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ส่วนราชการ ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี โทร.3343

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เรื่อง ขออนุมัติค่าตอบแทนการตีพิมพ์ผลงาน

เรียน รองคณบดีฝ่ายวิจัยและบริการ ผ่านหัวหน้าภาควิชาวิศวกรรมเคมี

อ้างถึงประกาศมหาวิทยาลัยอุบลราชธานี ประกาศ ณ วันที่ 26 ตุลาคม 2555 เรื่อง "หลักเกณฑ์การจ่ายค่าตอบแทนการตีพิมพ์ผลงานวารสารวิชาการ คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี" ตามความทราบแล้วนั้น เนื่องด้วยดิฉัน ผศ.ดร. สุมนา สิริพัฒนากุล อาจารย์ประจำภาควิชาวิศวกรรมเคมี ได้ตีพิมพ์บทความวิชาการดังรายละเอียดต่อไปนี้

<u>บทความตีพิมพ์ในวารสารวิชาการระดับนานาชาติที่มี Impact Factor</u>

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ขอรับค่าตอบแทนของ ผศ.ดร.สุมนา สิริพัฒนากุล (ร้อยละ 100) และเงินเพิ่มในกรณีที่เป็นชื่อแรก คิดเป็นเงิน 12,500 บาท

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Entrapped cell system for decentralized hospital wastewater treatment: inhibitory effect of disinfectants

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This study aims to improve decentralized hospital wastewater treatment inhibited by disinfectants by using calcium alginate cell entrapment technique. The effects of disinfectant types (glutaraldehyde, povidone iodine (PI) and a potassium hydroxide solution) and disinfectant concentrations, cell entrapment conditions (cell-to-matrix ratios) and cell loadings were investigated. The batch experiments were conducted using synthetic wastewater with initial chemical oxygen demand (COD) of approximately 370 mg/L and acclimated activated sludge. Among three disinfectants, PI substantially affected the wastewater treatment efficiency (inhibition of 40%) while other disinfectants exhibited inhibition effects of less than 9%. The results also indicated that the entrapped cells obviously performed better than the free cells. The cell-to-matrix ratio of 1:20 (v/v) provided the highest treatment efficiency of 86% (inhibition of 9%) while the free cell system had inhibition of 47%. The system at the entrapped cell loading of 2000 mg/L performed the highest COD removal of 62% for ten-cycle sequencing batch operation. A scanning electron microscope image provided information on entrapped cell structure subjected to the disinfectant.

Keywords: calcium alginate; cell entrapment; disinfectant; inhibition; hospital wastewater treatment

Introduction

Hospitals are one of the service facilities having their own wastewater treatment systems. In Thailand, the hospital wastewater treatment systems can be categorized into two types, which are centralized and decentralized wastewater treatment systems. The centralized wastewater treatment plants are operated in large hospitals (more than 100 hospital beds) and normally are city or regional hospitals. Wastewater from several buildings in the hospital is collected and treated in one system. The typical centralized wastewater treatment systems are oxidation ditch or completely mixed activated sludge processes. On the other hand, the decentralized wastewater treatment systems are used in small hospitals (for less than 100 hospital beds) such as district or private hospitals. The decentralized wastewater treatment systems are compact on-site systems for individual buildings, and which is usually an extended-aeration activated sludge process. Based on information from the Ministry of Public Health, Thailand, there are approximately 10,000 district hospitals that have compact wastewater treatment systems. The size of the compact systems typically ranges from 0.5 to 20 m^3, which are designed for wastewater flow rates of approximately 2–60 m^3/d.

Commonly, the treated effluents from the decentralized systems of hospitals do not meet the regulatory standard for organic (biochemical oxygen demand (BOD) or chemical oxygen demand (COD)) removal. One of the potential major reasons was the presence of biocides, including antibiotics and disinfectants, in the wastewater [1,2]. It is known that large amounts and various types of disinfectants are used in hospitals, such as halogenated, aldehyde and phenolic compounds. These chemicals are washed down and finally end up in the wastewater treatment systems. The disinfectants do not only kill germs for medical purposes, but also inactivate the microorganisms in the wastewater treatment system, which in turn can cause system failure. For example, Bodik et al. [3] found that hypochlorite-based disinfectants inhibited municipal wastewater treatment efficiency up to 97%. To the best of our knowledge there has been no study on the effect of disinfectants on hospital wastewater treatment and the problems for abatement technology.

