เอกสารอ้างอิง

กระทรวงวิทยาศาสตร์ เทคโนโลยีและสิ่งแวดล้อม. 2537. *มาตรฐานควบคุมการระบายน้ำทิ้งจากอาคารบางประเภทและบางขนาด* [ออนไลน์]. www.pcd.go.th. เมื่อถึงเมื่อวันที่ 12 ธันวาคม 2553.

ธงชัย พรรณสวัสดิ์ และคณะ. 2530. *น้ำเสียชุมชนและปัญหามลภาวะทางน้ำในเขต กทม. และปริมณฑล* [ออนไลน์]. www.pcd.go.th. เมื่อถึงเมื่อวันที่ 12 ธันวาคม 2553.

รุ่งทิพย์ ชวนชื่น. 2552. *สารฆ่าจุลชีพ: ยาระงับเชื้อโรคและยาฆ่าเชื้อโรค*. กรุงเทพฯ: บริษัทตีรณสาร.

สุรพล สายพานิช. 2538. *ระบบบำบัดน้ำเสียแบบ Activated Sludge*. ใน: คณาจารย์ภาควิชาวิศวกรรมสิ่งแวดล้อม จุฬาลงกรณ์มหาวิทยาลัย. การควบคุมระบบบำบัดน้ำเสีย. พิมพ์ครั้งที่ 2. กรุงเทพฯ: โรงพิมพ์ จุฬาลงกรณ์มหาวิทยาลัย.

Anderson, M. J., Horn, M.E., Lin, Y., Parks, P. J., Peterson, M.L. 2010. Efficacy of concurrent application of chlorhexidine gluconate and povidone iodine against six nosocomial pathogens. *American Journal of Infection Control*. 38(10), 826-831.

APHA, AWWA, WEF. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th ed, American Public Health Association, Washington DC, USA.

Bickerstaff G.F. 1997. In: Bickerstaff G.F. (Ed.), *Immobilization of Enzymes and Cells*, Humana Press, New Jersey, 1-11.

Bodik, I., Gasparikova, E., Dancova, L., Kalina, A., Hutnan, M., Drtil, M. 2008. Influence of disinfectants on domestic wastewater treatment plant performance. *Bioresource Technology*. 99, 532-539.

Dulieu, C., Poncelet, D., Neufeld, R.J. 1999. In: Kuhtreiber, W.M., Lanza, R.P., Chick, W.L. (Eds.), *Cell Encapsulation Technology and Therapeutics*, Birkhauser, Boston, 3-17.

Durani, P., Leaper, D. 2008. Povidone-iodine: use in hand disinfection, skin preparation and antiseptic irrigation. *International Wound Journal*. 5, 376-387.

Fraser, J.E., Bickerstaff, G.F. 1997. In: Bickerstaff, G.F. (Ed.), *Immobilization of Enzymes and Cells*, Humana Press, New Jersey, 61-66.

Gentry, T.J., Rensing, C., Pepper, I.L. 2004. New approach for bioaugmentation as a remediation technology. *Critical Reviews in Environmental Science and Technology*, 34, 447-494.

Guiot, S.R., Tawfiki-Hajji, K., Lepine, F. 2000. Immobilization strategies for bioaugmentation of anaerobic reactors treating phenolic compounds. *Water Science and Technology*, 42, 245-250.

Ha, J., Engler, C.R., Wild, J.R. 2009. Biodegradation of coumaphos, chlorferon, and diethylthiophosphate using bacteria immobilized in Ca-alginate gel beads. *Bioresource technology*, 100, 1138-1142.

Hajji, K.T., Lepine, F., Bisaillon, J.G., Beaudet, R., Hawari, J., Guiot, S.R. 2000. Effects of bioaugmentation strategies in UASB reactors with a methanogenic consortium for removal of phenolic compounds. *Biotechnology and Bioengineering*, 67, 417-423.

Hill, C. B., Khan E. 2008. A Comparative Study of Immobilized Nitrifying and Co-Immobilized Nitrifying and Denitrifying Bacteria for Ammonia Removal of Sludge Digester Supertanant. *Water, Air, and Soil Pollution*. 195, 23-33.

Jen, A. C., Wake, M. C., Mikos, A. G. 1996. Review: hydrogels for cell immobilization. *Biotechnology and Bioengineering*. 50, 357-364.

Kim, K. J., Kim, S. K. and Kim, S. H. 2001. Characterization of immobilized denitrifying bacteria isolated from municipal sewage. *Journal of Microbiology and Biotechnology*. 11, 756-762.

Kok, F.N., Hasirci V. 2000 In: Wise, D.L., Trantolo, D.J., Cichon, E.J., Inyang, H.I., Stottmeieter U. (Eds.), Bioremediation of Contaminated Soils, Marcel Dekker, New York, 465-535.

Kourkoutas, Y., Bekatorou, A., Banat, I.M., Marchant, R., Koutinas, A.A. 2004. Immobilization technologies and support materials suitable in alcohol beverages production: a review. *Food Microbiology*, 21, 377-397.

Kraigher, B., Kosjek, T., Heath, E., Kompare, B., Mandic-Mulec, I. 2008. Influence of pharmaceutical residues on the structure of activated sludge bacterial communities in wastewater treatment bioreactors. *Water Research*, 42 (17) 4578-4588.

Leenen, E.J., Dos, S.V., Grolle, K.C., Tramper, J., Wijffels, R.H. 1996. Characteristics of and selection criteria for support materials for cell immobilization in wastewater treatment. *Water Research*, 30, 2985-2996.

Metcalf and Eddy. 1991. *Wastewater Engineering: Treatment, Disposal and Reuse.* 3rd ed. McGraw–Hill, Singapore.

Ochoa-Herrera, V., Banihani, Q., Leon, G., Khati, C., Field, J. A., Sierra-Alvarez, R. 2009. Toxicity of fluoride to microorganisms in biological wastewater treatment systems. *Water Research*. 43, 3177-3186.

Park, J.K. Chang, H.N. 2000. Microencapsulation of microbial cells. *Biotechnology Advances*, 18, 303-319.

Pramanik, S., Khan, E. 2008. Effects of cell entrapment on growth rate and metabolic activity of mixed cultures in biological wastewater treatment. *Enzyme and Microbial Technology*, 43, 245-251.

Siripattanakul, S. Khan, E. 2010. Fundamentals and Applications of Entrapped Cell Bioaugmentation for Contaminant Removal. In: Shah, V. *Emerging Environmental Technologies, Volume 2*. Springer, Germany, 147-169.

Siripattanakul, S., Pochant, C. J., and Khan, E. 2010. Nitrate removal from agricultural infiltrate by bioaugmented free and alginate entrapped cells. *Water Environment Research.* 82(7), 617-621.

Siripattanakul, S., Wirojanagud, W., McEvoy, J., M., Casey, F., X., M., Khan, E. 2009. Atrazine Removal in Agricultural Infiltrate by Bioaugmented Polyvinyl Alcohol Immobilized and Free *Agrobacterium radiobacter* J14a: A Sand Column Study. *Chemosphere*, 74, 308-313.

Siripattanakul, S., Wirojanagud, W., McEvoy, J., Khan E. 2008. Effect of cell-to-matrix ratio in polyvinyl alcohol immobilized pure and mixed cultures for atrazine degradation. *Water Air Soil Pollution: Focus*. 8, 257-266.

Smidsrod, O., Skjak-Braek, G. 1990. Alginate as immobilization matrix for cells. *Trends in Biotechnology*. 8, 71-78.

Takeno, K., Yamaoka, Y., Sasaki, K. 2005. Treatment of oil-containing sewage wastewater using immobilized photosynthetic bacteria. *World Journal of Microbiology and Biotechnology*, 21, 1385-1391.

Vancov, T., Jury, K., van Zwieten, L. 2005. Atrazine degrading *Rhodococcus erythropolis* NI86/21 cells encapsulated in alginate beads. *Journal of Applied Microbiology*, 99, 767-775.

van Veen, J.A., van Overbeek, L.S., van Elsas, J.D. 1997. Fate and activity of microorganism introduced into soil. *Microbiology and Molecular Biology Reviews*, 61, 121-135.

Verlicchi, P., Galletti, A., Petrovic, M., Barcelo, D. 2010. Hospital effluents as a source of emerging pollutants: An overview of micropollutants and sustainable treatment options. *Journal of Hydrology*. 389, 416–428.

Yang, C.F., Lee, C.M. 2008. Pentachlorophenol contaminated groundwater bioremediation using immobilized *Sphingomonas* cells inoculation in the bioreactor system. *Journal of Hazardous Materials*, 152, 159-165.

Yang, H., Wright, J.R. 1999. In: Kuhtreiber, W.M., Lanza, R.P., Chick, W.L. (Eds.), Cell Encapsulation Technology and Therapeutics, Birkhauser, Boston, 79-89.Humana Press, New Jersey, 61-66.

