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An Application of Sequencing Batch Reactors in Microbial Degradation of Benzene, Toluene & Xylene under Anoxic and Micro Aerobic Condition

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Abstract This paper mainly focuses on treatment process for Benzene, Toluene & Xylene (BTX) containing wastewater by using an alternating anoxic/micro aerobic sequencing batch reactor to overcome the stripping of BTX compounds into the atmosphere, as often occurs in conventional aerobic treatment processes. An ORP probe was successfully used to monitor different electron acceptor conditions in sequencing batch reactors whereas a DO probe failed to detect the low DO concentration under micro aerobic conditions. Toluene and *m*-xylene were amenable to anoxic (denitrifying) metabolism while benzene, *o*-, and *p*-xylene were biodegradable under micro aerobic conditions. Compared to conventional aerobic treatment processes, this approach can eliminate vigorous aeration and significantly reduce stripping of BTX (and other volatile contaminants amenable to anoxic/micro aerobic biodegradation) caused by aeration.

Keywords: *anoxic, BTX, microaerobic, ORP, SBR*

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1. Introduction

One of the major concerns associated with using sequencing batch reactors for wastewater treatment is the uneven oxygen demands encountered throughout a reaction cycle, with much higher oxygen demands at the beginning. Such an oxygen demand profile leads to either a complicated operation strategy or high peak energy consumption. This particular problem associated with sequencing batch reactors can be overcome by incorporating an anoxic environment into the reaction cycle. This paper presents a study on the biological treatment of a wastewater stream containing benzene, toluene, *o*-, *m*-, and *p*-xylene (BTX) by using sequencing batch reactors with alternating anoxic and microaerobic conditions. BTX are a group of toxic and volatile aromatic compounds prevalent in many industrial wastewaters and gasoline or petroleum contaminated subsurfaces. Traditionally, BTX containing wastewater is treated by conventional aerobic wastewater treatment processes; however, the removal mechanisms are controversial since these compounds tend to be readily stripped from the aqueous phase to the atmosphere due to their volatile nature [Chin, 1994; Dold, 1989]. Therefore, alternative treatment strategies are needed. Studies with pure cultures grown under anoxic conditions demonstrated the biodegradation abilities of toluene [Biegert, 1995; Evan, 1991; Fries, 1994; Rabus, 1995; Su JJ, 1994], as well as *o*-(5), *m*- [Biegert, 1995; Fries, 1994; Rabus, 1995; Su JJ, 1994], and *p*xylene [Biegert, 1995; Su JJ, 1994]. Studies on mixed cultures grown under anoxic conditions exhibited similar patterns. Batch incubation tests [Alvarez, 1995; Ball, 1996] or *in situ* tests [Reinhard, 1997] with subsurface cultures showed the anoxic biodegradation abilities of toluene, *m*- and *p*-xylene. In a study on activated sludge [Fettig, 1998], the biodegradation of toluene and *m*-xylene was observed. The transformation of *o*-xylene in the presence of toluene biodegradation might be a common feature under anoxic conditions [Alvarez, 1995; Ball, 1996; Fettig, 1998]. The potential for benzene biodegradation under anoxic conditions is debatable. Most studies have shown that benzene is recalcitrant to anoxic biodegradation [Alvarez, 1995; Ball, 1996; Fettig, 1998; Langenhoff, 1996], although a recent study suggested otherwise [Burland, 1999]. In recent years, there have been several reports of BTX metabolism under oxygenlimited, or microaerobic conditions, in the presence of alternative electron acceptors such as nitrate-N (NO3 --N) or nitrite-N (NO2--N) [Alvarez, 1995; Kukor, 1996; Leahy, 1997; Su JJ, 1994]. Unfortunately, the definition of microaerobic is vague and the extent of oxygen limitation varies from study to study due to differences in the methods used to both control microaerobic conditions and to monitor residual dissolved oxygen concentration. Nevertheless, those studies showed enhanced BTX biodegradation abilities under microaerobic conditions in

the presence of alternative electron acceptors. Most importantly, Alvarez and Vogel [Alvarez, 1995] showed that benzene biodegradation was significantly improved in the presence of nitrate under oxygen-limiting conditions. One of the significant features of this work is to take full advantage of BTX biodegradation potentials under anoxic conditions and microaerobic conditions so that BTX stripping into the atmosphere is minimized and a low yet uniform oxygen demand profile throughout a reaction cycle can be achieved. Finally, we investigate oxidation-reduction potential (ORP) as a monitoring device for process control of microaerobic conditions.