An entrapped cell system is a potential alternative to address this problematic issue. Microorganisms entrapped in a porous polymeric material are known to be an effective technique for several environmental applications including drinking water treatment, wastewater treatment and site remediation [4–7]. The technique has proved that it can be applied to alleviate the limitations associated with traditional (free) cell treatment. The system provides high cell loading and toxic protection resulting in better treatment efficiency [4]. Most previous works reported the tolerance

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of the entrapped cells in phenol-contaminated environments [4]. It has also been discovered that entrapped cells could be applied to applications with other toxic substances as well. For example, entrapped *Micrococcus roseus* was more tolerant to pH, temperature changes and heavy metals compared to free cells during surface water remediation [5]. Siripattanakul et al. reported that *Agrobacterium radiobacter* J14a and mixed cultures could stand and well degrade atrazine, a widely used herbicide [7]. Based on this rationale, the cell entrapment technique could be applicable for disinfectant-contaminated hospital wastewater. Moreover, the entrapped cell matrix has a large size and its density is higher than that of the free cell. Therefore, it is easy to settle the entrapped cells from wastewater, leading to less cell washout compared to the free cell system (activated sludge). This feature of the entrapped cells means that the system does not require the sedimentation process. In addition, the entrapped cells can be applied directly in the existing decentralized wastewater treatment facility.

This study aims to examine the effect of disinfectants on decentralized hospital wastewater treatment performance and the use of the cell entrapment technique to lessen inhibition from disinfectants. The effects of disinfectant types and concentrations were investigated. Three disinfectants including glutaraldehyde (GA), povidone iodine (PI) and potassium hydroxide (eco-friendly biocide (EB)) were chosen. The optimum entrapped cell preparation and entrapped cell loading conditions for hospital wastewater treatment were also determined. Calcium alginate (CA), a widely used cell entrapment matrix, was selected for this study.

Materials and methods

Synthetic hospital wastewater

Synthetic hospital wastewater was prepared following wastewater characteristics from a district hospital near to the university in Warinchamrap, Ubon Ratchathani, Thailand. Based on information from a three-year chemical inventory from the hospital, three disinfectants (GA, PI and EB) were reported as the three most utilized disinfectants. The working concentrations of GA and PI used in the hospital were 2% and 10%, respectively. A cleaning product containing potassium hydroxide of 3.2–4.0% (Metri Clean 2, Metrex Research Corporation, MI, USA), which was commercially claimed to be a eco-friendly biocide, was applied in this study. The decentralized wastewater treatment system in the hospital was an extended-aeration activated sludge process with hydraulic retention time of 6 h. Average COD value of the influent was 350 mg/L.

Normally, the hospital wastewater contains similar constituents to municipal wastewater except that there are some chemicals from medical treatments. The synthetic wastewater was prepared from $C_{12}H_{22}O_{11}$, $CO(NH_2)_2$ and $Ca(H_2PO_4).H_2O$ at approximately COD:N:P of 100:5:1 to obtain general characteristics of municipal wastewater [8]. It is noted that the commercial chemicals obtained from local distributers (Bangkok, Thailand) were used to prepare the wastewater. The COD and pH values of the synthetic wastewater were approximately 370 mg/L and 6.5–7.0, respectively. Three types of commercial disinfectants (GA, PI and EB) with different concentrations were then added to the synthetic wastewater (Table 1).

Table 1. Descriptions of components in wastewater treatment inhibition kinetic tests by the free cells.

Test description[(1)]	Reactor ID	Disinfectant: Type	Disinfectant: Concentration (% v/v)[(2)]
(1) Effect of disinfectant types	TYPE-GA	GA	0.1
	TYPE-PI	PI	0.1
	TYPE-EB	EB	0.1
	TYPE-ND	No disinfectant	0.0 (control)
(2) Effect of disinfectant concentrations	CONC-0.1	The most inhibiting disinfectant selected from test 1	0.1
	CONC-0.2		0.2
	CONC-0.3		0.3
	CONC-0.0		0.0 (control)

Notes: [(1)] Activated sludge concentration in reactors was 1000 mg SS/L. [(2)] Percentage (v/v) was percentage of the working volume (at each disinfectant working concentration) to synthetic wastewater volume. Tested concentrations were calculated from the quantity of the disinfectant used and wastewater in the district hospital.

Activated sludge cultivation and acclimatization

Municipal activated sludge was used in this study to avoid the residue of disinfectant in the hospital activated sludge. The activated sludge was cultivated and acclimated using synthesized wastewater (without disinfectants) 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.

Activated sludge preparation

Free activated sludge

To prepare the cell for the experiment, 1000 mL of the activated sludge from the 30 L reactor was centrifuged at 7000 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.

Entrapped activated sludge

The activated sludge was entrapped in CA according to a technique adapted from a protocol by Smidsrod and

Skjak-Braek [9]. The technique was chosen because of several successful applications [4,6,9]. Sodium alginate (Fluka, Singapore) was dissolved into sterile DI to prepare a sodium alginate solution at a concentration of 2% (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 2. The mixture was manually dropped into a calcium chloride solution (3.5% (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.

Wastewater treatment inhibition kinetic test

Effect of disinfectant types

This study focused on the effect of disinfectant types on wastewater treatment inhibition. Based on information from a three-year inventory given by the model hospital, the three highest utilized disinfectants (GA, PI and EB) were selected. The most inhibiting disinfectant, based on wastewater treatment performance, was chosen to investigate the effect of disinfectant concentrations.

