Effects of combined growth of biogenic and xenobiotic substrates on degradation of xenobiotic by activated sludge

Nguyen Phuc Thien[1,a], Hoang Thi Hoai[1], Doan Quang Tri[2,3] and Chen Yi Ching[1]

[1]*Department of Environmental Engineering, Da-Yeh University, Changhua 51591, Taiwan*
[2]*Lowland Technology International (LTI) Member, Saga University, Japan*
[3]*National Center for Hydrometeorological Forecasting (NCHMF), Vietnam*

Abstract. The purpose of this study was to research about supplementation of different concentrations of the substrate on the degradation rate of xenobiotic and to determine the optimal concentrations of the auxiliary substrates that are most beneficial of xenobiotic degradation rate. 2,4-dichlorophenol acid (2,4-D) was used representative xenobiotic organic compounds, while peptone and sugar used for auxiliary substrates. The activated sludge was completely break down 100 mg/l of 2,4-D for three consecutive times. The different concentrations between biogenic substracts of sucrose and peptone were fed separately or combined into the medium containing 200 mg/l of 2,4-D and 140 mg SS/l of activated sludge. The results showed that sugar and peptone could affect 2,4-D degradation rate to several different degree at different concentrations. In separate supplementation, 2,4-D degradation completed within 25 hours, 40 mg/l sugar and 150 mg/l peptone concentrations were found to be the optimal concentrations. In combined case, 2,4-D was consumed totally within 20 hours and the optimal concentration of the combined sugar and peptone concentrations were 40 and 150 mg/l, respectively.

1 Introduction

Xenobiotic organic compounds such as phenoxy acid herbicides are foreign to most indigenous microorganisms, including those in activated sludge. In addition to their hard-to-treat nature, xenobiotics can be uncoupled of the microorganisms' metabolism of biogenic substrates. The uncoupling, or the energy-spilling reactions, occurs when catabolism energy is dissipated or diverted away from anabolism through the metabolism of a substrate in bacterial cells [1]. Although xenobiotics containing wastewater can be suitably treated using biological methods [2,3,4,5], the xenobiotic nature of the pollutant requires the treatment plant microorganisms (typically activated sludge) to go through an acclimation phase before the microorganisms evolve the degradation capability for treating the influent xenobiotics. Thus, biodegradation method is used widely to treat xenobiotics in water treatment methods. One of the popular xenobiotics is 2,4-Dichlorophenoxyacetic acid (2,4-D), a common joint systemic herbicide used in the control of broadleaf weeds. Therefore, the target xenobiotic organism pollutant was 2,4-D and activated sludge was an agent treatment in this study. The activated sludge process reduced or eliminated wastewater toxicity from a variety of sources along a wastewater collection system [6,7]. Eckenfielder [8] suggested that aquatic toxicity

[a] Corresponding author : d0405601@cloud.dyu.edu.tw

data on specific organic chemicals should not be applied to industrial wastewater effluents because many of these compounds were removed during biological treatment. Several authors [9,10] suggested that the fed-batch technique could evaluate toxicity to activated sludge with substrate continuously added to a batch reactor and accumulation of substrate followed with time. Toxic or inhibitory substrates exhibited an upward slope in the substrate accumulation curve, and nonlinear curve fitting techniques were used to determine inhibition constants.

Activated sludge biomass grown on the feed of biogenic substrate must be in a healthy physiological condition [11]. However, inconsistent results are found in literature about the effects of biogenic organics on the degradation of man-made xenobiotics or hydrocarbons. There have been some study cases of both beneficial and adverse effects of biogenic organics on xenobiotic degradation. The beneficial good cases include: citrate on toluene [12]; natural amino acids on mono-substituted phenol [13]; natural organics such as manure on dichloro two chloros and a nitro-herbicides [14]; fatty acids on soil hydrocarbon [15]; pyruvate on naphthalene [16]. Conversely, adverse cases are also found: glucose or amino acids in on xylene and toluene [17]; ethanol in on benzene, toluene and xylene [18]; glycolic acid and glucose on p-cresol [19]; yeast extract and milk on 3-nitrobenzoate, 4-chlorobenzoate, 4-chlorophenol [20]. Diauxic growth is a phenomenon occurs when microorganism is grown on cultural medium with the presence of two substrates, one of which is easier for the microorganism to metabolize. Sugar is consumed first, and it leads to a rapid growth. Only after the easier more natural substrate has been exhausted, the cells switch to the second, resulting in two separate growth phases. There are some previous studies which the effects of diauxic growth on 2,4-D acclimation and degradation [21]. The results showed that: If we feed sugar with a proper concentration and at the proper time, it can make 2,4-D acclimation lag time shorten. On the contrary, it can make the acclimation lag time longer. This is explained that sugar is an easier substrate than 2,4-D for the microbial cell to utilize, and thus the biomass temporarily ignores or escapes from the xenobiotic acclimation stress. This pause in of the process lets the xenobiotic still be intact until sugar is consumed entirely completely, thereby causing the elongation of the acclimation lag time.

