Municipal Wastewater Treatment Using Barium Alginate Entrapped Activated Sludge: Adjustment of Utilization Conditions

Sumana Siripattanakul-Ratpukdi and Thitiporn Tongkliang

***Abstract*—The objective of this research is to treat municipal wastewater by barium alginate (BA)-entrapped activated sludge. This study divided into 2 parts including 1) adjustment of operating conditions for municipal wastewater treatment by the BA-entrapped cells in batch experiment and 2) utilization of the BA-entrapped cells in sequencing batch experiment. In the batch experiment, optimization of cell entrapment preparation condition (cell-to-matrix (BA) ratios of 1:5, 1:10, and 1:20 by volume), determination of optimum dissolved oxygen (DO) concentration (DOs of 2, 4, and 6 mg/L), and determination of optimum entrapped cell loading (1,000, 3,000, and 5,000 mg dry cells/L) were performed. The result showed that the cell-to-matrix ratio, DO concentration, and cell loading of 1:10, 6 mg/L, and 1,000 mg/L, respectively performed highest treatability (COD removal of 47-86%). In the sequencing batch mode experiment, the free and BA-entrapped activated sludge reactors operated for 30 runs were performed and microscopically observed. The entrapped cells could treat wastewater for 54% while the free cells treated wastewater of 33%. The micro-structural observation indicated that the BA-entrapped cells slightly damaged after utilization and numerous microbial cells perforated all over the matrix.**

***Index Terms*—Barium alginate, Cell entrapment, Cell-to-matrix ratio, Cell loading, Dissolved oxygen, Municipal wastewater.**

I. Introduction

It is generally known that biological treatment by suspended activated sludge is a basic process for municipal wastewater treatment. The process with the suspended cells is efficient but a few drawbacks of the process are also concerned. Washed-out of the suspended activated sludge during the treatment process was previously reported [1]-[2]. Also, inactivation of the suspended microbial cells by environmental stresses, such as contamination of toxic substances in the systems was found [3].

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Cell entrapment, cell immobilization in a porous polymeric matrix, has been applied to lessen the problems [4-5]. The cell entrapment technique was successfully applied for both municipal and industrial wastewater treatment applications [3]-[6]. Typical polymers used as an entrapment matrix including calcium alginate, carragenan, polyvinyl alcohol, and cellulose triacetate were reported [3].

Calcium alginate is the most utilized entrapment matrix in environmental applications [3]-[4]. This is due to calcium alginate is a natural polymer which is non-toxic to microorganisms and environment. However, based on prior studies, calcium alginate was not durable [3]-[7]. The matrix produced by calcium alginate likely to break or swell during use affecting lower wastewater treatment performance. Barium alginate (BA) entrapment was developed and applied to solve the problem [8]-[10]. It was found that the BA-entrapped cells well performed and were durable in environmental pressures. However, most previous studies focused on utilization of the BA-entrapped cells for treating different types of wastewater [8]-[10].Thus far, there is no publication emphasizing on operating factors related to performance of the wastewater treatment by the BA-entrapped cells.

The aim of this study is to treat municipal wastewater by the barium alginate (BA)-entrapped activated sludge. The work included determination of optimum wastewater operating condition for the BA-entrapped cells and demonstration of the wastewater treatment by the BA-entrapped cells in sequencing batch reactor (SBR) experiment. In addition, scanning electron microscopic (SEM) observation was performed along with the wastewater treatment for insight information.

II. Materials and Methods

A. Wastewater Source and Characteristics

Wastewater from university cafeteria was obtained (Ubon Ratchathani, Thailand). The wastewater characteristics were as shown in Table I.

B. Activated Sludge Cultivation

Activated sludge was cultivated and acclimated using commercial synthetic medium in a 30-L reactor for 2 months before use in the experiments. The reactor was operated in sequencing batch reactor (SBR) mode with hydraulic and solid retention times of 1 and 30 days, respectively. Dissolved oxygen concentration of higher than 1 mg/L was continuously maintained.

TABLE I: WASTEWATER CHARACTERISTICS

Parameter	Range
Biochemical Oxygen Demand	260-760 mg/L
Chemical Oxygen Demand (COD)	360-840 mg/L
Suspended solids (SS)	155-400 mg/L
Total kjeldahl nitrogen	198-243 mg/L
Ammonia-nitrogen (as N)	Not detectable
Nitrate-nitrogen (as N)	0.14-0.58 mg/L
pH	5.53-6.38

C. Free and BA-Entrapped Cell Preparation

To prepare the cell for the experiment, 1,000 mL of the activated sludge from the 30-Liter reactor was centrifuged at 7,000 rpm for 10 min to obtain concentrated cells. The concentrated cells were vigorously re-suspended in 10 mL of sterile de-ionized water (DI). The re-suspended cells were used for the free activated sludge (described below) and also for preparing the entrapped cells.