Three reactors to which were added GA, PI, EB and one control reactor (no disinfectant) designated TYPE-GA, TYPE-PI, TYPE-EB and TYPE-ND, respectively were set up (Table 1). All experiments are duplicated. The synthetic wastewater (250 mL) with disinfectant concentration of 0.1% (by volume) and the concentrated acclimatized activated sludge (or sterile DI) filled the reactors. Final concentration of the activated sludge (measured as suspended solids (SS)) in the reactors was 1000 mg/L. All reactors were shaken at 150 rpm and 30 °C for 8 h. The wastewater samples (10 mL) were taken at 1 h intervals for the entire experiment to measure the soluble COD. The wastewater treatment kinetics and the wastewater treatment efficiencies were determined. Inhibition effect of wastewater treatment was then calculated as shown in Equation (1) [10].

$$Inhibition\ (\%) = \left(1 - \frac{\begin{matrix} Average\ treatment \\ activity\ of\ the\ reactor \end{matrix}}{\begin{matrix} Average\ treatment \\ activity\ of\ the\ control \end{matrix}}\right) \times 100 \tag{1}$$

Effect of disinfectant concentrations

This part emphasized the effect of disinfectant concentrations on wastewater treatment inhibition. The most inhibiting disinfectant from above was selected. The experiment preliminarily determined the relationship of the disinfectant concentrations and inhibition.

Duplicate experiments consisted of four reactors. These included three reactors (adding the selected disinfectant) at concentrations of 0.1%, 0.2% and 0.3 % (v/v) and one control reactor, designated CONC-0.1, CONC-0.2, CONC-0.3 and CONC-0.0, respectively (Table 1). The setup of the reactors, reactor operation and wastewater sampling were similar to the earlier experiment. The wastewater treatment kinetics, the wastewater treatment efficiencies and the wastewater treatment inhibition were also determined.

Wastewater treatment enhancement using entrapped cell system

Optimization of cell entrapment preparation

Generally, the effect of the cell-to-matrix (CA) ratios on substrate diffusivity and contaminant removal ability by the entrapped cells was one of the major concerns in previous studies [5,11]. The purpose of this part was to investigate the optimum cell-to-matrix ratio for treating wastewater containing disinfectant. In this experimental section, eight reactors containing different contents of the cells and the matrix were prepared to determine the effect of cell-to-matrix ratios (Table 2).

Duplicate 8 h batch experiments were performed. 250 mL of synthetic wastewater with the selected disinfectant (PI) at the selected concentration (0.1% v/v) was put into a reactor. Details of the inoculation are shown in Table 2. All reactors were shaken at 150 rpm and kept at 30 °C. During the 8 h experiment wastewater samples of 10 mL were taken every hour for measuring the soluble COD. The wastewater treatment kinetics, the wastewater treatment efficiency and the wastewater treatment inhibition were determined. The CA matrices were taken for microstructure observation using scanning electron microscopy (SEM) for further insight.

Optimization of cell loadings

Microbial cell loading is known to be one of the most important factors for contaminant removal by either free or entrapped cells [12]. The effect of entrapped cell loadings on treatment performance was studied and compared to that of the free cells. Also, the long-term performances of both systems were investigated.

Duplicate experiments were conducted in SBR mode. Six reactors of 250 mL labelled EC-1000, EC-2000, EC-3000, FC-1000, FC-2000 and FC-3000 were studied (Table 3). Note that the optimum cell preparation (cell-to-matrix ratio) as obtained above was applied. The reactors were consecutively run for ten cycles. Each cycle took 9 h and included five periods of the traditional SBR cycle:

(1) Fill for 0.25 h,
(2) React for 6 h,
(3) Settle for 2 h,
(4) Draw of 0.25 h,
(5) Idle for 0.50 h.

Table 2. Descriptions of components in the investigation of the optimum cell-to-matrix ratio.

			Cell-to-matrix ratio (mL of cells: mL of calcium alginate)		
Test	Reactor	Cell type	Volume of cells (or DI) (mL)	Volume of CA (mL)	Total inoculated volume (mL)
(3) Optimization of cell-to-matrix ratios	CM-1:05	Entrapped cells	10(1)	50	60
	CM-1:10		10(1)	100	110
	CM-1:20		10(1)	200	210
	FC-1:00	Free cells	10(1)	0	10
	CA-0:05	Only CA matrices	10(2)	50	60
	CA-0:10		10(2)	100	110
	CA-0:20		10(2)	200	210
	NC-0:00	Control	10(2)	0	10

Notes: All conditions were spiked with PI at concentration 0.1% by volume. (1) Concentrated activated sludge at the final concentration in reactor of 1000 mg SS/L. (2) Sterile DI.

Table 3. Descriptions of components in the investigation of the optimum cell loading.