2. Methods

2.1. Reactor Setup

Two activated sludge sequencing batch reactors (SBRs), each with a working volume of 2 liters and a headspace of 0.5 liter were used. The glass reactors were maintained at 25 ºC and continuously mixed at 200 rpm during react and feed phases. The pH in the reactors was controlled at neutral with pH controllers. Both of the SBRs were operated with a 24-hour cycle including fill (0.5 hour), react (21.5 hours), settle (1.5 hours) and draw (0.5 hour). One SBR (ANX) was operated so that the 21.5 hr react phase remained anoxic (denitrifying) while the second SBR (ANX/MA) was operated with 9 hr of an anoxic and 12.5 hr of a micro aerobic condition. Biomass for the ANX SBR was acquired from an industrial activated sludge wastewater treatment facility, whereas the ANX/MA SBR received biomass from the same industrial activated sludge wastewater facility (3/4 volume) as well as a local domestic wastewater treatment facility (1/4 volume). Sludge retention time (SRT) and hydraulic retention time (HRT) were maintained at 15 days and 4 days, respectively, in each of the reactors. The reactor off gas was collected into a 1.5L tedlar air sampling bag during the reaction phase and vented to a fume hood during the fill phases. The tedlar bags were filled at least half way with 99.998% N2 gas every day before the start of the reaction phase and served as a N2 gas reservoir in order to maintain positive gas pressure in the reactors during extensive sampling periods. The N2 reservoirs also helped to ensure an oxygen-free headspace for the ANX SBR, and for the anoxic phase of the ANX/MA SBR. The reactors were purged with N2 gas via spargers during the feeding phase in order to maintain anoxic conditions. To avoid accumulation of BTX and possible problems of floating sludge during settling caused by gas entrained in the sludge flocs, the reactors were also purged with N2 at the end of each reaction phase. Therefore, the effective residence time of any non biodegraded BTX compounds was less than one day. To encourage the growth of a diverse microbial community while maintaining BTX degrading bacteria in the mixed liquid suspended solids (MLSS), the SBRs were fed with 600 mg/l COD of a biogenic organic substrate including proteins, sugars, and organic acids (3), and amended with 5 mg/l of each BTX compound at the beginning of each cycle. Mineral salts medium was prepared to provide essential nutrients for biomass growth and nitrate as an electron acceptor under anoxic conditions. The concentration of components in the stock mineral salts solution was as following: 0.144 mM CaCl2, 0.044 mM FeCl3.6H2O, 0.005 mM CoCl2.6H2O, 0.009 mM ZnCl2, 0.002 mM CuCl2.2H2O, 0.002 mM H3BO3, 0.487 mM MgSO4.7H2O, 0.021 mM MnSO4.H2O, 0.002 mM Na2MoO4.2H2O, 4 mM KH2PO4, and 48 mM NO3--N. The concentration of NO3--N was slightly reduced for the ANX/MA SBR to avoid the accumulation of NO2--N or NO3--N. Half of the NO3--N provided was from concentrated nitric acid while the rest was from KNO3. The mineral salts solution was diluted by 1:4 in the SBRs. The volatile nature of BTX compounds prevented use of diffused air to provide oxygen for the micro aerobic conditions. In order to provide oxygen to the laboratory ANX/MA SBR without agitation, we used hydrogen peroxide coupled with excess catalase to generate dissolved oxygen directly. Therefore, the micro aerobic zone was oxygenated by adding 2% (w/w) hydrogen peroxide for 8.5 hours following by 1% hydrogen peroxide for 4 hours, coupled with 1.47´104 units/Ml catalase (Sigma chemicals, St. Louis, MO) whenever hydrogen peroxide was added. All solutions were added at a flow rate of 0.03 ml/min. The low flow rate was used to avoid significantly changing of the reactor volume. Abiotic tests conducted in the reactor under anoxic and hydrogen peroxide-catalase generated micro aerobic conditions showed that the BTX constituents remained in solution for the duration of the reaction phase.