ภาคผนวก ก

วิธีการวิเคราะห์

ภาคผนวก ก1
วิธีวัดค่าซีโอดีละลายใช้วิธีการย่อยด้วย
สารละลายโปแตสเซียมไดโครเมต (รีฟลักซ์แบบปิด)

1. เครื่องมือและอุปกรณ์

1) หลอดย่อย (digestion vessel) ที่มีฝาสลักเกลียว
2) ขวดรูปชมพู่ ขนาด 125 มิลลิลิตร
3) ขวดปรับปริมาตร ขนาด 1,000 มิลลิลิตร
4) เตาอบ (oven) สามารถควบคุมอุณหภูมิให้อยู่ประมาณ 150 ± 2 องศาเซลเซียส
5) บิวเรต
6) ตาชั่งละเอียด

2. การเตรียมสารเคมี

1) <u>สารละลายมาตรฐานโปแตสเซียมไดโครเมต เข้มข้น 0.1 นอร์มัล</u> อบสารโปแตสเซียมไดโครเมต 4.913 กรัม ที่อุณหภูมิ 103 องศาเซลเซียส เป็นเวลา 2 ชั่วโมง จากนั้นละลายสารในน้ำกลั่น 500 มิลลิลิตร แล้วเติมกรดซัลฟิวริกเข้มข้น 167 มิลลิลิตร และปรอทซัลเฟต 33.3 กรัม คนให้ละลายและปล่อยทิ้งไว้ให้เย็น แล้วเจือจางด้วยน้ำกลั่นจนได้ปริมาตร 1000 มิลลิลิตร
2) <u>สารละลายกรดซัลฟิวริกเติมซิลเวอร์ซัลเฟต</u> ชั่งซิลเวอร์ซัลเฟต (Ag_2SO_4) 8.8 กรัม และเติมลงในกรดซัลฟิวริกเข้มข้น 1 ลิตร ตั้งทิ้งไว้ 1-2 วัน เพื่อให้ซิลเวอร์ซัลเฟตละลายได้ทั้งหมด ก่อนนำไปใช้ต่อไป
3) <u>สารละลายมาตรฐานเฟอรัสแอมโมเนียมซัลเฟต (Ferrous Ammonium Sulfate; FAS) 0.05 นอร์มัล</u> ละลายเฟอรัสแอมโมเนียมซัลเฟต ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) 19.6 กรัม ในน้ำกลั่น แล้วจึงเติมกรดซัลฟิวริกเข้มข้น 20 มิลลิลิตร จากนั้นปรับปริมาตรเป็น 1000 มิลลิลิตรด้วยน้ำกลั่น
4) <u>สารละลายเฟอโรอีนอินดิเคเตอร์</u> ละลายเฟอรัสซัลเฟต (Ferrous Sulfate , $FeSO_4 7H_2O$) 695 มิลลิกรัม และ 1,10 ฟีแนนโทรลีนโมโนไฮเดรต (1,10-Phenanthroline Monohydrate , $C_{12}H_8N_2 \cdot H_2O$) 1.485 กรัม ในน้ำกลั่นแล้วเจือจางเป็น 100 มิลลิลิตร

3. วิธีวิเคราะห์

1) <u>การเลือกหลอดแก้วและฝาปิดสำหรับต้มซีโอดี COD ให้เหมาะสม</u> ถ้าตัวอย่างน้ำมีค่าซีโอดีต่ำให้เลือกใช้หลอดขนาด 25×150 มิลลิเมตร (ปริมาตรน้ำตัวอย่าง 10 มิลลิลิตร) ถ้าค่าซีโอดีค่อนข้างสูงให้ใช้หลอดขนาด 20×150 มิลลิเมตร(ปริมาตรน้ำตัวอย่าง 5 มิลลิลิตร) และถ้าค่าซีโอดีสูง สามารถใช้หลอดแก้วขนาด 16×100 มิลลิเมตร (ปริมาตรน้ำตัวอย่าง 2 มิลลิลิตร) สำหรับกรณีค่าซีโอดีสูงมากควรเจือจากตัวอย่างน้ำก่อน

2) <u>การเลือกปริมาตรตัวอย่างน้ำ</u> ถ้าเป็นน้ำสะอาดที่มีค่าซีโอดีต่ำๆ (< 40 มิลลิกรัมต่อลิตร) ควรใช้ตัวอย่างน้ำ 10 มิลลิลิตร โดยใช้หลอดขนาด 25×150 มิลลิเมตร แต่ถ้าค่าซีโอดีค่อนข้างสูงควรใช้ปริมาตรตัวอย่างน้ำ 5 มิลลิลิตร หรือน้อยกว่าแล้วเติมน้ำกลั่นให้เป็น 5 มิลลิลิตร โดยใช้หลอดขนาด 20×150 มิลลิเมตร และถ้าตัวอย่างน้ำที่มีค่าซีโอดีสูงมากต้องเจือจางก่อนนำมาใช้ ควรประมาณค่าซีโอดีของตัวอย่างน้ำคร่าว ๆ ก่อนเพื่อเลือกปริมาณตัวอย่างได้อย่างเหมาะสม การเลือกขนาดตัวอย่างน้ำที่จะใช้วิเคราะห์ได้เหมาะสมอาจดูได้จากตารางที่ ก1 ในทางปฏิบัติควรเลือกใช้ปริมาณตัวอย่างน้ำให้ผลต่างของสารละลายเอฟเอเอส (ไตแตรนท์) ที่ใช้ในการไตเตรตแบลงค์และตัวอย่างน้ำอยู่ระหว่าง 1-5 มิลลิลิตร

ตารางที่ ก1 ขนาดตัวอย่างและอัตราเจือจางที่เหมาะสม

ช่วงซีโอดี	ขนาดตัวอย่าง (มิลลิลิตร)*	อัตราเจือจาง
< 200	5	1:1
200 - 400	4	1:1
400 - 800	2	1:1
800 - 1,600	1	1:1
1,600 - 3,200	5	1:10
2,700 - 5,300	3	1:10
4,000 - 8,000	4	1:20
8,000 - 16,000	2	1:20
13,000 - 26,500	3	1:50
20,000 - 40,000	2	1:50
40,000 - 80,000	2	1:100
80,000 - 160,000	1	1:100

* เมื่อใช้ FAS ความเข้มข้น 0.05 N และ $K_2Cr_2O_7$ 0.1 N

3) <u>การเติมน้ำตัวอย่าง</u> เติมน้ำตัวอย่างลงในหลอดแก้วขนาดเหมะสม เติมสารละลายโปรแตสเซียมไดโครเมต ตามด้วยกรดซัลฟิวริกอย่างช้า ๆ ในปริมาณที่แสดงดังตารางที่ ก2 (ถ้าใช้ปริมาณตัวอย่างน้ำน้อยกว่าที่แสดงไว้ในตารางให้เติมน้ำกลั่นให้ครบตามจำนวน) ปิดฝาให้แน่นแล้วเขย่าผสมกันให้ดี สำหรับแบลงค์ใช้น้ำกลั่นแทนน้ำตัวอย่าง วางหลอดแก้วในตู้อบ ตั้งอุณหภูมิไว้ที่ 150 ± 2 องศาเซลเซียส เป็นเวลา 2 ชั่วโมง เมื่อครบเวลาแล้วนำออกจากตู้อบปล่อยทิ้งให้เย็น

ตารางที่ ก2 ขนาดของหลอดแก้ว ปริมาตรตัวอย่างน้ำและสารเคมีที่เหมาะสม

ขนาดหลอดแก้ว (มิลลิเมตร)	ปริมาตรน้ำตัวอย่าง (มิลลิลิตร)	สารละลายไดโครเมต (มิลลิลิตร)	สารละลายกรดซัลฟิวริก (มิลลิลิตร)	ปริมาตรทั้งหมด (มิลลิลิตร)
16×100	2.5	1.5	3.5	7.5
20×150	5.0	3.0	7.0	15.0
25×150	10.0	6.0	14.0	30.0

4) <u>การไตเตรชัน</u> เทสารละลายออกจากหลอดแก้วลงในขวดรูปชมพู่ ใช้น้ำกลั่นฉีดล้างสารละลายในหลอดแก้วให้หมด แล้วเทรวมลงในขวดรูปชมพู่ เติมเฟอโรอีนอินดิเคเตอร์ 2-3 หยด แล้วไตเตรตด้วยสารละลายมาตรฐานเอฟเอเอส สีของสารละลายค่อย ๆ เปลี่ยนจากเหลือง $\rightarrow$ เขียวอมเหลือง $\rightarrow$ ฟ้า $\rightarrow$ น้ำตาลแดง ซึ่งแสดงว่าถึงจุดยุติ จดปริมาณเอฟเอเอสที่ใช้ไตเตรต

4. การคำนวณ

$$\text{ซีโอดี, mg } O_2\text{/L} = \frac{(A-B) \times N \times 8000}{\text{มิลลิลิตรของตัวอย่างน้ำ}}$$

เมื่อ A = มิลลิลิตรของเอฟเอเอสที่ใช้ในการไตเตรตแบลงค์

B = มิลลิลิตรของเอฟเอเอสที่ใช้ในการไตเตรตตัวอย่างน้ำ

N = ความเข้มข้นของเอฟเอเอส (นอร์มัล)

ภาคผนวก ก2

วิธีวัดค่าของแข็งแขวนลอย

1. อุปกรณ์และสารเคมี

1) กระดาษกรองใยแก้ว GF/C เส้นผ่าศูนย์กลาง 4.7 เซ็นติเมตร
2) ปิเปต ขนาด 25 มิลลิลิตร
3) อุปกรณ์ชุดกรอง
4) เครื่องดูดอากาศ
5) เตาอบที่สามารถควบคุมอุณหภูมิได้ 103-105 องศาเซลเซียส
6) โถทำแห้ง (desiccator) พร้อมสารดูดความชื้น
7) เครื่องชั่งอย่างละเอียด 4 ตำแหน่ง
8) กระดาษอะลูมิเนียม เพื่อทำเป็นภาชนะสำหรับใส่กระดาษกรอง
9) กระบอกตวง
10) คมหนีบ
11) น้ำตัวอย่าง
12) น้ำดีไอ