Test	Reactor ID	Cell type	Cell loading (mg SS/L)
(4) Optimization of cell loading	EC-1000	Entrapped cells	1000
	EC-2000		2000
	EC-3000		3000
	FC-1000	Free cells	1000
	FC-2000		2000
	FC-3000		3000

Influent and effluent samples from each cycle were taken for COD and pH measurement.

Analytical procedures

COD, SS and pH were measured according to standard methods [13]. After filtering the water sample using GF/C filter glass paper, the soluble COD was measured by potassium dichromate digestion method. pH was measured using a pH meter (inoLab pH level 1, WTW GmBH, Weilheim, Germany).

For SEM observation, the procedure described in a previous study was applied [14]. The entrapped cell beads were rinsed in 0.1 M $CaCl_2$ for 15 min twice and fixed in a solution containing 2.5% glutaraldehyde and 0.1 M $CaCl_2$ for 1 h. The samples were rinsed in 0.1 M $CaCl_2$ for 15 min twice. The beads underwent dehydration by storing the beads in five solutions for 30 min, successively. These solutions were:

(1) 30% ethanol and 0.07 M $CaCl_2$,
(2) 50% ethanol and 0.05 M $CaCl_2$,
(3) 70% ethanol and 0.03 M $CaCl_2$,
(4) 90% ethanol and water,
(5) 100% ethanol.

The dehydrated beads were critical-point dried using an autosamdri-810 drier with liquid carbon dioxide as a transitional fluid. After that, the beads were cut and attached to aluminium mounts by silver paint. Then the beads were coated with gold using a Balzers SCD 030 sputter coater and examined using a JEOL JSM-6300 scanning electron microscope.

Results and discussion

Wastewater treatment inhibition kinetic test

Effect of disinfectant types

The effect of the disinfectant type on wastewater treatment inhibition was determined. Figure 1(a) presents the normalized COD remaining of the synthetic wastewater during 8 h of testing. An average initial COD from the duplicate experiment was 370 mg/L. The trends of COD reduction from the tests with different disinfectants and without disinfectant were similar. The COD values rapidly decreased within the first 4–5 h and slightly decreased in a later period. At the end of the experiments (8 h), COD removals were 64%, 42%, 67% and 70% for the TYPE-GA, TYPE-PI, TYPE-EB and TYPE-ND reactors, respectively. This indicated that different types of disinfectants could inhibit the wastewater treatment activities differently. The TYPE-ND reactor, which was a control (no disinfectant), removed 70% of COD while the other reactors, containing disinfectants, removed COD less than the control by 1–20%.

The wastewater treatment inhibition (calculated by Equation (1)) and wastewater treatment kinetics are shown in Table 4. The inhibition by the disinfectants ranged from 4–40% of the control (Table 4). The removals of COD by all reactors were well fitted with the first-order kinetic reaction at the rate constants of 0.09–0.16 h^{-1}. This obviously proved that the disinfectants played an important role in wastewater treatment performance. The result was similar to a previous study, which reported the damage

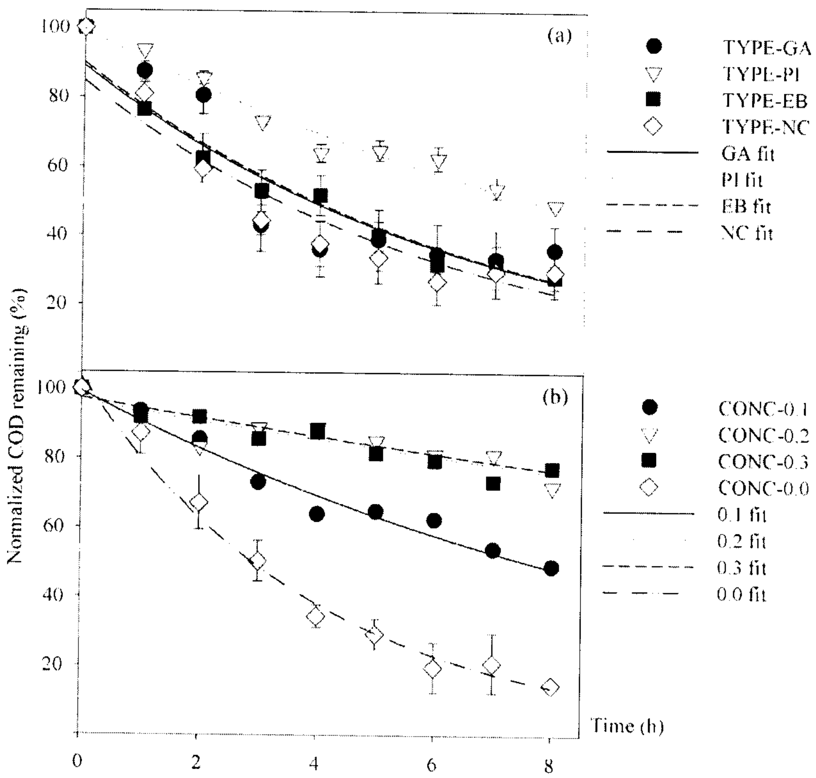

Figure 1. Normalized COD remaining and kinetic curve fitting: the effects of disinfectant types and concentrations.

to microorganisms by disinfectants [15,16]. The disinfectants could injure the cell wall, membrane and cytoplasm, resulting in inactivation of the cells.