The purpose of this study, therefore, was to determine effects of auxiliary substrates on 2,4-D degradation and detail profiles of ATP that contained in activated sludge cells during the cells' degradation of a model xenobiotic compound 2,4- dichloro-phenoxyacetic acid (2,4-D). Sugar and peptone were supplemented with into cultural medium contained 2,4-D to determine the optimal concentrations of each the auxiliary substrates in separate or double. Generally, sugar and peptone are used as primary major carbon and nitrogen sources in a medium of microbial cultivation. However, 2,4-D plays a role as a carbon source in this study. Thus, sugar and peptone were used as the representation of the auxiliary substrates. In this study case, sugar sucrose and feed of 2,4-D, peptone and feed of 2,4-D with peptone are fed transferred into the cultural medium at the same time. Therefore, we can find out which concentration is the most effective on 2,4-D degradation. Results of this study can supplement for the knowledge about 2,4-D biodegradation, and apply to actual real water treatment system. The efficiency and applicability consist of economic efficiency when the methods are applying to actual xenobiotic treatment systems. However, this study can be used as references for studies of relative compounds and other xenobiotic organics.

2 Materials and methods

As mentioned above, 2,4-D was used to be target substrate, and activated sludge was used to be agent treatment. Peptone and sugar are considered as auxiliary substrates that could affect 2,4-D degradation by activated sludge.

2.1 Suspended solid (SS) measurements

Suspended solid is considered as a representation of microbe biomass in samples. It is measured one time per day during 2,4-D degradation in this study. The procedure is used, including the following

steps: 1. The filter papers (pore size 0.45 µm) are washed by using distilled water and vacuum filtration; 2. After washing, the papers are dried about 4-5 hours at 105^0 C in a drying cabinet; 3. Scaling the dried the papers before using to filter samples, getting paper weight (M_0 - g); 4. Putting V (ml) sample on the dried filter paper and filtering by vacuum filtration to separate the solid from the liquid; 5. Drying again about 4- 5 hours at 105^0 C in the drying cabinet; 6. Scaling again the dried sample to get paper and SS weight (M_1 - g); 7. Calculating SS (mg/l) is showed in Equation 1:

$$SS = \frac{(M_1 - M_0)x100}{0.001\,xV} \quad (1)$$

where: SS is suspended solid weight (mg/l); M_0 is the weight of the initial dried paper (g); M_1 is a weight of the dried paper after filtering sample (g); V is a volume of the sample (ml).

2.2 Experimental method to obtain acclimated activated sludge

Activated sludge was fed in separate mediums corresponding to different periods as follows: The first period was to cultivate activated sludge in a sequence batch reactor with supplementing daily sucrose 100mg/l, peptone 18mg/l, $FeCl_3$ 1mg/l, NH_4Cl 30mg/l, K_2HPO_4 200mg/l, KH_2PO_4 156mg/l and $MgSO_4$ 31.26mg/l. The second period was to make the activated sludge acclimate to 2,4-D. After feeding in the continuous medium, 140 mg/l of the activated sludge was transferred to batch medium with 100 mg/l 2,4-D as sole carbon resource until the activated sludge degraded completely 2,4-D in three times. The original activated sludge spent about seven days degrading completely 100 mg/l 2,4-D in the first time. However, it only spent about two days in the second time, and one day for in the third time on this process. The obtained activated sludge was called A. A would be used major experiments with sugar and peptone supplementation.