2.2. Analytical Methods

BTX compounds were analyzed with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a 30 m poly(alkylene glycerol), 0.25 mm i.d. capillary column, with a film thickness of 0.25 µm, and a 5 m guard column. A split injecton was used at a ratio of 1:30. The injection port temperature was 250 ºC, and the FID temperature was 260 ºC. Helium was used as a carrier gas at a column head pressure of 15 psi and a column flow rate of 1.46 ml/min. The initial column temperature was 70 ºC for 10 minutes, and then the temperature was ramped at 10 ºC/min to a final temperature of 120 ºC. BTX analytical external standards were made using hexanes as solvent. The BTX concentration in mixed liquid suspended solids samples collected from the reactors was determined by directly extracting with hexanes for 2 hours on a rotary mixer at an extraction ratio of 7 parts sample to 1 part hexanes in a 16 ml vial equipped with a teflon-lined cap. After extraction, the liquid and solvent mixture was separated by centrifugation (1900 ´g) and the extract was transferred to a 2 ml GC auto sampler vial and stored at 4 ºC until analyzed. Anions including nitrate-N, nitrite-N, and sulfate were analyzed by a Dionex 2010I ion chromatograph (IC) with an IONPAC AS4A-SC column and an electrochemical conductivity detector. The eluent for the IC was 1.7 mM sodium bicarbonate-1.8 mM sodium carbonate. External anion standards were used. Mixed liquid suspended solids samples were collected from the reactors and centrifuged at 12,100 ´g for 10 minutes and the supernatant was filtered through 0.2 µm supor filters prior to analysis. ORP probes and pH/mV meters were used to monitor the ORP in both SBRs. The probes were standardized with Light's solution according to Standard Methods [APHA, 1995]. The ORP data

reported below are referenced to the silver/silver chloride electrode.

3. Results & Discussion

3.1. Toxicity Assessment

To evaluate the possible toxicity caused by using hydrogen peroxide coupled with catalase to provide oxygen in the micro aerobic phase of the ANX/MA SBR, specific oxygen uptake rates (SOUR) were measured using biomass from a local domestic wastewater treatment plant that was not acclimated to hydrogen peroxide. Two oxygen supply methods, conventional diffused aeration and hydrogen peroxide coupled with catalase, were employed in the tests. SOUR data were collected from 6 independent tests for each oxygenation method (data not shown). At a test level of 5%, a pooled student t test (2 tails, assume equal variance) indicated that the mean of SOUR from each oxygenation method was not significantly different. Therefore, it is concluded that hydrogen peroxide did not impose toxicity on biomass in the ANX/MA SBR.

3.2. BTX Biodegradation in the ANX SBR

The ANX SBR was maintained for approximately 6 months after stable effluent quality was observed. Figure 1 shows the effluent BTX collected during this stable operating period. It can be concluded from Figure 1 that toluene and *m*-xylene were biodegradable under denitrifying conditions except on the days immediately after intensive samplings, which consumed 1/3 of the biomass volume. Benzene, *o*-, and *p*-xylene were recalcitrant to anoxic biodegradation throughout the study. A typical performance profile is shown in Figure 2. ORP was maintained between -200 mV and -100 mV under anoxic conditions as shown in Figure 2a. This range concurs with the typical values observed under anoxic conditions [Koch, 1985]. Nitrite-N and nitrate-N were used as electron acceptors, as shown in Figure 2b. The presence of nitrite-N at time zero is due to the oxidation of biogenic substrates as the substrates and mineral salts were pumped into the reactor. The accumulation of nitrite occurred due to the influent COD: NO3--N ratio and is typical in denitrifying systems [Glass, 1998]. Figure 2c shows that it took approximately 6 and 9 hours to completely biodegrade toluene and *m*-xylene in the ANX SBR, respectively. The concentration of benzene, *o*-xylene and *p*-xylene remained relatively constant throughout the reaction cycle, confirming that these compounds were recalcitrant to anoxic biodegradation.