2. วิธีการ

1) <u>การเตรียมกระดาษกรอง</u> นำกระดาษกรองพรมน้ำดีไอปริมาณ 10 มิลลิลิตร ไปใส่ในถ้วยอะลูมิเนียมที่ทำสัญลักษณ์ไว้ จากนั้นอบถ้วยอะลูมิเนียมพร้อมกระดาษกรองที่อุณหภูมิ 103-105 องศาเซลเซียส เป็นเวลา 1 ชั่วโมง ทิ้งให้เย็นในโถทำแห้ง แล้วชั่งน้ำหนักอะลูมิเนียมพร้อมกระดาษกรอง
2) <u>การวิเคราะห์</u> เตรียมน้ำตัวอย่างสำหรับนำไปกรอง โดยถ้าค่าของแข็งแขนลอยโดยประมาณ 2.5-200 มิลลิกรัม ใช้ปริมาตรน้ำตัวอย่าง 20 มิลลิลิตร ในกรณีที่เก็บตัวอย่างแช่เย็นไว้ให้ทำให้ตัวอย่างมีอุณหภูมิห้องก่อน จากนั้นใช้คีมหนีบคีบกระดาษกรองที่ชั่งแล้วมาวางลงบนกรวยในชุดกรอง ซึ่งต่อเข้ากันกับเครื่องดูดอากาศโดยให้ด้านขรุขระของกระดาษอยู่ด้านบน เขย่าตัวอย่างให้เข้ากันดี แล้วใช้ปิเปตดูดตัวอย่างจากขวดเก็บตัวอย่างใส่ชุดกรอง เปิดเครื่องดูดอากาศ แล้วใช้น้ำดีไอปริมาณ 10 มิลลิลิตร ฉีดล้างของแข็งที่อาจติดอยู่ในปิเปต และชุดกรองจนหมด รอจนกว่ากระดาษกรองแห้ง ปิดเครื่องดูดอากาศ ใช้คีมหนีบกระดากรองใส่ถ้วยอะลูมิเนียม แล้วนำไปอบในตู้อบที่อุณหภูมิ 103-105 องศา

เซลเซียส เป็นเวลา 1 ชั่วโมง ทิ้งให้เย็นในโถทำแห้ง แล้วชั่งหาน้ำหนักอะลูมิเนียมพร้อมกระดาษกรองอีกครั้ง

3. การคำนวณ

$$\textbf{ของแข็งแขวนลอย (mg/l)} = \frac{(B-A)*10^6}{\text{ปริมาณของน้ำตัวอย่างที่ใช้,มิลลิลิตร}}$$

เมื่อ A คือ น้ำหนักถ้วยอะลูมิเนียมพร้อมกระดาษกรองก่อนการกรอง (มิลลิกรัม)

B คือ น้ำหนักถ้วยอะลูมิเนียมพร้อมกระดาษกรองหลังการกรอง (มิลลิกรัม)

ภาคผนวก ข

ข้อมูลการทดลอง

ภาคผนวก ข1

ผลการศึกษาผลของอิทธิพลของชนิดสารฆ่าเชื้อต่อการบำบัดน้ำเสีย

ตารางที่ ข1 ค่าซีโอดีละลายคงเหลือในชุดทดลอง TYPE-GA

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	208	368	288	100.00
1	162	342	252	87.50
2	128	336	232	80.56
3	32	216	124	43.06
4	16	192	104	36.11
5	16	208	112	38.89
6	8	192	100	34.72
7	8	184	96	33.33
8	24	184	104	36.11

ตารางที่ ข2 ค่าซีโอดีละลายคงเหลือในชุดทดลอง TYPE-PI

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	442	520	481	100.00
1	421	480	450.5	93.66
2	360	464	412	85.65
3	312	392	352	73.18
4	256	360	308	64.03
5	256	368	312	64.86
6	240	362	301	62.58
7	208	312	260	54.05
8	208	265	236.5	49.17

ตารางที่ ข3 ค่าซีโอดีละลายคงเหลือในชุดทดลอง TYPE-EB

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	200	480	340	100.00
1	152	368	260	76.47
2	56	368	212	62.35
3	48	312	180	52.94
4	48	304	176	51.76
5	40	232	136	40.00
6	40	176	108	31.76
7	56	168	112	32.94
8	24	168	96	28.24

ตารางที่ ข4 ค่าซีโอดีละลายคงเหลือในชุดทดลอง TYPE-ND

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	216	376	296	100.00
1	152	328	240	81.08
2	120	232	176	59.46
3	64	200	132	44.59
4	32	192	112	37.84
5	16	184	100	33.78
6	8	152	80	27.03
7	8	168	88	29.73
8	8	168	88	29.73

ภาคผนวก ข2

ผลการศึกษาผลของอิทธิพลของความเข้มข้นสารฆ่าเชื้อต่อการบำบัดน้ำเสีย

ตารางที่ ข5 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CONC-0.1

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลอง (มก./ล.)	ร้อยละค่าซีโอดีละลายคงเหลือ
0	481	100.00
1	450.5	93.66
2	412	85.65
3	352	73.18
4	308	64.03
5	312	64.86
6	301	62.58
7	260	54.05
8	236.5	49.17

ตารางที่ ข6 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CONC-0.2

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลอง (มก./ล.)	ร้อยละค่าซีโอดีละลายคงเหลือ
0	432	100.00
1	400	92.59
2	360	83.33
3	384	88.89
4	384	88.89
5	368	85.19
6	352	81.48
7	352	81.48
8	312	72.22

ตารางที่ ข7 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CONC-0.3

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลอง (มก./ล.)	ร้อยละค่าซีโอดีละลายคงเหลือ
0	392	100.00
1	360	91.84
2	360	91.84
3	336	85.71
4	344	87.76
5	320	81.63
6	312	79.59
7	288	73.47
8	304	77.55

ตารางที่ ข8 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CONC-0.0

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	216	128	172	100.00
1	152	120	136	79.07
2	120	96	108	62.79
3	64	72	68	39.53
4	32	40	36	20.93
5	16	32	24	13.95
6	11	16	14	7.85
7	14	16	15	8.72
8	12	9	11	6.10

ภาคผนวก ข3

ผลการศึกษาการหาสภาวะการเตรียมเซลล์ดักติดที่เหมาะสม

ตารางที่ ข9 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CM-1:05

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	613	226	419.5	100.00
1	520	213	366.5	87.37
2	440	213	326.5	77.83
3	400	146	273	65.08
4	373	120	246.5	58.76
5	360	93	226.5	53.99
6	293	66	179.5	42.79
7	280	93	186.5	44.46
8	266	87	176.5	42.07

ตารางที่ ข10 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CM-1:10

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	520	333	426.5	100.00
1	240	253	246.5	57.80
2	186	173	179.5	42.09
3	146	173	159.5	37.40
4	93	173	133	31.18
5	106	173	139.5	32.71
6	80	80	80	18.76
7	80	106	93	21.81
8	80	40	60	14.07

ตารางที่ ข11 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CM-1:20

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	440	466	453	100.00
1	106	373	239.5	52.87
2	173	266	219.5	48.45
3	146	186	166	36.64
4	106	226	166	36.64
5	200	133	166.5	36.75
6	213	146	179.5	39.62
7	106	186	146	32.23
8	106	200	153	33.77

ตารางที่ ข12 ค่าซีโอดีละลายคงเหลือในชุดทดลอง FC-1:00

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	442	520	481	100.00
1	421	480	450.5	93.66
2	360	464	412	85.65
3	312	392	352	73.18
4	256	360	308	64.03
5	256	368	312	64.86
6	240	362	301	62.58
7	208	312	260	54.05
8	208	265	236.5	49.17

ตารางที่ ข13 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CA-1:05

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	666	306	486	100.00
1	426	266	346	71.19
2	444	320	382	78.60
3	453	306	379.5	78.09
4	386	293	339.5	69.86
5	426	240	333	68.52
6	413	306	359.5	73.97
7	440	333	386.5	79.53
8	453	266	359.5	73.97

ตารางที่ ข14 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CA-1:10

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	316	296	306	100.00
1	240	240	240	78.43
2	220	232	226	73.86
3	245	235	240	78.43
4	306	306	306	100.00
5	249	237	242	78.76
6	226	226	226	73.86
7	236	224	230	75.16
8	222	234	226	73.86

ตารางที่ ข15 ค่าซีโอดีละลายคงเหลือในชุดทดลอง CA-1:20

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	546	320	433	100.00
1	453	280	366.5	84.64
2	453	240	346.5	80.02
3	440	200	320	73.90
4	440	146	293	67.67
5	440	146	293	67.67
6	440	160	300	69.28
7	440	93	266.5	61.55
8	453	186	319.5	73.79

ตารางที่ ข16 ค่าซีโอดีละลายคงเหลือในชุดทดลอง NC-0:00

เวลา (ชั่วโมง)	ค่าซีโอดีละลายคงเหลือในการทดลองครั้งที่ (มก./ล.)			ร้อยละค่าซีโอดีละลายคงเหลือ
	1	2	เฉลี่ย	
0	386	413	399.5	100.00
1	373	440	406.5	101.75
2	533	440	486.5	121.78
3	493	440	466.5	116.77
4	373	440	406.5	101.75
5	400	453	426.5	106.76
6	373	440	406.5	101.75
7	373	426	399.5	100.00
8	346	400	373	93.37

ภาคผนวก ข4

ผลการศึกษาการหาปริมาณเซลล์ดักติดที่เหมาะสม

ตารางที่ ข17 ค่าซีโอดีละลายคงเหลือในชุดทดลอง EC-1,000

วัฏจักรที่	ค่าซีโอดีละลายในการทดลองครั้งที่ (มก./ล.)						ร้อยละค่าซีโอดีละลายที่กำจัดได้เฉลี่ย
	1			2			
	เข้า	ออก	ร้อยละกำจัด	เข้า	ออก	ร้อยละกำจัด	
1	160	112	30.00	240	120	50.00	40.00
2	160	80	50.00	200	120	40.00	45.00
3	240	80	66.67	120	40	66.67	66.67
4	136	84	38.24	136	96	29.41	33.82
5	152	103	32.24	160	103	35.63	33.93
6	152	84	44.74	160	97	39.38	42.06
7	120	80	33.33	160	96	40.00	36.67
8	80	56	30.00	160	104	35.00	32.50
9	160	80	50.00	112	48	57.14	53.57
10	160	92	42.50	160	60	62.50	52.50