For the entrapped cell preparation, sodium alginate (Fluka, Singapore) was dissolved into sterile DI to prepare a sodium alginate solution at a concentration of 3% (w/v). The re-suspended activated sludge as prepared above was uniformly mixed with the sodium alginate solution. The cell-matrix mixture contents (cell-to-matrix ratios) are described in Table II. The mixture was manually dropped into a calcium chloride solution of 5% (w/v) using a sterile syringe to form spherical beads with a size of 3-5 mm. The beads remained in the solution for 2 h for hardening.

TABLE II: DESCRIPTIONS OF REACTORS IN BATCH EXPERIMENT

Reactor	Cell-to-matrix ratio*	DO (mg/L)	Final cell loading in reactor (mg SS/L)
Optimization of entrapment matrix preparation			
F-CTM1:00	1:00	6	1,000
E-CTM1:05	1:05	6	1,000
E-CTM1:10	1:10	6	1,000
E-CTM1:20	1:20	6	1,000
Optimization of DO concentration			
E-DO2	Selected ratio	2	1,000
E-DO4	from previous	4	1,000
E-DO6	experiment	6	1,000
Optimization of entrapped cell loading			
E-1000	Selected ratio	Selected	1,000
E-3000	from previous	concentration from	3,000
E-5000	experiment	previous experiment	5,000

* mL of cells : mL of BA

D. Batch Experiment

In the batch experiment, optimization of entrapment preparation condition (cell-to-matrix (BA) ratios of 1:5, 1:10, and 1:20 by volume), determination of optimum dissolved oxygen (DO) concentration (DOs of 2, 4, and 6 mg/L), and determination of optimum entrapped cell loading (1,000, 3,000, and 5,000 mg dry cells/L) were focused. Description of the reactors used in the experiment was as shown in Table II. All experiments were triplicate.

The obtained wastewater of 400 mL and the free or entrapped cells were filled in the reactors. All reactors were aerated for 8 hr. The wastewater samples (10 mL) were taken at one-hour interval for the entire experiment to measure soluble COD. The wastewater treatment kinetics and the wastewater treatment efficiencies were determined.

E. Sequencing Batch Experiment

Triplicate experiments of SBR for the free and BA-entrapped activated sludge operated for 30 runs was demonstrated. The entrapped cell preparation and operating conditions were followed the optimum conditions based on the results from the batch experiment. Each cycle took 10 hr and included five periods of the traditional SBR cycle: 1) fill of 0.25 hr, 2) react of 8 hr, 3) settle of 1 hr, 4) draw of 0.50 hr, and 5) idle of 0.25 hr. Influent and effluent samples from each cycle were taken for soluble COD measurement. The entrapped cells before used (cycle no. 0) and after used at cycle no. 10 and 30 were observed using SEM.

F. Analytical Procedures

All parameters were measured according to standard methods [11]. After filtering water sample using GF/C filter glass paper, SS (solids retained on the filter paper) was measured. The soluble COD was measured by potassium dichromate digestion method.

For SEM observation, the entrapped cell beads were rinsed in 5% $BaCl_2$ solution for five times (15 min each) and fixed in a solution containing 2.5% glutaraldehyde at 4°C for 6 d. After that, the samples were rinsed in 5% $BaCl_2$ solution for three times (20 min each). The beads were undergone dehydration process by storing the beads in five ethanol solutions (30%, 50%, 70%, 90%, and absolute ethanol) for 20 min each, consecutively. The dehydrated beads were critical point dried with liquid carbon dioxide as a transitional fluid (Balzers, CPD 020, Liechtenstein). After that, the beads were cut and coated with gold using a Balzers SCD 040 sputter coater, and examined using a scanning electron microscope (JEOL, JSM-5410LV, Tokyo, Japan).