Among the three disinfectants, PI affected the treatment efficacies five and ten times higher than GA and EB, respectively. Even though EB chemical structure is confidential, the main ingredient of EB is potassium hydroxide at a low concentration. It is obvious that EB is an environmental friendly biocide; therefore, it just slightly affected the wastewater treatment activity. Between GA and PI, it is known that GA has better antimicrobial efficiency than PI [17]. However, the wastewater treatment inhibition and kinetics turned out contradictory. This could be from different working concentrations of the disinfectants in the commercial products. The PI disinfectant was normally used for treatment of skin infection and wounds, but GA was used for instrument disinfection. Therefore, the commercial PI concentration used in the hospital was higher than that of GA. In this experiment, the concentration of 0.1% (v/v) of each commercial product was tested. This resulted in a higher concentration of PI than GA in the synthetic wastewater.

The initial PI concentration used in the study was five times higher than GA while EB concentration was about two times higher than GA (following the concentrations in practice). After normalizing the concentrations, it was found that GA inhibition was slightly higher than that of PI (1.07 times higher) whereas EB inhibition was much lower than that of PI (3.74 times less). As expected, different disinfectants gave different magnitudes of wastewater treatment inhibition. In this case, GA and PI substantially affected the wastewater treatment while EB inadequately influenced the treatment. In the next section, the effect by PI, which is in practice used at a much higher concentration, was emphasized.

Effect of disinfectant concentrations

The effect of the disinfectant concentrations on the wastewater treatment inhibition (as normalized COD remaining) was shown in Figure 1(b). In the control reactor (CONC-0.0), the COD values rapidly decreased within the first 6 h and slightly reduced thereafter. The trends of COD reduction in all tests with disinfectants were similar. The COD values gradually decreased for the entirety of the experiments. At the end of the experiments (8 h), the COD removal efficiencies of 50%, 27%, 23% and 85% were observed from the CONC-0.1, CONC-0.2, CONC-0.3 and CONC-0.0 reactors, respectively. This indicated that lower treatment efficiencies were attributed to higher concentration of disinfectants. Similar results were also found in the previous study [15]. The previous study reported that high

Table 4. Wastewater treatment inhibitory kinetics.

Test	Reactor ID	COD removal (%)	Inhibition (% compared to control)	Wastewater treatment kinetics: Equation[1]	R^2	Rate constant (h^{-1})
(1) Effect of disinfectant types	TYPE-GA	64	8.57	$Y = -0.15X + 4.49$	0.79	0.15
	TYPE-PI	42	40.00	$Y = -0.09X + 4.60$	0.97	0.09
	TYPE-EB	67	4.28	$Y = -0.15X + 4.50$	0.97	0.15
	TYPE-ND	70	–[2]	$Y = -0.16X + 4.44$	0.87	0.16
(2) Effect of disinfectant concentrations	CONC-0.1	50	41.18	$Y = -0.09X + 4.60$	0.97	0.09
	CONC-0.2	27	68.24	$Y = -0.03X + 4.57$	0.77	0.03
	CONC-0.3	23	72.94	$Y = -0.03X + 4.58$	0.91	0.03
	CONC-0.0	85	–[2]	$Y = -0.25X + 4.64$	0.98	0.25
(3) Optimization of cell-to-matrix ratios	CM-1:05	57	39.36	$Y = -0.11X + 4.56$	0.96	0.11
	CM-1:10	58	38.30	$Y = -0.14X + 4.35$	0.92	0.21
	CM-1:20	86	8.51	$Y = -0.24X + 4.41$	0.83	0.24
	FC-1:00	50	46.81	$Y = -0.09X + 4.60$	0.97	0.09
	Control[2]	94	–[2]	$Y = -0.45X + 4.64$	0.98	0.25

Notes: [1] $Y = \ln \mathrm{COD}$; X = time. [2](-) indicates no inhibition because they are the control experiments (no disinfectant).

fluoride concentrations (5–300 mg/L) reduced wastewater treatment efficiency. This phenomenon could be from less viable activated sludge influenced by PI. Anderson et al. reported that several types of bacteria including *Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter baumannii, Pseudomonas aeruginosa and Escherichia coli* were susceptible to PI [18]. It was found that the number of microorganisms in contact with PI (0.2% w/v) decreased from 10^7 to 10^5 CFU/mL within only a few hours. This showed that PI obviously influenced the microbial viability.