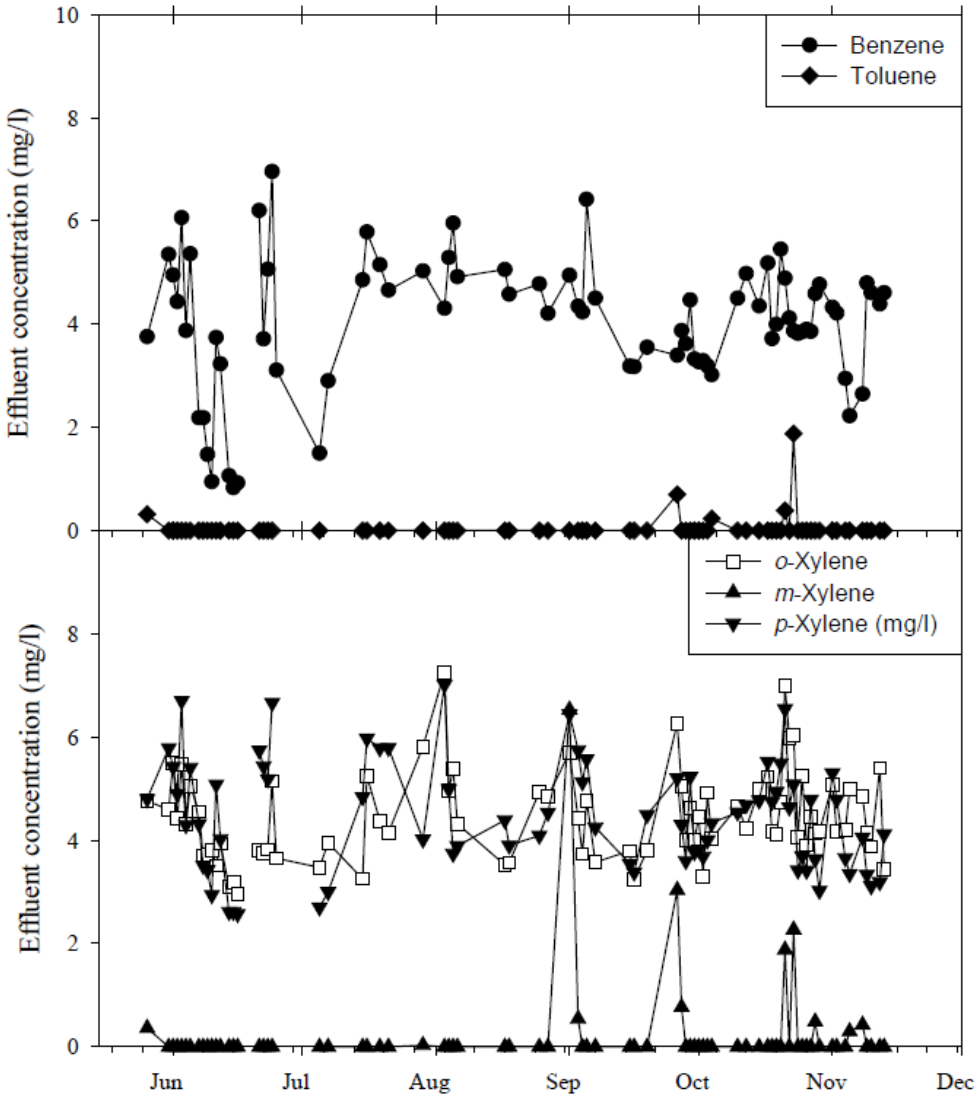

Figure 1. BTX biodegradation in anoxic SBR

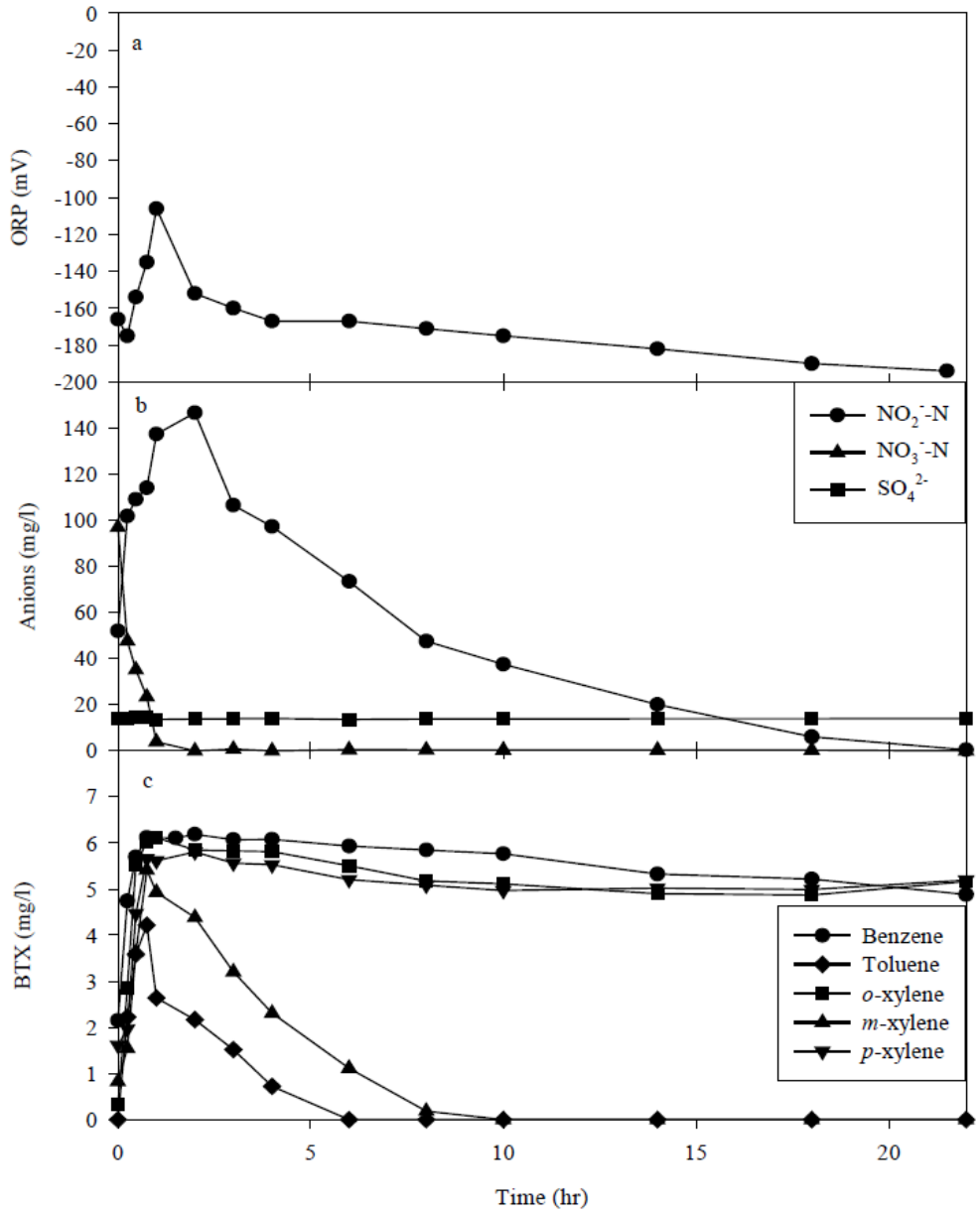

Figure 2. Profile of the anoxic SBR

3.3. BTX Biodegradation in the ANX/MA SBR

All five BTX compounds were biodegraded in the ANX/MA SBR since the effluent BTX concentrations were not detectable (data not shown). A typical profile is shown in Figure 3. Under anoxic conditions, the ANX/MA SBR showed the same pattern as the ANX SBR. When the reactor environment shifted to micro aerobic conditions, benzene, *o*-, and *p*-xylene, the compounds that were recalcitrant to anoxic metabolism, were biodegraded under micro aerobic conditions as shown in Figure 3c. The anions data in Figure 3b showed that nitrite-N was used as an alternative electron acceptor in addition to oxygen under micro aerobic conditions. Initially, we attempted to use a dissolved oxygen (DO) probe with a detection limit of 0.2 mg/l to monitor DO in the micro aerobic zone, but DO was routinely below the level of detection. However, Figure 3a shows that the two different redox environments can be distinguished by using an ORP probe. Under micro aerobic conditions, the ORP readouts were between -50 mV to -20 mV.

4. Conclusions

This study showed that only toluene and *m*-xylene were amenable to anoxic biodegradation while benzene, *o*-, and *p*-xylene were biodegradable under microaerobic conditions which were generated without agitation. We successfully used an ORP probe to distinguish between micro aerobic conditions and anoxic (denitrifying) conditions. Therefore, ORP probes may be used for process control purposes within SBR systems employing micro aerobic treatment strategies. The incorporation of anoxic conditions in sequencing batch reactors for treating BTX containing wastewater has several advantages. Firstly, nitrate-N serves as an electron acceptor for the biodegradation of biogenic substrates; therefore, high oxygen supplies at the beginning of reaction cycles that are commonly seen in SBRs can be eliminated. If nitrate is not generated internally through nitrification, a cost analysis of adding nitrate versus oxygen would have to be conducted to determine the feasibility of this approach.