2.3 Experimental methods with (sugar) sucrose and peptone

To determine effects of auxiliary substrates supplementation on 2,4-D degradation, the study conducted the experiments in separated and combined case. Experimental symbols: A stands for the reactor only includes A and 2,4-D; S_x stands for the reactor includes A, x mg/l Sugar and 2,4-D; P_y stands for the reactor includes A, y mg/l Peptone and 2,4-D; P_yS_x stands for the reactor includes A, y mg/l Peptone x mg/l Sugar and 2,4-D. All experiments were conducted with 200 mg/l 2,4-D and 140 mg/l A. In separated case: Each substrate was supplemented concurrently with 2,4-D into A medium with different concentrations. Supplemental sugar concentrations consisted of 20, 40, 60, 80, 100, 150 mg/l, while supplemental peptone concentrations were 20, 40, 100, 150, 200, 300 mg/l. In combined case: Both sugar and peptone are supplemented concurrently with 2,4-D into A medium with different couple concentrations. Supposing that: 0 stands for the best concentrations; "-"stands for the lower concentrations than the best concentrations; "+" stands for the higher concentrations than the best concentrations. From the supposition, the matrix of sugar and peptone concentrations is shown in Table 1.

Table 1. Matrix of sugar and peptone concentrations.

Sugar \ Peptone	-	0	+
-	- -	- 0	- +
0	0 -	0	0 +
+	+ -	+ 0	+ +

2,4-D concentration and SS were measured each 5 hours during the degradation process.

2.4 2,4-D degradation rate calculation

This study used Equation 2 to calculate 2,4-D degradation rate.

$$R = \frac{\Delta S}{\Delta t} = \frac{(S_i - S_{i+n})}{(t_{i+n} - t_i)} \quad (2)$$

where: R stands for 2,4-D degradation rate (mg 2,4-D/l.hr); S is 2,4-D concentration; t stands for time (hour); i stands for first time mark, the point was chosen at 0hr; i+n stands for the second time mark, the point was chosen at 20hrs or 15hrs for separate cases or combined case, respectively.

3 Results and discussion

3.1 Effects of different sugar concentrations on 2,4-D degradation rate

SS increased from 140 mg/l to 270 mg/l, depending on sugar concentration (figure 1a). Figure 1b showed that 2,4-D degradation completed within 25 hours. SS grew on higher sugar concentrations were always higher greater than the growth in on lower concentrations at the same time.

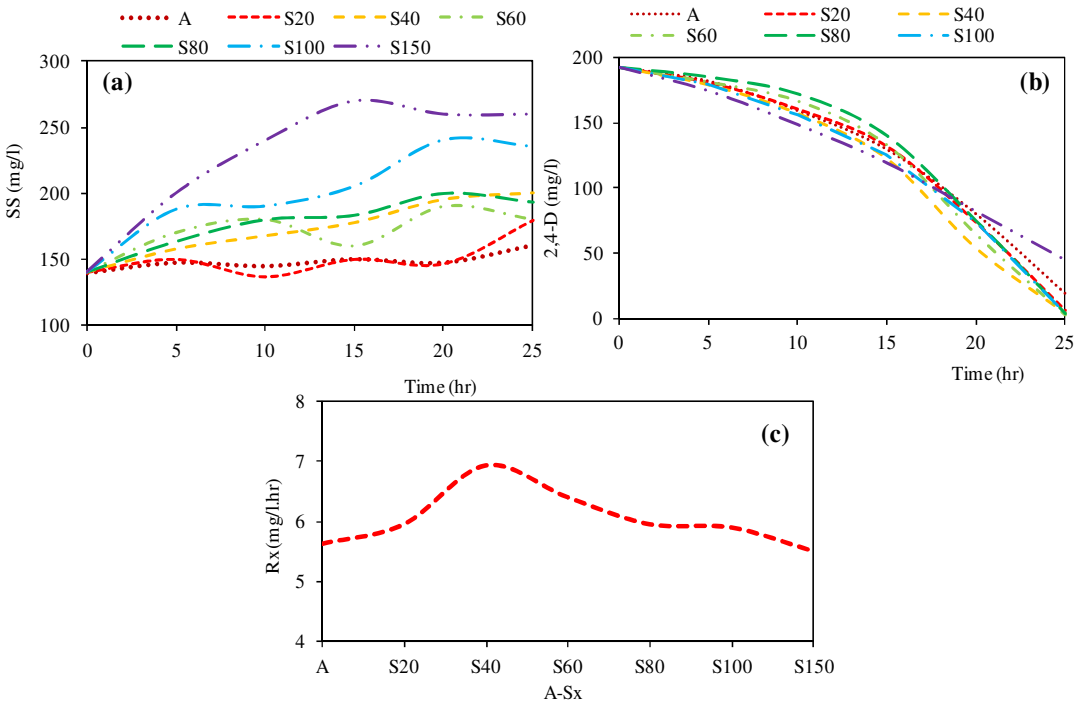

Figure 1. (a) 2,4-D degradation with sugar supplementation; (b) Activated sludge growth (SS) of sugar supplementation; (c) 2,4-D degradation rate of sugar supplementation.