III. RESULTS AND DISCUSSION

A. Batch Experiment

1) Optimization of entrapment matrix preparation

Aim of this section is to investigate the optimum condition of cell entrapment preparation. Four reactors with activated sludge prepared differently (free cells and BA-entrapped cells at the cell-to-matrix ratios of 1:5, 1:10, and 1:20 by volume designated F-CTM1:00, E-CTM1:05, E-CTM1:10, and E-CTM1:20, respectively) were tested. Fig. 1 presents the normalized COD remaining of the wastewater during the tests for 8 hr. The COD values continually decreased for entire of the experiment. At the end of the experiments (8 hr), COD remained 45, 25, 18, and 37% in F-CTM1:00, E-CTM1:05, E-CTM1:10, and E-CTM1:20 reactors, respectively. The COD removal kinetics in this case followed the first order kinetic reaction with the rate constants of 0.10-0.22 1/hr (Table III).

During this experiment, control tests which were only the BA matrices (no activated sludge) were also tested. These reactors were performed to determine effect of the BA matrix adsorption. The COD remaining in the control test was quite stable for entire of the experiment (data not shown). This clearly indicated that COD adsorption onto the BA matrices was not significant. The COD removal in this study was biological removal by activated sludge. This observation was similar to a previous study which reported insignificant adsorption of organic contaminant by the entrapment matrices [12]. The above results suggested that the BA-entrapped cells (COD removal of 63-82%) obviously performed better than the free cells (COD removal of 51%) as presented in Table III. This is because the entrapped cells generally tolerate and acclimate better than the free cells resulting higher wastewater treatment efficiency [4], [12]-[13].

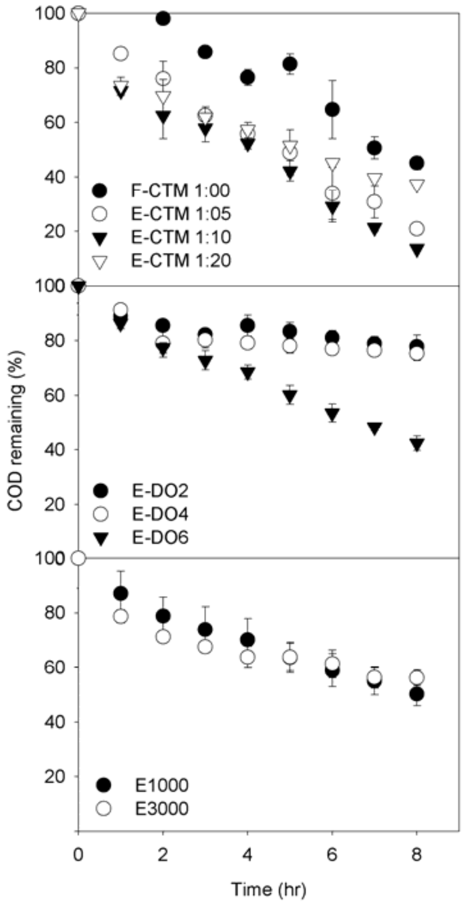

Fig. 1. Normalized COD remaining in the batch experiment.

TABLE III: COD REMOVAL KINETICS

Reactor	COD removal (%)	COD removal kinetics		
		Equation*	R^2	Rate constant (hr^{-1})
Optimization of entrapment matrix preparation				
F-CTM1:00	54.90	Y = -0.10X+4.75	0.90	0.10
E-CTM1:05	75.07	Y = -0.19X+4.68	0.97	0.19
E-CTM1:10	82.38	Y = -0.22X+4.65	0.94	0.22
E-CTM1:20	62.75	Y = -0.11X+4.50	0.97	0.11
Optimization of DO concentration				
E-DO2	22.22	Y = -0.02X+4.54	0.78	0.02
E-DO4	24.71	Y = -0.03X+4.52	0.71	0.03
E-DO6	57.60	Y = -0.10X+4.59	0.99	0.10
Optimization of entrapped cell loading				
E-1000	46.81	Y = -0.08X+4.56	0.99	0.08
E-3000	43.78	Y = -0.06X+4.46	0.85	0.06

* Y = ln COD an d X = time (hr)

For comparison of the cell entrapment preparation conditions, the cell-to-matrix ratios apparently influenced the treatment performance. In this study, the entrapped cells prepared at the cell-to-matrix ratio of 1:10 provided the best COD removal. Too high ratio (1:5) could lead to too high cell density inside the matrices resulting in not enough space for cells to grow. On the other hand, too low ratio (1:20) could limit substrate diffusion through the matrices. This result was similar to prior works which the entrapped cell preparation condition affecting wastewater treatment was stated [12]-[13]. However, the optimum entrapped cell preparation condition may not conclude for all cases since there are co-factors influencing wastewater treatment performance, such as wastewater characteristics, microbial species, and reactor operations. The precise entrapped cell preparation condition should be carried out for case by case.