ตารางที่ ข18 ค่าซีโอดีละลายคงเหลือในชุดทดลอง EC-2,000

วัฏจักรที่	ค่าซีโอดีละลายในการทดลองครั้งที่ (มก./ล.)						ร้อยละค่าซีโอดีละลายที่กำจัดได้เฉลี่ย
	1			2			
	เข้า	ออก	ร้อยละกำจัด	เข้า	ออก	ร้อยละกำจัด	
1	320	80	75.00	272	120	55.88	65.44
2	280	112	60.00	296	156	47.30	53.65
3	144	56	61.11	144	80	44.44	52.78
4	256	88	65.63	160	72	55.00	60.31
5	192	40	79.17	208	80	61.54	70.35
6	176	64	63.64	184	72	60.87	62.25
7	216	80	62.96	232	88	62.07	62.52
8	200	88	56.00	216	110	49.07	52.54
9	240	96	60.00	320	80	75.00	67.50
10	312	88	71.79	304	64	78.95	75.37

ตารางที่ ข19 ค่าซีโอดีละลายคงเหลือในชุดทดลอง EC-3,000

วัฏจักรที่	ค่าซีโอดีละลายในการทดลองครั้งที่ (มก./ล.)						ร้อยละค่าซีโอดีละลายที่กำจัดได้เฉลี่ย
	1			2			
	เข้า	ออก	ร้อยละกำจัด	เข้า	ออก	ร้อยละกำจัด	
1	320	218	31.88	352	232	34.09	32.98
2	192	152	20.83	152	84	44.74	32.79
3	224	144	35.71	228	106	53.51	44.61
4	200	120	40.00	184	112	39.13	39.57
5	264	120	54.55	184	104	43.48	49.01
6	192	108	43.75	176	60	65.91	54.83
7	192	112	41.67	120	62	48.33	45.00
8	200	88	56.00	192	60	68.75	62.38
9	280	130	53.57	200	126	37.00	45.29
10	256	96	62.50	248	96	61.29	61.90

ตารางที่ ข20 ค่าซีโอดีละลายคงเหลือในชุดทดลอง FC-1,000

วัฏจักรที่	ค่าซีโอดีละลายในการทดลองครั้งที่ (มก./ล.)						ร้อยละค่าซีโอดีละลายที่กำจัดได้เฉลี่ย
	1			2			
	เข้า	ออก	ร้อยละกำจัด	เข้า	ออก	ร้อยละกำจัด	
1	130	72	44.62	135	100	25.93	35.27
2	152	80	47.37	200	96	52.00	49.68
3	130	80	38.46	200	120	40.00	39.23
4	160	120	25.00	120	80	33.33	29.17
5	144	120	16.67	166	120	27.71	22.19
6	120	80	33.33	160	120	25.00	29.17
7	136	96	29.41	200	120	40.00	34.71
8	104	56	46.15	120	96	20.00	33.08
9	200	176	12.00	136	88	35.29	23.65
10	136	104	23.53	104	98	5.77	14.65

ตารางที่ ข21 ค่าซีโอดีละลายคงเหลือในชุดทดลอง FC-2,000

วัฏจักรที่	ค่าซีโอดีละลายในการทดลองครั้งที่ (มก./ล.)						ร้อยละค่าซีโอดีละลายที่กำจัดได้เฉลี่ย
	1			2			
	เข้า	ออก	ร้อยละกำจัด	เข้า	ออก	ร้อยละกำจัด	
1	240	126	47.50	272	124	54.41	50.96
2	280	114	59.29	280	122	56.43	57.86
3	168	80	52.38	192	88	54.17	53.27
4	144	80	44.44	120	64	46.67	45.56
5	144	120	16.67	120	88	26.67	21.67
6	208	120	42.31	200	128	36.00	39.15
7	200	112	44.00	184	120	34.78	39.39
8	232	208	10.34	232	156	32.76	21.55
9	224	178	20.54	240	166	30.83	25.68
10	232	184	20.69	240	208	13.33	17.01

ตารางที่ ข22 ค่าซีโอดีละลายคงเหลือในชุดทดลอง FC-3,000

วัฏจักรที่	ค่าซีโอดีละลายในการทดลองครั้งที่ (มก./ล.)						ร้อยละค่าซีโอดีละลายที่กำจัดได้เฉลี่ย
	1			2			
	เข้า	ออก	ร้อยละกำจัด	เข้า	ออก	ร้อยละกำจัด	
1	280	184	34.29	345	187	45.80	40.04
2	144	90	37.50	168	70	58.33	47.92
3	190	89	53.16	120	80	33.33	43.25
4	208	88	57.69	194	56	71.13	64.41
5	88	67	23.86	124	64	48.39	36.13
6	96	56	41.67	80	56	30.00	35.83
7	136	91	33.09	152	87	42.76	37.93
8	160	88	45.00	184	80	56.52	50.76
9	192	104	45.83	160	88	45.00	45.42
10	192	120	37.50	168	92	45.24	41.37

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ผลงานตีพิมพ์ภายใต้ทุนวิจัย

Optimum Alginate Cell Entrapment for Treating Hospital Wastewater in the Presence of Povidone Iodine Disinfectant

(Oral Presentation)

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(Initial page layout)

Optimum Alginate Cell Entrapment for Treating Hospital Wastewater in the Presence of Povidone Iodine Disinfectant

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Abstract
This study aims to investigate hospital wastewater treatment in presence of povidone iodine disinfectant using cell entrapment technique. Synthetic wastewater with initial chemical oxygen demand (COD) of approximately 370 mg/L with povidone iodine of 0.1% and acclimated activated sludge were used. Calcium alginate cell entrapment was selected for the study. To determine the optimum cell entrapment condition, the activated sludge was entrapped with different cell-to-matrix ratios of 1:5, 1:10, and 1:20 (volume:volume). The analogous tests with the free cells were performed for comparative purpose. The result indicated that the entrapped cells obviously performed better than the free cells. The cell-to-matrix ratio of 1:20 gave the highest treatment efficiency of 86%. The entrapped cell system at the optimum entrapment condition could reduce the effect of disinfectant (inhibition of 9%) while the free cell system acquired the inhibition of 47%. The result also showed the system at the entrapped cell loading of 2,000 mg/L performed the highest COD removal of 62%. In addition, the result indicated that the entrapped cell system had higher treatment performance than the free cell system.

Keywords
Calcium alginate; cell entrapment; disinfectant; inhibition; hospital wastewater treatment

INTRODUCTION

Hospital is one of service facilities having its own wastewater treatment system which generally is activated sludge system. It is commonly found that the treated effluent from the systems do not meet hospital wastewater standard for organic (Biochemical Oxygen Demand or Chemical Oxygen Demand (COD)) removal. One of potential major sources could be biocides including antibiotics and disinfectants contaminating in the wastewater (Chitnisa et al., 2004; Rezaee et al., 2005). It is known that a large amount and various types of disinfectants are used in hospital, such as halogenated, aldehyde, and phenolic compounds. Povidone iodine (PI) is one of common used disinfectants for wound treatment. The disinfectant did not only kill germ for medical purpose but also can cause failure in wastewater treatment system. However, to the best of our knowledge, there is no study on improvement technology to solve the problem.

Entrapped cell system is a potential alternative for answering this problematic issue. The microorganism entrapped in porous polymeric material is known as one of effective techniques for environmental applications (Hill and Khan, 2008; Siripattanakul et al., 2008; Siripattanakul and Khan, 2010). The technique was proved that it can be applied to alleviate the limitation associated with the traditional (free) cell wastewater treatment. The system provides high cell loading and toxic protection which result in better wastewater treatment efficiency. Numerous successful previous applications for wastewater treatment included carbon and nitrogen treatments as well as toxic substance treatments, such as toxic dyes and pesticides (Hill and Khan, 2008; Pramanik and Khan, 2008; Siripattanakul et al., 2008; Siripattanakul and Khan, 2010). In this context, the technique sounds applicable to the problem. Moreover, the system does not require the sedimentation process attributing to easy and flexible for operation. In this case, the entrapped cells can be used in the existing facility for practical.

Based on above information, the aim of this study is to examine performance of povidone iodine-contaminated wastewater treatment using cell entrapment technique. Optimum entrapped cell preparation and entrapped cell loading for hospital wastewater treatment were determined. Calcium alginate (CA), a widely used cell entrapment matrix, was selected for this study.

METHODS

Synthetic hospital wastewater

Synthetic hospital wastewater was prepared followed wastewater characteristics from a model district hospital in Warinchamrap, Ubonratchathani, Thailand. The wastewater synthesized from $C_{12}H_{22}O_{11}$, $CO(NH_2)_2$, and $Ca(H_2PO_4).H_2O$ at COD:N:P of 100:5:1. The COD and pH values were approximately 370 mg/L and 6.5 to 7.0, respectively. Commercial PI of 0.1% was added in the synthetic wastewater.

Activated sludge cultivation and acclimatization

Municipal activated sludge was used in this study to avoid residue of disinfectant in hospital activated sludge. The activated sludge was cultivated and acclimated in a 30-L reactor for 2 months before application. 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 (DO) of higher than 1 mg/L was continuously supplied.

Free and entrapped activated sludge preparation

The activated sludge from the 30-L reactor of 1,000 mL was centrifuged at 7,000 rpm for 10 min to obtain concentrated cells. The concentrated cells were vigorously resuspended in sterile de-ionized water (DI) of 10 mL. The resuspended cells were used as the free activated sludge (details described in the next section) and for entrapped cell preparation.

The activated sludge was entrapped in CA according to a technique adapted from Smidsrod and Skjak-Braek (1990). The technique was chosen because of several successful prior applications (Gentry et al., 2004; Hill and Khan, 2008; Siripattanakul and Khan, 2010). Sodium alginate (Fluka, Singapore) was dissolved into sterile DI at 2% (w/v). The resuspended activated sludge from earlier section was uniformly mixed with a sodium alginate solution. The mixture contents were described in the next section. The mixture was manually dropped into a calcium chloride solution of 3.5% (w/v) using a sterile syringe (bead size of 3-5 mm). The droplets remained in the solution for 2 hr to form and harden spherical beads.

Optimization of cell entrapment preparation

For typical entrapped cells, the effect of the cell-to-matrix (CA) ratios on substrate diffusivity and contaminant removal ability was one of major concerns previously reported in several studies (Kim et al., 2001; Siripattanakul et al., 2008). The aim of this part was to investigate the optimum cell-to-matrix ratio for treating wastewater contaminated with disinfectant. In the present experiment, 8 reactors containing different content of the cells and the matrix were prepared to test the effect of cell-to-matrix ratio (Table 1).

