original article

Anaerobic biodegradation of ethylene dichloride in an anaerobic sequential batch reactor

Asadollah Nadi, Ali Fatehizadeh[1,2], Amir Hesam Hassani[3], Mohammad Reza Marasy[1,4], Mohammad Mehdi Amin[1,2]

Department of HSE, Bandar Imam Petrochemical Industries, Khouzestan, Iran, [1]Environment Research Center, Isfahan University of Medical Sciences (IUMS), Isfahan, Iran, [2]Departments of Environmental Health Engineering, IUMS, Isfahan, Iran, [4]Biostatistics and Epidemiology, School of Health, IUMS, Isfahan, Iran, [3]School of Environment and Energy, Science and Research Branch, Islamic Azad University of Tehran, Tehran, Iran

Address for correspondence:
Dr. Mohammad Mehdi Amin,
Environment Research Center,
Isfahan University of Medical Sciences,
Hezar-Jerib Avenue, Isfahan, Iran.
E-mail: amin@hlth.mui.ac.ir

ABSTRACT

Aims: The efficiency of an anaerobic sequencing batch reactor (ASBR) in ethylene dichloride (EDC) and chemical oxygen demand (COD) removal at different operational conditions was evaluated.
Materials and Methods: Biological EDC and COD removal was studied in a laboratory scale ASBR. The ASBR was seeded at the start-up with granular anaerobic sludge of sugarcane industry and operated at different organic loading rates (OLR), EDC loading rates, and influent concentration of COD and EDC.
Results: During start-up period, COD removal efficiencies of above 80% were selected for reactor adaptation to achieve steady state during 48 days of operation. Maximum COD removal efficiency was 95% with an influent COD concentration of 1700 mg/L at 0.5 gCOD/L.d, and the efficiency rapidly dropped with increasing influent COD concentrations and OLR. When the EDC loading rate was adjusted between 0.6 to 4.7 gCOD/L.d, the EDC removal efficiencies were 95% and 46%, respectively, with influent EDC concentrations of 2000 and 16000 mg/L at the end of EDC loading stage. The kinetic study showed that the EDC and COD removal by ASBR followed the second order kinetic.
Conclusions: Based on the results of this study, the ASBR process is successfully applicable for biodegradation of the COD and EDC (>90%) in wastewater treatment. The kinetic study showed that, at same time, ASBR capable to removing COD rather than EDC.

Key words: Anaerobic biodegradation, ASBR, ethylene dichloride, kinetic, volatile fatty acids

INTRODUCTION

Ethylene dichloride (EDC) (Cl-CH_2-CH_2-Cl) or 1,2-dichloroethane is a synthetic chemical, which has no known natural sources. EDC is dominantly used as an intermediate in the synthesis of vinyl chloride and is also used in the production of chlorinated solvents such as trichloroethene, tetrachloroethene, and 1, 1, 1-trichloroethane.[1] EDC is one of the major halogenated organic pollutants that were detected in groundwater and industrial effluents.[2] EDC is a known carcinogen due to the conversion into chloroacetaldehyde, which is considered to have mutagenic properties.[2,3] Other health effect of EDC consists of strongly irritating to the eyes and upper respiratory tract of man and on the central nervous system (CNS).[4]

Access this article online	
Quick Response Code:	Website: www.ijehe.org
	DOI: 10.4103/2277-9183.100137

This article may be cited as:
Nadi A, Fatehizadeh A, Hassani AH, Marasy MR, Amin MM. Anaerobic biodegradation of ethylene dichloride in an anaerobic sequential batch reactor. Int J Env Health Eng 2012;1:35.

Due to persistence and toxicity of EDC, it is subjected to degradation by physico-chemical or biological methods rather than phase change (e.g., adsorption on sludge or gas stripping).[1]

Anaerobic digestion is a controlled biodegradation process that converts organic matter in wastewater into biogas. Conventional digesters used for wastewater treatment include continuously stirred tank reactors (CSTR) and plug-flow reactors.[5-7]In these two types of digesters, hydraulic retention time (HRT) equals solid retention time (SRT) and active biomass is removed from the digester in the effluent on a daily basis.[6] The HRT needs to be long enough to ensure a sufficient SRT in the digester so that a viable bacteria population necessary for complete anaerobic digestion process is maintained. The minimum SRT varies with the digester temperature and generally decreases with the increase of temperature.[6,8]

A conventional anaerobic sequential batch reactor (ASBR) is operated with intermittent cycles of four stages: Feeding or loading process of liquid influent, anaerobic biological reactions, biomass sedimentation, and effluent discharge with removal of sludge when necessary.[9,10] ASBR has been used for treating high-strength wastewaters (dairy, brewery, piggery, petrochemical and landfill leachate)[11-15] as well as for low-strength ones (domestic wastewater).[16,17] The main advantages of ASBR application are: (i) No short circuit, (ii) High efficiency for both chemical oxygen demand (COD) removal and gas production, (iii) No primary and secondary settles, and (iv) Flexible control.[13,18]

The purpose of this study was to evaluate the efficiency of an anaerobic sequencing batch reactor (ASBR) in COD and EDC removal at different operational conditions.