The reason is that sugar is easily consumed by microorganisms and releases a higher amount of energy. The energy could help the sludge grow faster. 2,4-D degradation rates were calculated, and shown in figure 1c. Figure 1c showed that the reactor included 40 mg/l sugar, had the highest rate of 2,4-D degradation at 6.94 mg/l.hr. For sugar concentrations that were less than 40 mg/l, the rate increased when sugar concentration increased. Because the microorganisms can easily metabolize sugar, and releases a large of energy. The energy could help the sludge grow faster. But the trend would be not similar for very high sugar concentrations such as 150 mg/l. This could be explained by

the diauxic growth process. Sugar is a better substrate than 2,4-D for microorganisms to utilize, and thus the biomass temporarily ignores or escapes from the xenobiotic degradation stress. This interference of the degradation lets the xenobiotic still being ignored until sugar is consumed completely. Thus, 40 mg/l sugar concentration is supposed the optimal sugar concentration to enhance 2,4-D degradation rate.

3.2 Effects of different peptone concentrations on 2,4-D degradation rate

Figure 2a showed that 2,4-D degradation completed within 25 hours. The growth of activated sludge also increased when peptone concentration increased. However, sugar made the activated sludge grow faster than peptone did at similar concentration. Therefore, sugar might release more energy than peptone did when microorganisms metabolized these substrates (figure 2b).

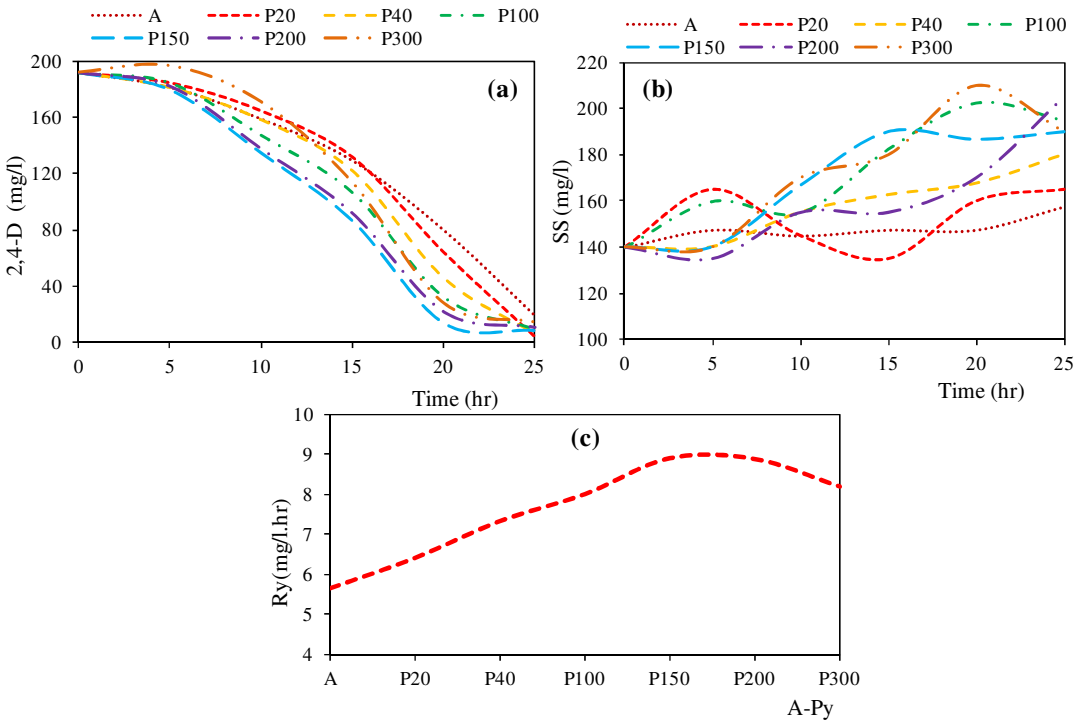

Figure 2. (a) 2,4-D degradation with peptone supplementation; (b) Activated sludge growth (SS) with peptone supplementation; (c) 2,4-D degradation rate of peptone supplementation.