Duplicate 8-hr batch experiment was performed. The synthetic wastewater of 250 mL with the selected disinfectant (PI) at the selected concentration (0.1%) was filled in a reactor inoculated with the content shown in Table 1. All reactors were shaken at 150 rpm and 30°C. Dissolved oxygen concentration of higher than 1 mg/L was continuously supplied. During the 8-hr experiment, wastewater samples of 10 mL were taken every hour for measuring soluble COD. Wastewater

treatment reaction kinetics and wastewater treatment efficiency were determined. Inhibition of wastewater treatment was then calculated as shown in equation 1 followed Ochoa-Herrera et al. (2009). The CA matrices were taken for observing microstructure using scanning electron microscopic (SEM) technique for insight information.

$$Inhibition\ (\%) = \frac{Average\ treatment\ activity\ of\ the\ reactor}{Average\ treatment\ activity\ of\ the\ control} \times 100 \qquad equation\ 1$$

Table 1. Descriptions of components in the optimum cell-to-matrix ratio investigation

Reactor	Cell type	Cell-to-matrix ratio (mL of cells: mL of calcium alginate)		
		Volume of cells (or DI) (mL)	Volume of CA (mL)	Total inoculated volume (mL)
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

[(1)] Concentrated activated sludge at the final concentration in reactor of 1,000 mg SS/L
[(2)] Sterile DI

Optimization of cell loadings

It is known that microbial cell loading is one of the most important factors for contaminant removal by either free or entrapped cells (Siripattanakul et al., 2009). This part intended to find the optimum entrapped cell loading compared to that by the free cells. Also, the long-term performance of the system was investigated.

Duplicate experiment was conducted in SBR mode. Six reactors of 150 mL including the entrapped cell reactors (EC-1,000, EC-2,000, and EC-3,000) and the free cell reactors (FC-1,000, FC-2,000, and FC-3,000) were tested for the cell loadings of 1,000, 2,000, and 3,000 mg SS/L. Note that optimum cell preparation (cell-to-matrix ratio) investigated in earlier section was applied. The reactors were consecutively run for 10 cycles. Each cycle included five periods followed traditional SBR cycle: 1) fill of 0.25 hr, 2) react of 6 hr, 3) settle of 2 hr, 4) draw of 0.25 hr, and 5) idle of 0.50 hr followed time period in the model hospital wastewater treatment system. Influent and effluent from each cycle were taken for COD measurement. Wastewater treatment performance was determined.

Analytical procedures

COD, SS, and pH were measured according to standard methods (APHA et al, 1998). After filtering water sample using GF/C filter glass paper, soluble COD was measured by potassium dichromate digestion method. The filtrate was used for measuring SS whereas pH was measured by using a pH meter (inoLab pH level 1, WTW GmBH, Weilheim, Germany).

For SEM observation, the method described earlier (Siripattanakul et al, 2010) was applied. 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 hr. The samples were rinsed in 0.1 M $CaCl_2$ for 15 min twice. The beads were then dehydrated in solutions (30 min each) containing 30% ethanol and 0.07

M $CaCl_2$, 50% ethanol and 0.05 M $CaCl_2$, 70% ethanol and 0.03 M $CaCl_2$, 90% ethanol and water, and 100% ethanol, respectively. 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, attached to aluminum mounts by silver paint, coated with gold using a Balzers SCD 030 sputter coater, and examined using a JEOL JSM-6300 scanning electron microscope.

RESULTS AND DISCUSSION

Optimization of cell entrapment preparation

Figure 1 presents normalized COD remaining in the synthetic wastewater during the test for 8 hr. In Figure 1a, the results from the control test (NC-0:00) and the tests with only CA at different entrapment preparation conditions (designated CA-0:05, CA-0:10, and CA-0:20) are shown. These reactors were performed to determine effect of matrix adsorption. The COD remaining in the control test was quite stable for entire of the experiment while the results from the CA-0:05, CA-0:10, and CA-0:20 reactors were similar. The COD value rapidly decreased within the first hour for 11 to 25% and remained stable thereafter. At the end of the experiment (8 hr), COD remaining of 95, 79, 75, and 73% from the NC-0:00, CA-0:05, CA-0:10, and CA-0:20 reactors, respectively were observed. This clearly indicated that COD was just slightly adsorbed by CA entrapment matrices for all entrapment conditions. This was similar to a previous study which reported insignificant adsorption of atrazine pesticide by entrapment matrices (Siripattanakul et al., 2008).

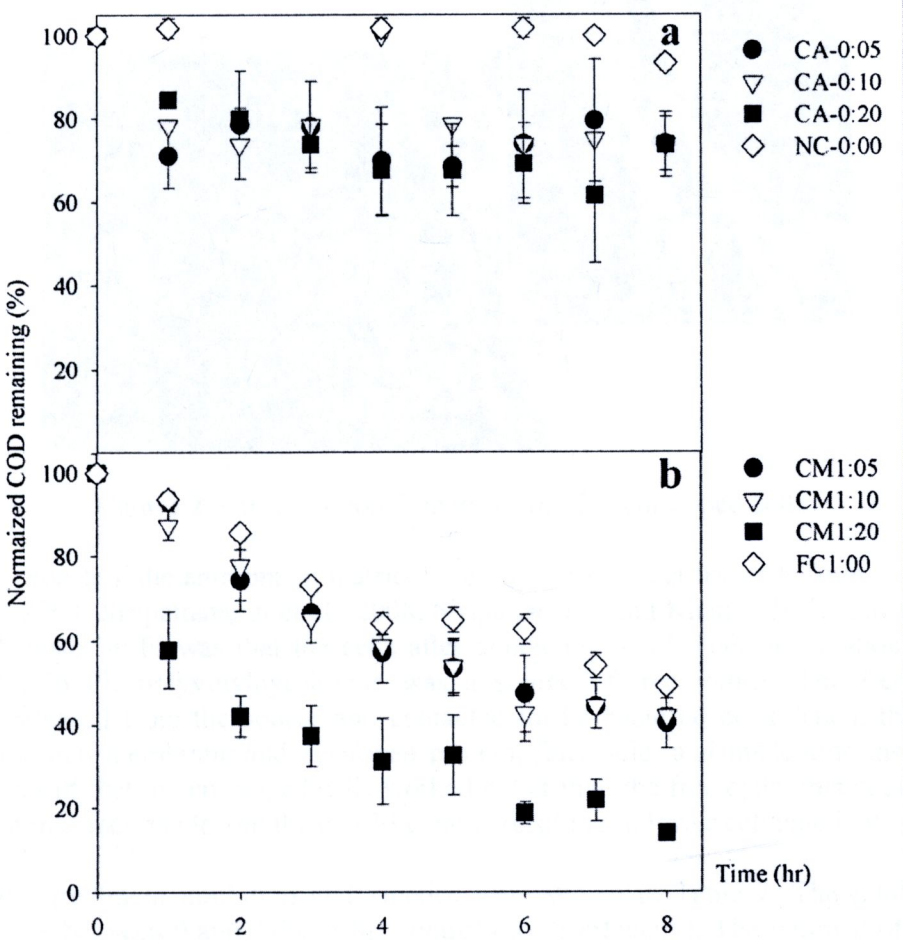

Figure 1. Normalized COD remaining in the optimization of cell entrapment preparation test

In Figure 1b, the results from the free cell test (FC-1:00) and the entrapped cell tests at different entrapment preparation conditions (designated CM-1:05, CM-1:10, and CM-1:20) are shown. The results from all reactors were similar. The COD value quickly decreased within the first 6 hr and gradually reduced thereafter. At the end of the experiment (8 hr), COD remaining of 50, 43, 42, and 14% from the FC-1:00, CM-1:05, CM-1:10, and CM-1:20 reactors, respectively were observed. This noticeably proved that entrapped cells performed better than the free cells. As expected, the cell entrapment condition played a role in enhancement of disinfectant-contaminated wastewater treatment performance. This was similar to prior studies (Kim et al., 2001; Siripattanakul et al., 2008). The studies reported that different cell-to-matrix ratios resulted in different cell densities inside the matrices. At the same cell mass (1,000 mg/L), the matrices in the CM-1:05 reactor were lower than those in the CM-1:10 reactor resulting in higher cell density in the CM-1:05 reactor.

Based on the result shown in Figure 1b, in this study, it is obvious that the lowest cell density (CM-1:20) provided in the best wastewater treatment efficiency. This could be because the entrapped cells at the low cell have enough space to grow and low substrate diffusion limitation. Figure 2 presents a cross-sectional image of the entrapped cells in micro-structural 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 really dense resulted in the calcium alginate sheets with a number of cells fixed inside the beads (Figure 2). The sheets were combined and caused numerous of voids attributing to high substrate and oxygen diffusion.

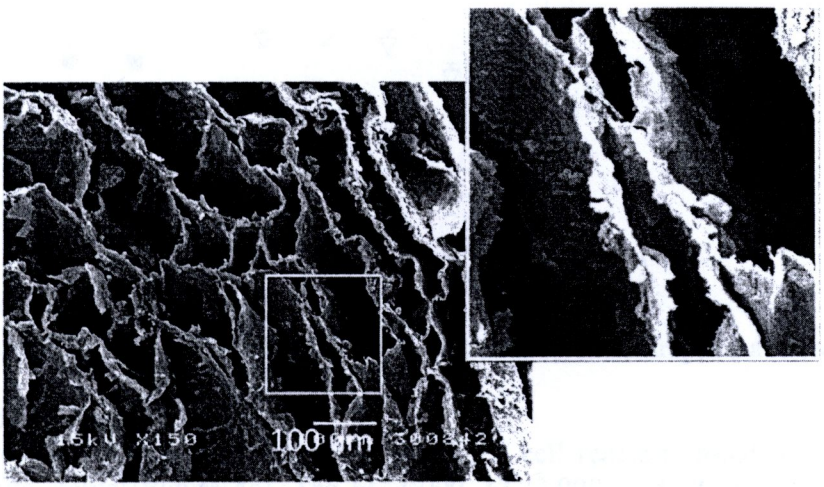

Figure 2. Cross-sectional image of the CA entrapped cells

Moreover, as expected, the entrapment matrices were able to protect the cells from toxic substance (Gentry et al., 2004; Siripattanakul et al., 2008; Siripattanakul and Khan, 2010). Generally, the cell damage mechanism by PI was that the cells after contacting to PI were inactivation (Durani and Leaper, 2008). In PI, polyvinylpyrolidone was a source of free iodine. The free iodine was continuously released from the source and contacted to the bacterial cells. Then, the free iodine diffused though cell membrane and destroyed protein, fatty acid, and nucleotide inside the cells. Based on the result that the entrapped cells worked better than the free cells; this could be because the entrapment matrices can lessen the cell-PI contact resulting in lower cell inactivation.