Secondly, since toluene and *m*-xylene are biodegradable under anoxic conditions, the stripping of BTX caused by aeration was reduced. In addition, this study showed that benzene, *o*- and *p*-xylene were amenable to micro aerobic metabolism. The stripping of BTX under micro aerobic conditions can be minimized by employing bubble less membrane aeration systems which will minimize vigorous aeration for full scale applications. Therefore, the alternating anoxic/micro aerobic SBR may provide an alternative process other than conventional aerobic process for treating wastewaters containing BTX (and possibly other volatile) compounds.

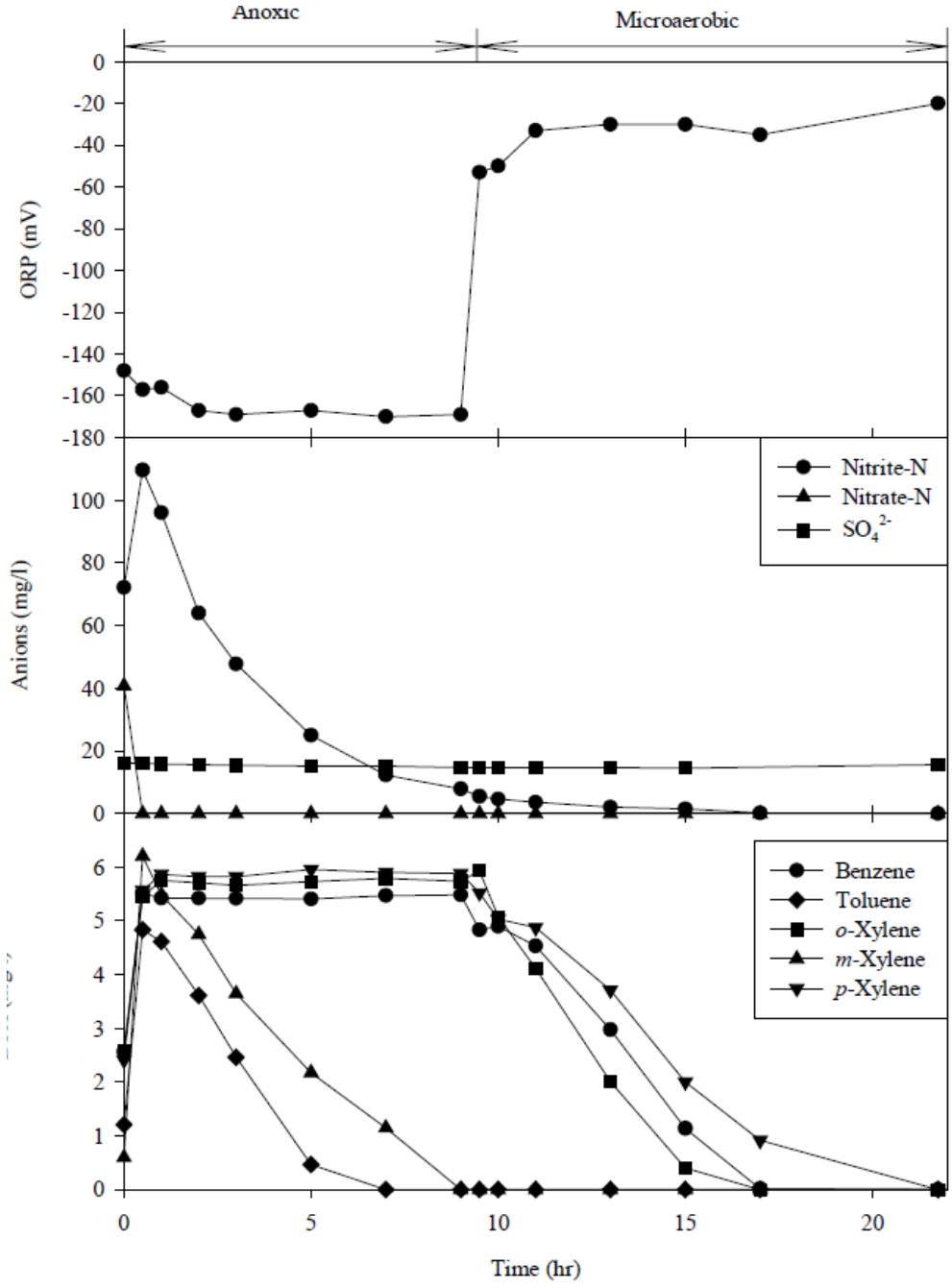

Figure 3. BTX biodegradation in ANX/MA SBR

References

[1] Alvarez, P. J. J., and T. M. Vogel. 1995. Degradation of BTEX and their aerobic metabolites by indigenous microorganisms under nitrate reducing conditions. *Wat. Sci. Tech.* 31: 15-28.