Figure 2c showed that 2,4-D degradation rate increased when peptone concentrations were increased until peptone concentration reached higher 150 mg/l. This case was not like sugar because there was a range of peptone concentrations between 150 and 200 mg/l which have similar effect on 2,4-D degradation. There is been an assumption that peptone does not cause diauxic growth as sugar. Hence, the optimal peptone concentration for the best 2,4-D degradation rate was 150 mg/l.

3.3 Effects of combined auxiliary substrates on 2,4-D degradation rate

From the results obtained previously, 40 mg/l sugar concentration and 150 mg/l peptone concentration were supposed to be the optimal concentrations. This section presents the combined case which used both substrates: sugar and peptone were supplemented concurrently with different couple concentrations. These different coupled concentrations were designed as shown in Table 2.

Table 2. Matrix of sugar and peptone concentrations.

Sugar (S) / Peptone (P)	S_1 (20 mg/l)	S_2 (40 mg/l)	S_3 (100 mg/l)
P_1 (100 mg/l)	P_1S_1	P_1S_2	P_1S_3
P_2 (150 mg/l)	P_2S_1	P_2S_2	P_2S_3
P_3 (200 mg/l)	P_3S_1	P_3S_2	P_3S_3

For combined sugar and peptone supplementation, 2,4-D degradation completed within about 20 hours (fugures 3a-3c); especially, the activated growth increases appreciably from 140 mg/l to 300 mg/l (figure 3d). It means reasonable effects of sugar and peptone on 2,4-D degradation and the activated sludge growth are significant. The growth was obviously resulted from the auxiliary substrates, shown in figure 3.

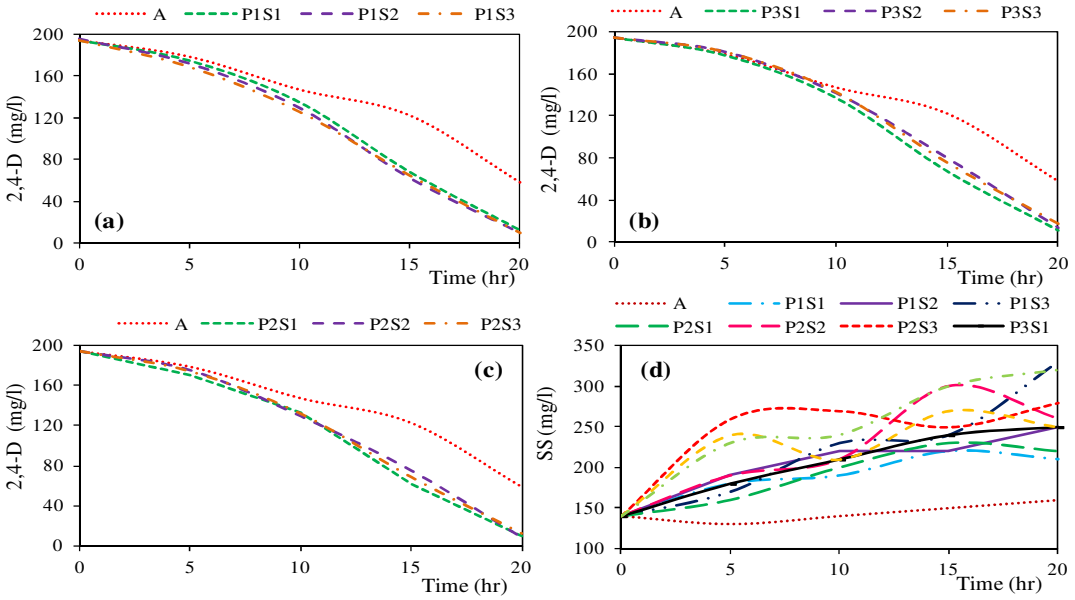

Figure 3. (a) 2,4-D degradation with sugar and peptone supplementation - P_1S_x; (b) 2,4-D degradation with sugar and peptone supplementation - P_3S_x; (c) 2,4-D degradation with sugar and peptone supplementation - P_2S_x; (d) Activated sludge growth (SS) with sugar and peptone supplementation.

Figure 4 showed that couple of 150 mg/l peptone and 40 mg/l sugar concentrations are supposed as the optimal concentrations with the rate is 8.99 mg/l.hr. In this case, it is very difficult to identify a certain trend of the rate because of the combination of two auxiliary substrates. Thus, this study only determines the optimal concentrations of combined auxiliary substrates.