The effects of disinfectant concentration on wastewater treatment inhibition and kinetics are shown in Table 4. The inhibition by the disinfectant (PI) was between 41% and 72% of the control (Table 4). The removal of COD from the reactors with the disinfectant followed the first-order kinetic reaction at the rate constants of 0.03–0.09 h^{-1}. This clearly indicated that the disinfectant concentrations affected wastewater treatment performance. Based on the results from the CONC-0.2 and CONC-0.3 reactors, it was noted that the PI concentration of 0.2% (v/v) was the lowest concentration that severely affected the wastewater treatment activity. Even though PI concentration was increased to 0.3%, the equal inhibition kinetic rate constants remained stable for both PI concentrations.

It is interesting that at the levels of PI being studied not all microorganisms were deactivated. The COD removal of approximately 20% in the CONC-0.2 and CONC-0.3 reactors may be due to the microorganisms' toleration of PI. To ensure that the reduction of COD was not from an abiotic process, an experiment with wastewater containing PI but without the activated sludge was conducted. The COD values were quite stable (± less than 5% removal) for the entirety of the test (data not shown). Therefore, the COD removal by an abiotic process did not significantly take place. Moreover, some previous studies reported a few species of microorganisms tolerant of PI [18,19].

Wastewater treatment enhancement using entrapped cell system

Optimization of cell entrapment preparation

The purpose of this section was to investigate the potential of entrapped cells for the disinfectant-containing wastewater treatment. The focus was on the optimum condition for cell entrapment preparation. The entrapped cells from eight different compositions were tested. Figure 2 presents the normalized COD remaining of the synthetic wastewater during the tests for 8 h. Figure 2(a) shows the results from the control test (NC-0:00) and the tests with only CA (no cells) for different entrapment preparation conditions (designated as CA-0:05, CA-0:10 and CA-0:20). These reactors were used to determine the effect of the CA matrix adsorption. The COD remaining in the control test was quite stable for the entire experiment while the results from the CA-0:05, CA-0:10 and CA-0:20 reactors were similar. The COD values rapidly decreased within the first hour from 11% to 25% and remained stable thereafter. At the end of the experiments (8 h), COD remained 95%, 79%, 75% and 73% from the NC-0:00, CA-0:05, CA-0:10 and CA-0:20 reactors, respectively. This clearly indicated that COD was just slightly adsorbed by the CA entrapment matrices for all entrapment conditions. This observation was similar to a previous study, which reported insignificant adsorption of atrazine (pesticide) by the entrapment matrices [7].

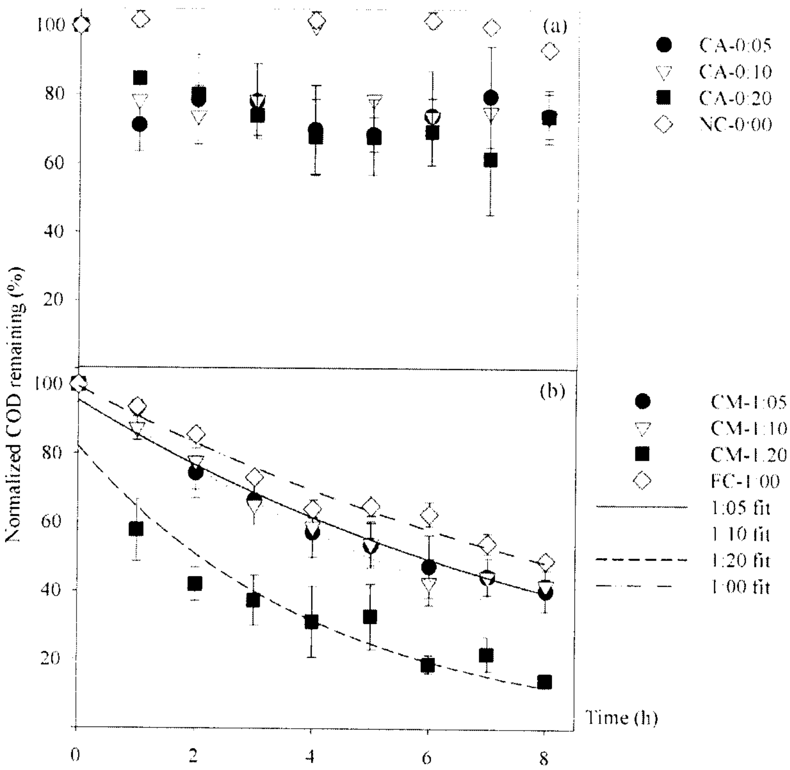

Figure 2. Normalized COD remaining and kinetic curve fitting: the optimization of cell entrapment preparation.

Figure 2(b) presents the results from the free cell test (FC-1:00) and the entrapped cell tests at different entrapment preparation conditions (designated as CM-1:05, CM-1:10 and CM-1:20). For all reactors, the trends of COD removal were similar. The COD values quickly decreased within the first 6 h and gradually reduced thereafter. At the end of the experiments (8 h), remaining COD of 50%, 43%, 42% and 14% for the FC-1:00, CM-1:05, CM-1:10 and CM-1:20 reactors, respectively, were observed. This noticeably proved that the entrapped cells performed better than the free cells. As expected, the cell entrapment conditions played an important role in enhancing the treatment performance of wastewater containing the disinfectant. The previous studies reported that different cell-to-matrix ratios resulted in the different cell densities inside the matrices [7]. Based on the results shown in Figure 2(b), in this study, it is obvious that the lowest cell density (CM-1:20) provided the best COD removal efficiency. This could be because the entrapped cells at the lowest cell density have enough space to grow and less substrate diffusion limitation.