The wastewater treatment inhibition and kinetics were shown in Table 2. 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 to

0.24 hr^{-1}. This remarkably signified that the entrapped cells at the optimum entrapment condition performed much better than the free cell. The treatment rate by the entrapped cells at the optimum condition was about the rate by the traditional wastewater treatment without the disinfectant. The treatment trend was similar to the control as shown in Figure 3. This obviously proved that the CA entrapped cells were really potential for treating hospital wastewater. The entrapped cells performed well and sounded applicable for the typical on-site hospital wastewater treatment system.

Table 2. Treatment kinetics and inhibition

Reactor name	COD removal (%)	Inhibition (% of control)	Wastewater treatment kinetics		
			Equation [1]	R^2	Rate constant (hr^{-1})
CM-1:05	57	39.36	Y = -0.11X+4.56	0.96	0.11
CM-1:10	58	38.30	Y = -0.21X+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	Control	Y = -0.25X+4.64	0.98	0.25

[1] Y = ln COD and X = time

[2] Control is the traditional (free cell) wastewater treatment system without the disinfectant.

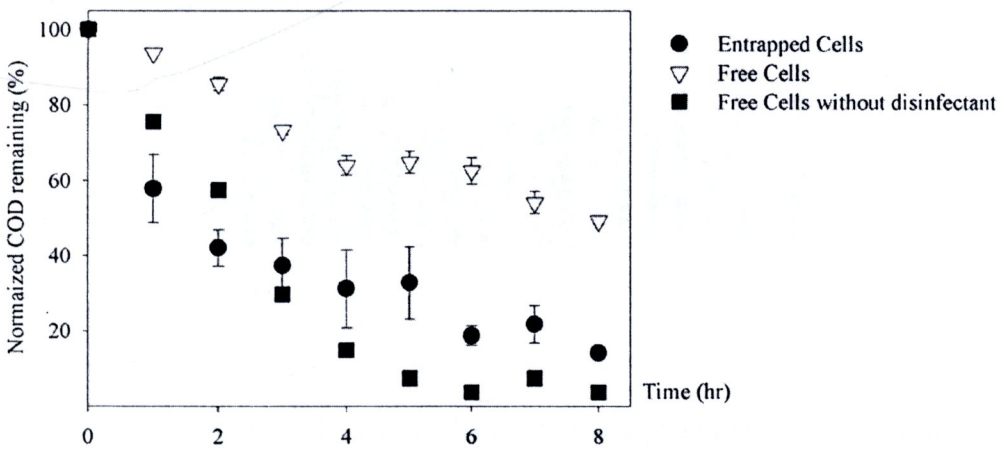

Figure 3. Comparison of the wastewater treatment by the entrapped and free cells

Optimization of cell loadings

The results were shown in Figure 4. For the entrapped cell reactors, trend of normalized COD removal was quite stable (Figure 4a). The EC-1,000, EC-2,000, and EC-3,000 reactors removed COD of 44, 62, and 47%, respectively. For the free cell reactors, trend of normalized COD removal concurrently decreased (Figure 4b). The FC-1,000, FC-2,000, and FC-3,000 reactors removed COD of 31, 38, and 44%, respectively.

Based on the trends, the entrapped cells performed more stable compared to the free cells. This should be because the entrapment matrices provided better environment for the cells (Siripattanakul et al., 2008). Besides protecting the cells for the toxic substance, the entrapped cell system had better cell separation in the settling period. This is apparent; the entrapped cells were much heavier than the free cells and were settled more than the free cells. Therefore, the entrapped cell system had less cell loss resulting in better performance.

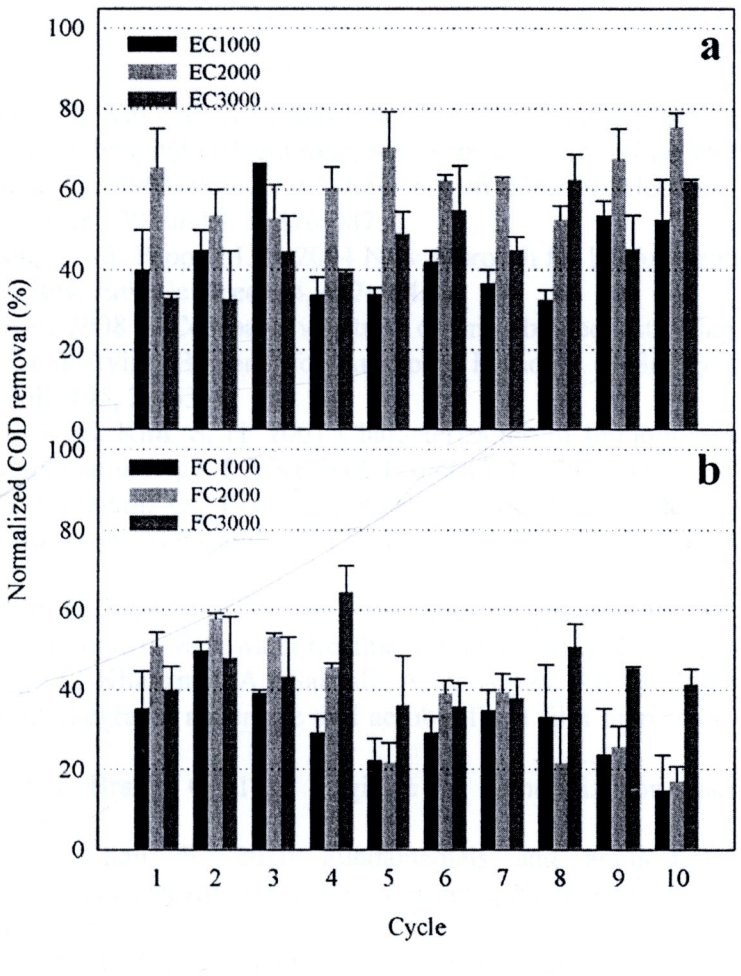

Figure 4. Normalized COD removal by the entrapped and free cells in SBR mode

CONCLUSIONS

It has been known that hospital wastewater treatment systems are not successfully operated. This could be from numerous chemicals used in hospitals including drugs, disinfectants, and laboratory chemicals. Povidone-iodine substantially inhibited the wastewater treatment efficiency. The entrapped cell system can apparently alleviate the problem. At the optimum cell entrapment condition (1:20), the entrapped cell system gave the treatment efficiency of 86% (only inhibition of 9%). In this case, the entrapped cells had much superior wastewater treatment. The continued work on disinfectant-tolerated microbial community was recommended for insight information.

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REFERENCES

Chitnisa, V., Chitnisa, S., Vaidyaa, K., Ravikanta, S., Patilb., S., and Chitnisa, D. S. 2004 Bacterial population changes in hospital effluent treatment plant in central India. Water Res.38, 441-447.

Durani, P. & Leaper, D. 2008 Povidone-iodine: use in hand disinfection, skin preparation and antiseptic irrigation. Int. Wound J. 5, 376-387.

Gentry, T. J., Rensing, C. & Pepper, I. L. 2004 New approach for bioaugmentation as a remediation technology. Crit. Rev. Env. Sci. Tec. 34, 447-494.

Hill, C. B. & Khan E. 2008 A Comparative Study of Immobilized Nitrifying and Co-Immobilized Nitrifying and Denitrifying Bacteria for Ammonia Removal of Sludge Digester Supertanant. Water Air Soil Poll. 195, 23-33.

Kim, K. J., Kim, S. K. & Kim, S. H. 2001 Characterization of immobilized denitrifying bacteria isolated from municipal sewage. J. Microbiol. Biotechn. 11, 756-762.

Ochoa-Herrera, V., Banihani, Q., Leon, G., Khati, C., Field, J. A. & Sierra-Alvarez, R. 2009 Toxicity of fluoride to microorganisms in biological wastewater treatment systems. Water Res. 43, 3177-3186.

Pramanik, S. & Khan, E. 2008 Effects of cell entrapment on growth rate and metabolic activity of mixed cultures in biological wastewater treatment. Enzyme Microb. Tech. 43, 245-251.

Rezaee, A., Ansari, M., Khavanin, A., Sabzali, A. & Aryan, M. M. 2005 Hospital wastewater treatment using as integrated anaerobic and aerobic fixed film bioreactor. Am. J. Environ. Sci. 1(4), 259-263.

Smidsrod, O. & Skjak-Braek, G. 1990 Alginate as immobilization matrix for cells. Trends biotechnal. 8, 71-78.

Siripattanakul, S. & Khan, E. 2010 Fundamentals and applications of entrapped cell bioaugmentation for contaminant removal. In: *Emerging Environmental Technologies, Volume 2. Shah, V. (ed.)* Springer.

Siripattanakul, S., Pochant, C. J. & Khan, E. 2010 Nitrate removal from agricultural infiltrate by bioaugmented free and alginate entrapped cells. Water Environ. Res. 82(7), 617-621.