[2] APHA, AWWA, and WEF. 1995. Standard methods for the examination of water and wastewater. 19th ed. Washington, D. C.

[3] Bailey, E. L., and N. G. Love. 1999. Treatment of a wastewater containing nitrification inhibiting oximes using a single-sludge nitrogen removal treatment system. *Wat. Environ. Res.* 71: 94-101.

[4] Ball, H. A., and M. Reinhard. 1996. Monoaromatic hydrocarbon transformation under anaerobic conditions at seal beach, California: laboratory studies. *Environ. Toxicol. Chem* 15: 114-122.

[5] Biegert, T., and G. Fuchs. 1995. Anaerobic oxidation of toluene (analogues) to benzoate (analogues) by whole cells and by cell extracts of a denitrifying *Thauera* sp. *Arch. Microbiol.* 163: 407-417.

[6] Burland, S. M., and E. A. Edwards. 1999. Anaerobic benzene degradation linked to nitrate reduction. *Appl. Environ. Microbiol.* 65: 529-533.

[7] Chin, K. K. 1994. Evaluation of treatment efficiency of processes for petroleum refinery wastewater. *Wat. Sci. Tech.* 29: 47-50.

[8] Dold, P. L. 1989. Current practice for treatment of petroleum refinery wastewater and toxics removal. *Wat. Poll. Res. J. Canada* 24: 363-390.

[9] Evans, P. J., D. T. Mang, K. S. Kim, and L. Y. Young. 1991. Anaerobic degradation of toluene by denitrifying bacterium. *Appl. Environ. Microbiol.* 57: 1139-1145.

[10] Fettig, J. D. 1998. Stoichiometry and kinetics of BTX degradation by a denitrifying activated sludge consortium receiving a mixed waste. Master thesis. Virginia Polytechnic Institute and State University

[11] Fries, M. R., J. Z. Zhou, J. Chee-Sanford, and J. M. Tiedje. 1994. Isolation, characterization, and distribution of denitrifying toluene degraders from a variety of habitats. *Appl. Environ. Microbiol.* 60: 2802-2810.

[12] Glass, C., and J. Silverstein. 1998. Denitrification kinetics of high nitrate concentration water: pH effect on inhibition and nitrite accumulation. *Wat. Res.* 32: 831-839.

[13] Koch, F. A., and W. K. Oldham. 1985. Oxidation-reduction potential - A tool for monitoring, control and optimization of biological nutrient removal systems. *Wat. Sci. Tech.* 17: 259-281.

[14] Kukor, J. J., and R. H. Olsen. 1996. Catechol 2, 3-dioxygenases functional in oxygen limited (hypoxic) environments. *Appl. Environ. Microbiol.* 62: 1728-1740.

[15] Langenhoff, A. A. M., A. J. B. Zehnder, and Schraa G. 1996. Behavior of toluene, benzene and naphthalene under anaerobic conditions in sediment columns. *Biodegradation* 7: 267-274.

[16] Leahy, J. G., and R. H. Olsen. 1997. Kinetics of toluene degradation by toluene-oxidizing bacteria as a function of oxygen concentration, and the effect of nitrate. *FEMS Microbiol. Ecol.* 23.

[17] Rabus, R., and F. Widdel. 1995. Anaerobic degradation of ethyl benzene and other aromatic hydrocarbons by new denitrigying bacteria. *Arch. Microbiol.* 163: 96-103.

[18] Reinhard, M., S. Shang, P. K. Kitanidis, E. Orwin, G. D. Hopkins, and C. A. Lebron. 1997. *In situ* BTEX biotransformation under enhanced nitrate- and sulfate-reducing conditions. *Environ. Sci. Technol.* 31: 28-36.

[19] Su, J. J., and D. Kafkewitz. 1994. Utilization of toluene and xylenes by a nitrate reducing strain of *Pseudomonas maltophilia* under low oxygen and anoxic conditions. *FEMS Microbiol. Ecol.* 15: 249-258.