Figure 3 presents a cross-sectional image of the entrapped cells at the microstructure level. The CA entrapment is a cross-linking reaction between entrapment material (sodium alginate) and salt (calcium chloride). It was found that the cross-linking network was dense, resulting in the calcium alginate sheets with a number of cells fixed inside the beads (Figure 3). The sheets were corrugated structures and caused numerous macro voids attributed to high substrate and oxygen diffusion. This led to high wastewater treatment performance in the entrapped cells.

Numerous previous studies reported that the entrapment matrices were able to protect the cells from toxic substances [4,7,10,19]. It means that the entrapped cells apparently had higher lethal concentration levels compared to the free cells. In a previous study, it was proved that the immobilized cells had pentachlorophenol lethal concentration levels of more than 2000 mg/L, which was more than 20 times higher than that of the free cells [19].

Generally, the cell damage mechanism by PI occurs after the cells come into contact with PI [20]. In the case of PI, polyvinylpyrolidone is a source of free iodine. The free iodine is slowly released from the source and contacts the bacterial cells. Then, the free iodine diffuses though the cell membrane and destroys protein, fatty acid and nucleotides inside the cells. Based on the result that the entrapped cells worked better than the free cells; this could be because the entrapment matrices could lessen the cell–PI contact, resulting in lower cell inactivation. Also, it has been reported that numerous organic contaminants could be adsorbed on CA matrix [4]. Even though the organic compound adsorption capacity was in some cases not high [7], some portion of PI may get adsorbed on the matrices attributing in lower PI concentration passed through the cells inside the matrices.

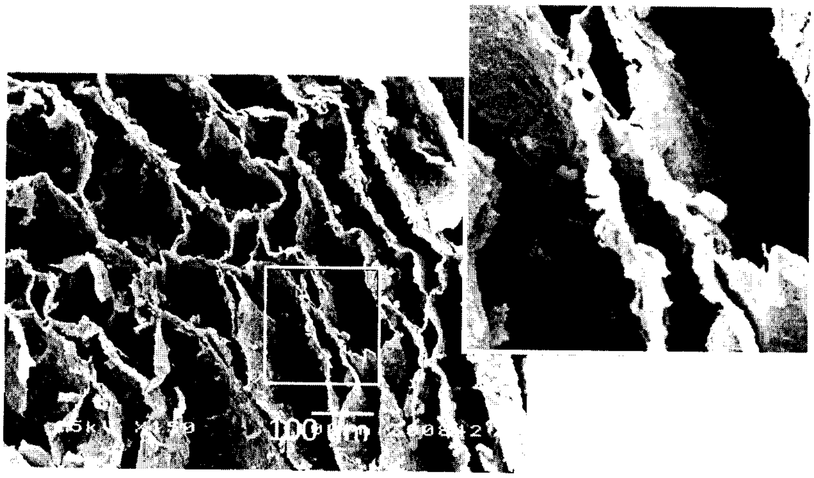

Figure 3. Cross-sectional image of the CA entrapped cells.

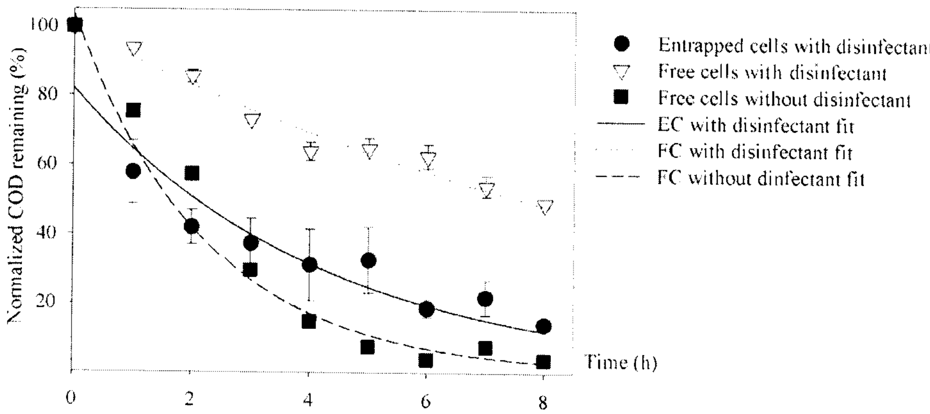

Figure 4. Comparison of the wastewater treatment by the entrapped and free cells.