Siripattanakul, S., Wirojanagud, W., McEvoy, J., M., Casey, F., X., M. & Khan, E. 2009 Atrazine Removal in Agricultural Infiltrate by Bioaugmented Polyvinyl Alcohol Immobilized and Free *Agrobacterium radiobacter* J14a: A Sand Column Study. Chemosphere. 74, 308-313.

Siripattanakul, S., Wirojanagud, W., McEvoy, J. & Khan E. 2008. Effect of cell-to-matrix ratio in polyvinyl alcohol immobilized pure and mixed cultures for atrazine degradation. Water Air Soil Poll.: Focus. 8, 257-266.

Standard Methods for the Examination of Water and Wastewater. 1998. 20th edn, American Public Health Asscociation/ American Water Works Association/ Water Environment Federation, Washington DC, USA

Entrapped Cell System for Decentralized Hospital Wastewater Treatment: Inhibitory effect of Disinfectants

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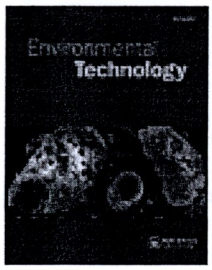

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Entrapped cell system for decentralized hospital wastewater treatment: inhibitory effect of disinfectants

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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].

$$\textit{Inhibition}\ (\%) = \left(1 - \frac{\textit{Average treatment activity of the reactor}}{\textit{Average treatment activity of the control}}\right) \times 100 \quad (1)$$

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.

Test	Reactor	Cell type	Cell-to-matrix ratio (mL of cells: mL of calcium alginate) 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

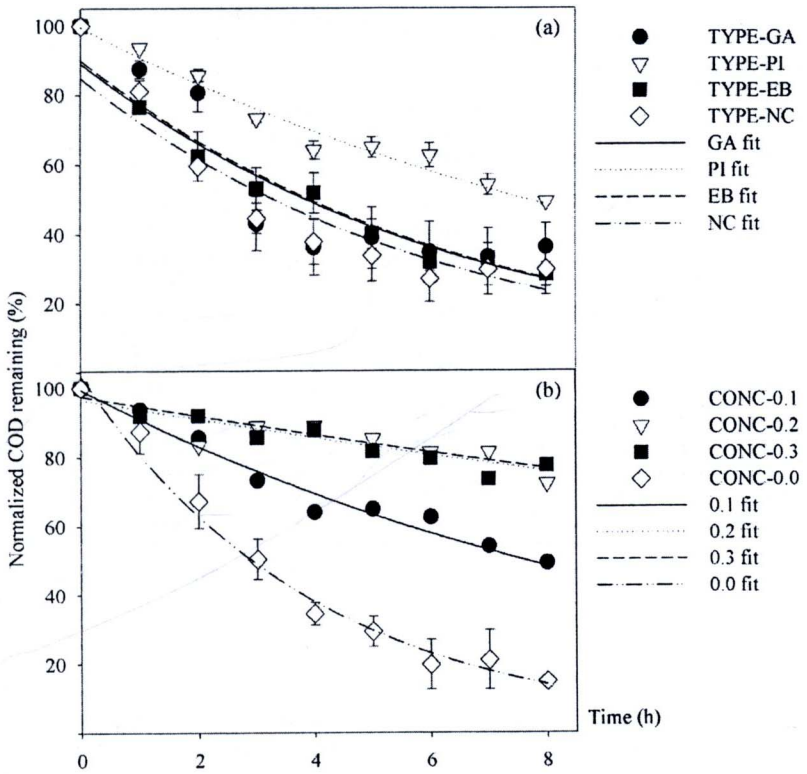

Figure 1. Normalized COD remaining and kinetic curve fitting: the effects of disinfectant types and concentrations.

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].

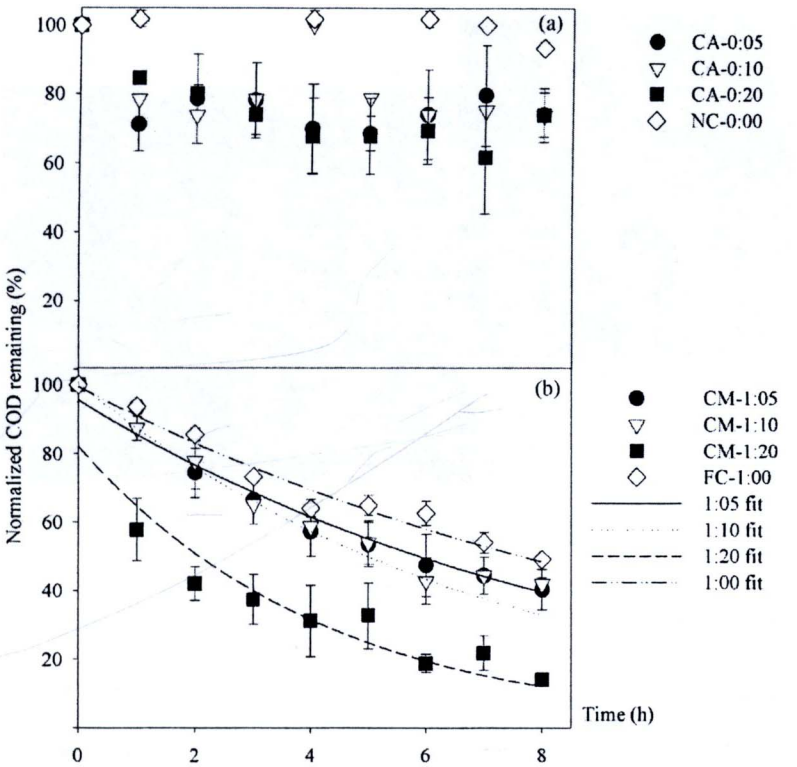

Figure 2. Normalized COD remaining and kinetic curve fitting: the optimization of cell entrapment preparation.

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.

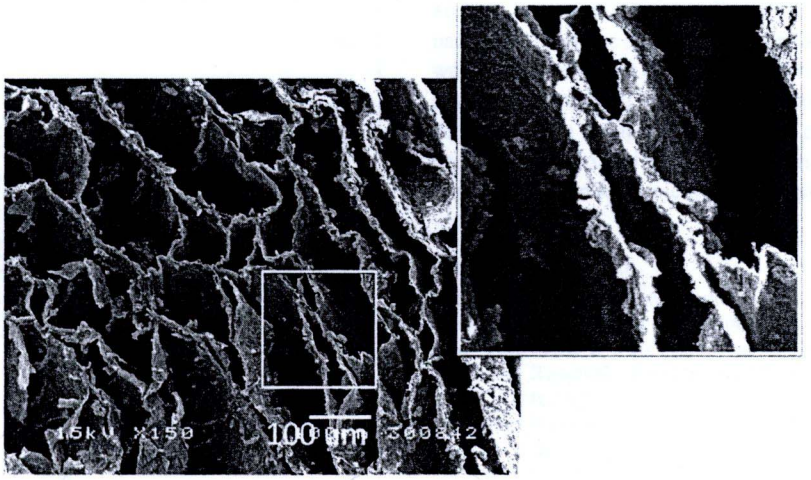

Figure 3. Cross-sectional image of the CA entrapped cells.

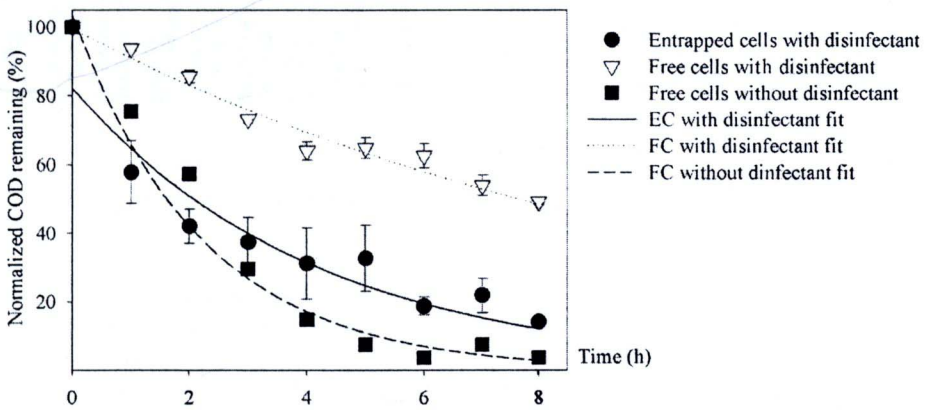

Figure 4. Comparison of the wastewater treatment by the entrapped and free cells.

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,

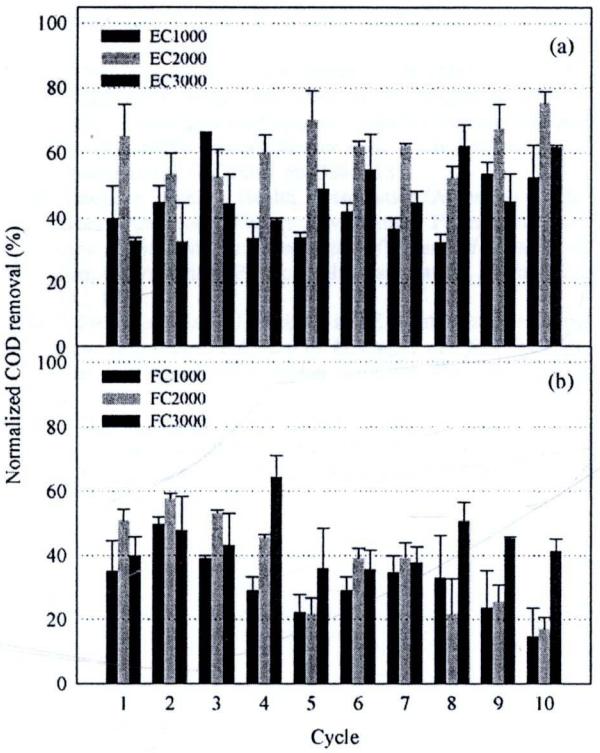

Figure 5. Normalized COD removal by the entrapped and free cells in SBR mode.

