E-002-110
計畫名稱	膜過濾結垢機制及預防之研究--薄膜生物顆粒反應器新程序之開發及機制(3/3)
出國人員姓名 服務機關及職稱	李篤中，台大化工系教授
出國時間地點	Singapore, 2007 年 09 月 05 日至 2007 年 09 月 07 日
國外研究機構	南洋理工大學

工作記要：本次國科會國合計畫(NSC96-2221-E-002-110)資助本人赴 Singapore 進行考察訪問。行程自 2007 年 09 月 05 日至 2007 年 09 月 07 日，計 3 日。

9 月 5 日　　搭乘 SQ875 自中正機場赴 Singapore，行程共計 4.5 小時，南洋理工大學 TC Pan 院長前來接機，並下榻南洋理工大學會館 Nanyang Executive Centre。

9 月 6 日　　本日與南洋理工大學 NEWRI 執行長 WJ Ng 與 TC Pan 院長會談討論未來雙方合作發展方向，NEWRI 一年經費約為二十億元，正尋求與中國大陸之全面合作。

本日進行二場專題演講，並約定十一月初台大化工系組團赴粵參訪事宜。

9 月 7 日　　自 Singapore 搭乘 SQ28 於下午到達中正機場，行程共計 5 小時。