2) Optimization of DO concentration

This section is to investigate the optimum DO concentration for wastewater treatment by the BA-entrapped cells (at the ratio of 1:10). Three reactors designated E-DO2, E-DO4, and E-DO6 (DOs of 2.1-2.3, 3.7-4.5, and 6.0-6.6 mg/L, respectively) were tested. Fig. 1 presents the normalized COD remaining of the wastewater during the tests for 8 hr. The COD values continually decreased for entire of the experiment. At the end of the experiments (8 hr), COD remained 88, 75, and 43% in E-DO2, E-DO4, and E-DO6 reactors, respectively. The COD removal kinetics followed the first order kinetic reaction with the rate constants of 0.02-0.10 1/hr (Table III).

The results clearly indicated that DO played an important role on wastewater treatment by the BA-entrapped cells. Based on the results of E-DO2 and E-DO4, the COD removal efficiencies (22-25%) were similar whereas the COD removal efficiency of almost 60% was found in E-DO6. The result suggested that optimum DO concentration for wastewater treatment by the BA-entrapped cells were 6 mg/L.

It is known that DO is a major factor influencing wastewater treatment performance by suspended activated sludge. In traditional (suspended) activated sludge, DO of 2 mg/L is sufficient. For the entrapped cells, structure of the cells is dense net of matrices. This limits the oxygen diffusion resulting in higher DO concentration required.

3) Optimization of entrapped cell loading

This section is to investigate the optimum BA-entrapped cell loading for wastewater treatment. Three reactors designated E-1000, E-3000, and E-5000 (which represented the reactors with cell masses of 1,000, 3,000, and 5,000 mg dry cells/L, respectively) were tested. Note that the E-5000 reactor contained too much of the BA-entrapped cells which caused the mixing and aeration problems. The E-5000 reactor was not continued for the experiment.

Fig. 1 presents the normalized COD remaining of the wastewater during the tests for 8 hr. The COD values continually decreased for entire of the experiment. At the end of the experiments (8 hr), COD remained 55 and 54% in E-1000 and E-3000 reactors, respectively. The COD removal kinetics followed the first order kinetic reaction with the rate constants of 0.06-0.08 1/hr (Table III).

The results showed that BA-entrapped cell loading slightly influenced wastewater treatment. Normally, the cell loading should obviously affect the treatment efficiency. Higher cell loading should provide better wastewater treatment efficiency. But in this study, E-1000 performed slight better than E-3000. This could be because the reactors contained a number of the BA-entrapped cells (also a number of the matrices) had mixing and aeration problems decreasing the treatment performance. The result suggested that the optimum BA-entrapped cell loading for the wastewater treatment were 1,000 mg/L.

B. Sequencing Batch Experiment

1) Performance of entrapped and free cell systems

In this section, the experiment was conducted to compare long term performance (30 cycles) between the BA-entrapped and free cells in SBR. Fig. 2 presents the COD remaining in the systems (FC_{inf} and EC_{inf} are the influent of the free and entrapped cell reactors while FC_{eff} and EC_{eff} are the effluent of the free and entrapped cell reactors). Average COD of the influent of the reactor was 612±18 mg/L (average ± standard deviation). The treatment efficiencies by both systems were quite stable. The BA-entrapped cells could remove COD for 54±2% while the free cells treated COD of 33±2%.

The results showed that the BA-entrapped cells had higher treatment performance compared to those of the free cells. Moreover, the BA-entrapped cell system was stable. Therefore, the BA-entrapped cells are promising to apply for municipal wastewater treatment.

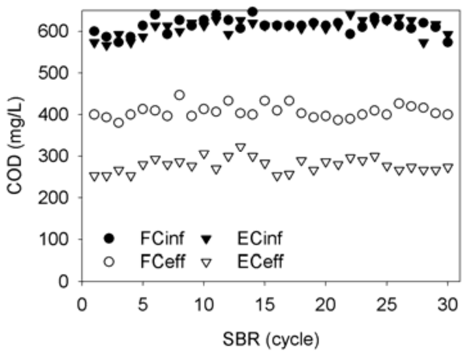

Fig. 2. COD of influents (FC_{inf} and EC_{inf}) and effluents (FC_{eff} and EC_{eff}) in the SBR experiment.