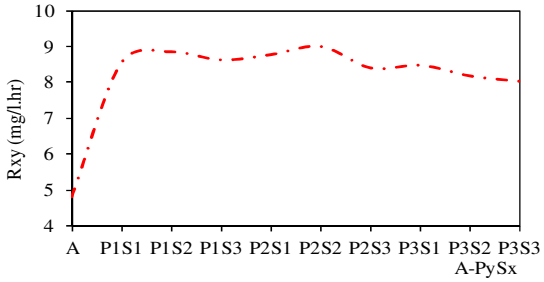

Figure 4. 2,4-D degradation rate with sugar and peptone supplementation.

4 Conclusions

1. For separate supplementation, both sugar and peptone could help 2,4-D degradation rate. 2,4-D degradation completed within 25 hours. The optimal concentrations of sugar and peptone were supposed 40 mg/l and 150 mg/l with 6.94 mg/l.hr and 150 mg/l.hr of 2,4-D degradation rate, respectively. These rates were higher than the rate of un-supplemented reactor (A).
2. For combined supplementation, 2,4-D degradation completed within 20 hours. The optimal concentration of the combined sugar and peptone supplementation is 40 mg/l and 150 mg/l for sugar and peptone, respectively. 2,4-D degradation rate at the optimal rate was 8.99 mg/l.

Acknowledgements

We are thankful to Prof.Chong, Nuyk-Ming for giving us this opportunity and facilities to carry out this study. The first author would like to give special thanks to Da-Yeh University, who provides financial support for this study.

References

1. J.B. Russell, The energy spilling reactions of bacteria and other organisms, Mol. Microbiol. Biotechnol., **13** (1-3), 1-11 (2007)
2. N.P. Hill, A.E. MacIntyre, R. Perry, and J.N. Lester, Water Res., **20**, 45-52 (1986)
3. M. Ettala, J. Koskela, and A. Kiesila, Water Res., **26**, 797-804 (1992)
4. S. Meric, G. Eremektar, F. Ciner, and O. Tünay, J. Hazard. Mater., **101** (2), 147-155 (2003)
5. H. Chin, P. Elefsiniotis, and N. Singhal, J. Environ. Eng. Sci., **4** (1), 57-63 (2005)
6. C.L. Logue, et al., J. Water Pollut. Control Fed., **61**, 632 (1989)
7. S. Joann, O.M. Richard, H.S. Joseph, A.S. Weber, and D.A. Michael, Activated Sludge, Res. J. Water Pollut. Control Fed., **62** (4), 398-406 (1990)
8. W.W. Jr. Eckenfelder, *Proceedings of The 43rd Ind. Waste Conf.*, *Purdue Univ.*, *West Lafayette*, *Ind.*, **1**, 73-78 (1989)
9. J. Patoczka, et al., *Proc. 43rd Ind. Waste Conf.*, *Purdue Univ.*, *West Lafayette*, *Ind.*, **51**, 256-265 (1989)
10. A.T. Watkin and W.W.Jr. Eckenfielder, Water Sci. Technol. (G.B.), **21**, 593 (1989)
11. N.M. Chong, M.L. Luong, and C.S. Hwu, Bioresour. Technol., **104**, 181-186 (2012)
12. E.M. Harrison and J.F. Barker, J. Contam. Hydrol., **1**, 349-373 (1987)
13. R.J. Shimp and F.K. Pfeander, Appl. Environ. Microbiol., **49**, 394-401 (1985)
14. T.B. Moorman, J.K. Cowan, E.L. Arthur, and J.R. Coats, Biol. Fertil. Soils, **33**, 541-545 (2001)
15. E.C. Nelson, M.V. Walter, I.D. Bossert, and D.G. Martin, Environ. Sci. Technol., **30**, 2406-2411 (1996)
16. K. Lee, J. Hazard. Mater., **105** (1-3), 157-167 (2003)
17. C.M. Swindoll, C.M. Aelion, and F.K. Pfaender, Appl. Environ. Microbiol., **54**, 212-217 (1988)
18. H.X. Corseuil, C.S. Hunt, R.D.S. Ferreira, and P.J.J. Alvarez, Water Res., **32**, 2065-2072 (1998)
19. D.L. Lewis, H.P. Kollig, and R.E. Hodson, Appl. Environ. Microbiol., **51**, 598-603 (1986)
20. Z. Hu, R.A. Ferrainab, J.F. Ericsonb, and B.F. Smetsa, Water Res., **39**, 3501-3510 (2005)
21. N.M. Chong, Bioresour. Technol., **100** (23), 5750-5756 (2009)