Table 4 shows the effect of cell-to-matrix ratio on wastewater treatment inhibition and kinetics. The inhibition by the disinfectants was between 9% and 47% of the control (no disinfectant). The removal of COD by the reactors with disinfectants followed the first-order kinetic reaction at the rate constants of 0.09–0.24 h^{-1}. This remarkably signified that the entrapped cells at the optimum entrapment condition performed much better than free cells. The COD removal rate by the entrapped cells at the optimum condition was close to the rate of the traditional wastewater treatment system without the disinfectant. The treatment trend was similar to the control as shown in Figure 4. This obviously proved that the CA-entrapped cells had a real potential for treating hospital wastewater. The entrapped cells performed well and should be applicable for the typical decentralized hospital wastewater treatment system.

Optimization of cell loading

The results of optimum entrapped cell loading for the disinfectant-containing wastewater treatment are shown in Figure 5. Three entrapped cell reactors (EC-1000, EC-2000 and EC-3000) and three free cell reactors (FC-1000, FC-2000 and FC-3000) contained 1000, 2000 and 3000 mg/L, respectively, of cells. For the entrapped cell reactors, the trend of the normalized COD removals were relatively stable (Figure 5(a)). The average COD removals of the EC-1000, EC-2000 and EC-3000 reactors for ten cycles were 44%, 62% and 47%, respectively. For the free-cell reactors, the trend of the normalized COD removals concurrently decreased (Figure 5(b)). The FC-1000, FC-2000 and FC-3000 reactors removed COD by 31%, 38% and 44%, respectively.

Normally, the reactor with higher cell loading should perform better than one with low cell loading. The free-cell reactors gave the COD removal following the theory (Figure 5(b)). However, the results from the entrapped cell reactors were contradictory. The EC-2000 reactor obviously performed better than the others. This was because the EC-1000 reactor had fewer cells, causing lower COD removal performance. For the EC-3000 reactor, it was noticed that,

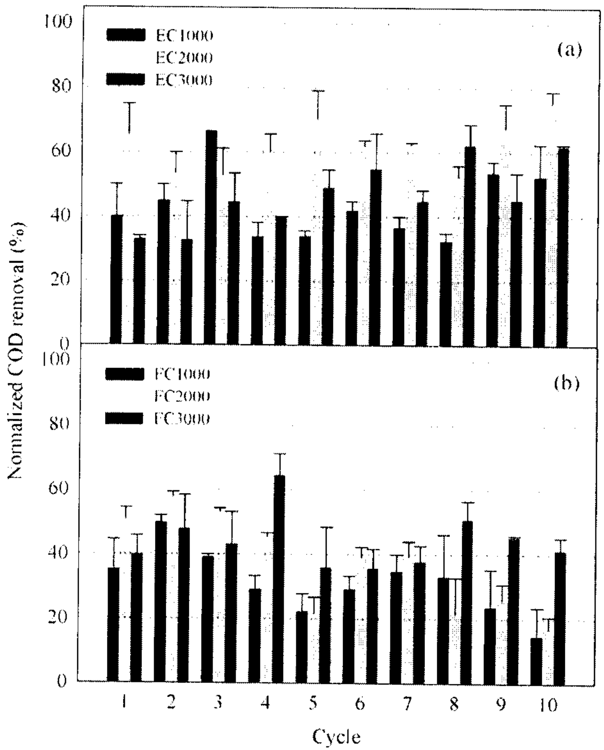

Figure 5. Normalized COD removal by the entrapped and free cells in SBR mode.

during the experiment, the reactor contained a number of the entrapped cells leading to a limitation of mixing. Therefore, this could cause the substrate and oxygen limitation.

Based on the trends (Figure 5), the entrapped cells performed in a more stable manner compared to the free cells. This might be due to the entrapment matrices providing a better environment for the cells, leading to better metabolic activity [4,5]. Besides protecting the cells from the toxic substance, the entrapped cell system had better cell separation in the settling period. Apparently, the entrapped cells were much heavier than the free cells and settled more than the free cells. Therefore, the entrapped cell system had less cell loss during the draining step in SBR mode resulting in better performance.

Conclusions

It has been known that decentralized hospital wastewater treatment systems do not operate successfully. This could be from disinfectants used in hospitals. Povidone iodine at working concentration substantially inhibited the wastewater treatment efficiency (inhibition of 40%). Higher concentrations resulted in more adverse effects. The entrapped cell system can alleviate the problem. Both cell entrapment conditions and cell loadings affected the wastewater treatment. At the optimum cell entrapment condition, the entrapped cell system provided the treatment efficiency of 86% (only 9% inhibition). During ten-cycle sequencing batch operation, the optimum entrapped cell loading yielded wastewater treatment efficiency of 62%. The entrapped cell system performed in a more stable manner and with better cell separation compared to the free cell system. Continued work on a disinfectant-tolerant microbial community is recommended to elucidate the insight information.

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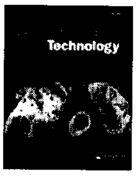

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