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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References

[1] V. Chitnisa, S. Chitnisa, K. Vaidyaa, S. Ravikanta, S. Patilb, and D.S. Chitnisa, *Bacterial population changes in hospital effluent treatment plant in central India*, Water Res. 38 (2004), pp. 441–447.

[2] A. Rezaee, M. Ansari, A. Khavanin, A. Sabzali, and M.M. Aryan, *Hospital wastewater treatment using as integrated anaerobic and aerobic fixed film bioreactor*, Am. J. Environ. Sci. 1 (2005), pp. 259–263.

[3] I. Bodik, E. Gasparikova, L. Dancova, A. Kalina, M. Hutnan, and M. Drtil, *Influence of disinfectants on domestic wastewater treatment plant performance*, Bioresource Technol. 99 (2008), pp. 532–539.

[4] M.B. Cassidy, H. Lee, and J.T. Trevors, *Environmental applications of immobilized microbial cells: A review*. J. Ind. Microbiol. 16 (1996), pp. 79–101.

[5] S.X. Liu, S.W. Hermanowicz, and M. Peng, *Nitrate removal from drinking water through the use of encapsulated microorganisms in alginate beads*, Environ. Technol. 24 (2003), pp. 1129–1134.

[6] H. Li, P. Li, T. Hua, Y. Zhang, X. Xiong, and Z. Gong, *Bioremediation of contaminated surface water by immobilized* Micrococcus roseus, Environ. Technol. 26 (2005), pp. 931–939.

[7] S. Siripattanakul, W. Wirojanagud, J. McEvoy, and E. Khan, *Effect of cell-to-matrix ratio in polyvinyl alcohol immobilized pure and mixed cultures for atrazine degradation*, Water Air Soil Poll. Focus 8 (2008), pp. 257–266.

[8] T.D. Reynolds and P.A. Richards, *Unit Operations and Processes in Environmental Engineering*, 2nd ed., PWS Publishing, Boston, 1996.

[9] O. Smidsrod and G. Skjak-Braek, *Alginate as immobilization matrix for cells*, Trends Biotechnol. 8 (1990), pp. 71–78.

[10] T.J. Gentry, C. Rensing, and I.L. Pepper, *New approach for bioaugmentation as a remediation technology*, Cri. Rev. Environ. Sci. Technol. 34 (2004), pp. 447–494.

[11] K.J. Kim, S.K. Kim, and S.H. Kim, *Characterization of immobilized denitrifying bacteria isolated from*

municipal sewage, J. Microb. Biotechnol. 11 (2001), pp. 756–762.

[12] S. Siripattanakul, W. Wirojanagud, J.M. McEvoy, F.X.M. Casey, and E. Khan, *Atrazine removal in agricultural infiltrate by bioaugmented polyvinyl alcohol immobilized and free* Agrobacterium radiobacter *J14a: A sand column study*, Chemosphere 74 (2009), pp. 308–313.

[13] American Public Health Association/American Water Works Association/Water Environment Federation, *Standard Methods for the Examination of Water and Wastewater*. 20th ed., American Public Health Association, Washington, DC, 1998.

[14] S. Siripattanakul, C.J. Pochant, and E. Khan, *Nitrate removal from agricultural infiltrate by bioaugmented free and alginate entrapped cells*, Water Environ. Res. 82 (2010), pp. 617–621.

[15] V. Ochoa-Herrera, Q. Banihani, G. Leon, C. Khati, J.A. Field, and R. Sierra-Alvarez, *Toxicity of fluoride to microorganisms in biological wastewater treatment systems*, Water Res. 43 (2009), pp. 3177–3186.

[16] P. Verlicchi, A. Galletti, M. Petrovic, and D. Barcelo, *Hospital effluents as a source of emerging pollutants: An overview of micropollutants and sustainable treatment options*, J. Hydrol. 389 (2010), pp. 416–428.

[17] T. Das, S. Sharma, J. Singh, V. Rao, and K.V. Chalam, *Evaluation of glutaraldehyde and povidone iodine for sterilization of wide-field contact vitrectomy lenses*, Ophthalmic Surg Laser 32 (2001), pp. 300–304.

[18] M.J. Anderson, M.E. Horn, Y. Lin, P. J. Parks, and M.L. Peterson, *Efficacy of concurrent application of chlorhexidinegluconate and povidone iodine against six nosocomial pathogens*, Am. J. Infect. Control 38 (2010), pp. 826–831.

[19] M.A. Heitkamp, V. Camel, T.J. Reuter, and W.J. Adams, *Biodegradation of* p*-nitrophenol in an aqueous waste stream by immobilized bacteria*, Appl. Environ. Microbiol. 56 (1990), pp. 2967–2973.

[20] P. Durani and D. Leaper, *Povidone-iodine: Use in hand disinfection, skin preparation and antiseptic irrigation*, Inter. Wound J. 5 (2008), pp. 376–387.

ภาคผนวก ง

สรุปผลการดำเนินการวิจัย

ผลสรุปแผนการดำเนินงานและผลการปฏิบัติงานจริง

กิจกรรม	ช่วงเวลา (เดือน)								ผู้รับผิดชอบ
	2	4	6	8	10	12	14	15	
1.ศึกษารวบรวมงานวิจัยที่เกี่ยวข้อง									ดร.สุมนา
2.ศึกษาสารฆ่าเชื้อที่ใช้ในโรงพยาบาล									ดร.สุมนา ผู้ช่วยวิจัย
3.ศึกษาผลของชนิดสารฆ่าเชื้อ									ดร.สุมนา ผู้ช่วยวิจัย
4.ศึกษาการเตรียมเซลล์ดักติดที่เหมาะสม									ดร.สุมนา ผู้ช่วยวิจัย
5.ศึกษาผลของความเข้มข้นสารฆ่าเชื้อ									ดร.สุมนา ผู้ช่วยวิจัย
6.วิเคราะห์ผลการทดลอง									ดร.สุมนา
7.สรุปผลการศึกษา									ดร.สุมนา
8.จัดทำเอกสารงานวิจัย/เผยแพร่ผลงาน									ดร.สุมนา

แผนการดำเนินงาน

การปฏิบัติงานจริง

รายงานสรุปการใช้งบประมาณ

หมวด/รายการ	จำนวนเงิน (บาท)		
	ตามแผนเดิม	ตามแผนใหม่	ใช้จ่ายจริง
1. หมวดค่าตอบแทน			
• ค่าทำการนอกเวลาราชการ	13,000	13,000	13,000.00
2. หมวดค่าใช้สอย			
• ค่าจ้างเหมาผู้ช่วยนักวิจัย	95,280	95,280	95,280.00
• ค่าเดินทางเข้าร่วมการประชุมถ่ายทอดงานวิจัย	26,220	-	-
• ค่าจ้างวิเคราะห์ SEM	20,000	-	-
3. หมวดค่าวัสดุ			
3.1 ค่าวัสดุวิทยาศาสตร์	95,000	141,220	142,708.05
3.2 ค่าวัสดุสำนักงาน	5,000	5,000	4,342
3.3 ค่าวัสดุคอมพิวเตอร์	5,000	5,000	4,740
4. หมวดค่าสาธารณูปโภค			
• ค่าไปรษณีย์	500	500	0
• ค่าสาธารณูปโภคสำหรับคณะและมหาวิทยาลัย	26,000	26,000	26,000
รวมทั้งสิ้น	**286,000**	**286,000**	**286,070.05**

ตารางเปรียบเทียบวัตถุประสงค์และผลที่ได้รับจากการดำเนินโครงการ

วัตถุประสงค์	ผลที่ได้รับ
ศึกษาผลกระทบของชนิดและความเข้มข้นสารฆ่าเชื้อที่ใช้ภายในโรงพยาบาลต่อประสิทธิภาพการบำบัดน้ำเสีย	• ข้อมูลสารสารฆ่าเชื้อหลักที่ใช้ภายในโรงพยาบาล • ผลการยับยั้งการบำบัดน้ำเสียจากสารฆ่าเชื้อชนิดต่าง ๆ และจลนพลศาสตร์การบำบัดน้ำเสียในสภาวะนั้น ๆ • ผลการยับยั้งการบำบัดน้ำเสียจากสารฆ่าเชื้อความเข้มข้นต่าง ๆ และจลนพลศาสตร์การบำบัดน้ำเสียในสภาวะนั้น ๆ
ศึกษาสภาวะที่เหมาะสมในการเตรียมเซลล์ดักติดเพื่อบำบัดน้ำเสียจากโรงพยาบาลเปรียบเทียบกับเซลล์อิสระ	• สภาวะที่เหมาะสมในการเตรียมเซลล์ดักติดเพื่อบำบัดน้ำเสียจากโรงพยาบาล • การบำบัดน้ำเสียที่ปนเปื้อนสารฆ่าเชื้อด้วยเซลล์ดักติดและเซลล์อิสระ • จลนพลศาสตร์การบำบัดน้ำเสียปนเปื้อนสารฆ่าเชื้อด้วยเซลล์ดักติดและเซลล์อิสระในสภาวะนั้น ๆ
ศึกษาปริมาณเซลล์ดักติดที่เหมาะสมเพื่อบำบัดน้ำเสียจากโรงพยาบาลเปรียบเทียบกับเซลล์อิสระ	• ปริมาณเซลล์ดักติดที่เหมาะสมเพื่อบำบัดน้ำเสียจากโรงพยาบาล • การบำบัดน้ำเสียที่ปนเปื้อนสารฆ่าเชื้อด้วยเซลล์ดักติดและเซลล์อิสระ • จลนพลศาสตร์การบำบัดน้ำเสียปนเปื้อนสารฆ่าเชื้อด้วยเซลล์ดักติดและเซลล์อิสระในสภาวะดังกล่าว
ศึกษาลักษณะโครงสร้างระดับจุลภาคของเซลล์ดักติดที่ประยุกต์ใช้ในการบำบัดน้ำเสียจากโรงพยาบาล	• ลักษณะโครงสร้างระดับจุลภาคของเซลล์ดักติดด้วยแคลเซียมแอลจิเนต