2) Microscopic observation of the entrapped cells

The microstructures of the BA-entrapped cells were investigated by SEM (Fig. 3). In Fig. 3a, the BA-entrapped cells before use, a dense network contained abundant fine pores was observed. The dense cross-linking was network of barium and alginate. The SEM image supported the results in earlier sections. Numerous pores could limit DO diffusion resulting high required DO in the system with the entrapped cells.

After use, the SEM images indicated that the BA-entrapped cells were slight abrasion during use (Fig. 3b and Fig. 3c). The macro-pores inside the matrices were observed. This could be from gases produced from the aerobic wastewater treatment process, such as carbon dioxide. Moreover, the macro-pores locating at the center of the entrapped cells were noticed. This should be from inner layer of the entrapped cells are not as strong as the outer layer resulting in damage (pore) taking place at the inner layer. Basically, barium and alginate cross-linking reaction begins at the outer layer of the entrapped cell droplets to form beads. Then, barium ion passes through the beads. The limitation of diffusion may cause the difficulty of barium transport which results in lower reaction and bead strength at the inner layer of the beads. However, based on the SEM image of the entrapped cells after use for up to 30 cycles, the outer layer of the entrapped cells was not damaged. Therefore, this indicated that the BA-entrapped cells were durable.

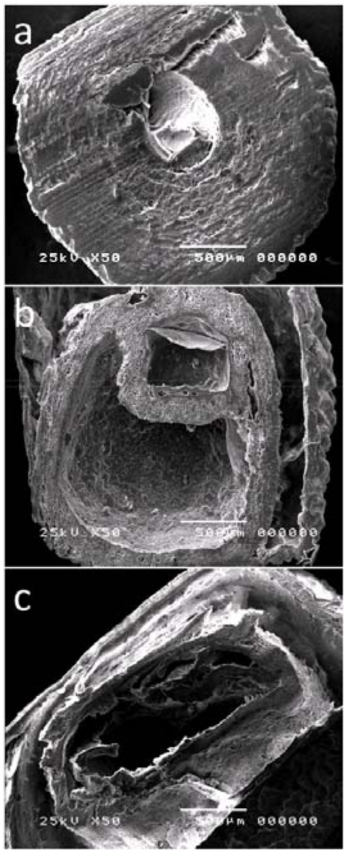

Fig. 3. SEM images of the cross-sectional BA-entrapped cells at 50× (a) before use, (b) after use for 10 cycles, and (c) after use for 30 cycles.

Wastewater treatment by the calcium alginate-entrapped cells was previously reported [7]. In the previous study, the entrapped cells used for only 4 cycles were obviously damaged. This indicated that the BA-entrapped cells were much stronger than the calcium alginate-entrapped cells.

Fig. 4a presents the cells occupied inside the BA matrix at the beginning (before use). After use, numerous cells proliferated in the alginate matrices (Fig. 4b). These SEM results suggested that the BA matrices were durable and the cells inside the matrices well grew and successfully performed wastewater treatment. Hence, the BA-entrapped cells are potential for real wastewater treatment applications.

IV. Conclusion

The BA-entrapped cells are potential for treating municipal wastewater. The entrapped cell preparation condition, DO, and cell loading affected the treatment performance. The wastewater treatment kinetics followed the first-order reaction mechanism. In sequencing batch mode, the BA-entrapped cells (COD removal of 54%)

performed better than the free cells (COD removal of 33%). After use for 30 cycles, the BA-entrapped cells were slightly damaged. For future work, change of microbial community of the entrapped cells during wastewater treatment should be performed.

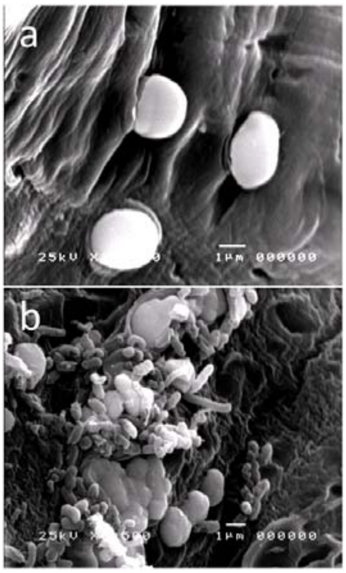

Fig. 4. SEM images of the microbial cells at 7,500× (a) before use, (b) after use for 10 cycles.

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