MATERIALS AND METHODS

Anaerobic Sequence Batch Reactor Set-Up

A glass ASBR with a working volume of 2.5 L, an internal diameter of 10 cm, and height of 32 cm was used to carry out the experiments. A schematic of the ASBR set-up is given in Figure 1.

According to previous studies, all the experiments were performed at 35 ± 0.5°C by circulating warm oil around the reactors.[19]

Substrate and Seed Sludge

The synthetic substrates consist of EDC and acetic acid as main and auxiliary substrate, respectively. The nutrients and trace elements with following composition were used: $CaCl_2$ (0.008 mg/L), $CoCl_2$ (0.051 mg/L), $FeCl_3.6H_2O$ (2.465 mg/L), $NaHPO_4.7H_2O$ (2.237 mg/L), KH_2PO_4 (0.874 mg/L), $NaHCO_3$ (25.7 mg/L), $FeSO_4.7H_2O$ (5.1 mg/L) and $MgSO_4.7H_2O$ (3.6 mg/L). The system was inoculated with granular anaerobic sludge obtained from the anaerobic digester of the sugarcane industry wastewater treatment plant (KeshtoSanate Ahwaz, Iran). The sludge is typically dispersed or granulates with 20120 mg/L of volatile suspended solids (VSS), 28750 mg/L of suspended solids (SS) and VSS/SS ratio of 0.7.

Reactor Start-Up and Operation

The phase duration and operation condition of ASBR are set according to previously described details by Ghasemian, *et al.*,[20] demonstrated in Table 1. For adaptation of microorganisms, achieving an acceptable COD removal (>80% removal) and obtaining the steady state condition, the start-up phase consisting of two stages: First, the reactor was operated with an OLR of 0.5 gCOD/L.d for 30 days of operation. At second stage (31–48 days), the OLR was sharply augmented and ASBR was operated at OLR level of 1 gCOD/L.d. In this time, the input EDC concentration to the reactor was zero. Table 2 shows the main operating parameters of the ASBR system.

Chemicals

The EDC was obtained from Merck Co, Germany. All other chemicals were of analytical grade and used without further purification.

Analytical Methods

The pH and COD were analyzed according to the Standard Methods for the Examination of Water and Wastewater. [21] EDC was determined by gas chromatograph (Tekmar Dohrmann 3800). The liquid sample was filtered through a 0.45 μm membrane filter and injection into the column and directly analyzed in gas chromatograph equipped with capillary column (Thermo TR-VI 30 m × 0.32 mm × 1.8 mm). The purge and trap (P and T) was used in order to water removal from samples. Nitrogen was used as a carrier gas (20 mL/min) with electron capture detector (ECD) as a detector. Injector temperature and detector temperature were 250°C and 280°C, respectively. The column oven temperature program was initially 70°C, ramped up at 10°C/min to 150°C, holding for 1 minute, and ramped up at 25°C/min to 280°C. Volatile fatty acids (VFA) in the effluent were measured by injecting 2 mL of filtered acidified samples through gas chromatograph (Agilent Techno 7890A. 5975C) equipped with flame ionization detector (GC-FID) using a 10% free fatty acid phase. The analysis was carried out at an oven temperature of 150°C, injector temperature of 180°C and detector temperature of 250°C. Nitrogen was applied as a carrier gas at a flow rate of 20 mL/min.

RESULTS

Start-Up and Acclimation

The start-up and acclimation stage was prolonged to 48 days with OLR of 0.5 to 1 gCOD/L.d and EDC concentration equal to zero. This stage was performed to achieve stable condition and acceptable COD removal efficiency. Figure 2 shows the results of acclimation stage at various OLR. The maximum COD removal efficiency was obtained with 1 gCOD/L.d at the end of 48 days' period.

Chemical Oxygen Demand (COD) Removal

The experiment lasted for 107 days. The time courses of COD in influent and effluent and OLR are shown in Figure 3. Four distinct phases are indicated with 1.7, 3.4, 6.8, 10.21 and 13.6 g/L of COD in the influent. The results showed that with increasing initial concentration of COD, the COD removal efficiency was decreased.

Ethylene Dichloride Removal

The results on EDC removal via ASBR investigated as a function of initial concentration of EDC and EDC loading rate are depicted in Figure 4. The results showed that with increasing initial concentration of EDC and EDC loading rate, the COD removal efficiency declined. After 43 days of ASBR operation, the concentration of EDC in the synthetic wastewater was decreased to 0.083 g/L (95% removal efficiency) when EDC initial concentration of 2 g/L and EDC loading rate of 0.59 gEDC/L.d was used.

Volatile Fatty Acid Profile

The effect of OLR on the variation of VFA content and pH solution is plotted in Figure 5. At the beginning of the changing in OLR, the pH decreased to <7 and then was augmented to >8. At this time, the VFA concentration was descended. In addition, the high OLR resulted in the enhancement of VFA concentration.

Typical patterns of VFA concentration profile as function of pH solution in ASBR are shown for all OLRs in Figure 6. According to Figure 6, with increasing pH up to 7.5, the VFA content was increased. After this point, VFA concentration was significantly decreased and then arises.

Table 1: Details of working cycle in the ASBR system

Stage	Fill	React	Settle	Decant
HRT (min)	10	1360	60	10
Feed pump	On	Off	Off	Off
Mixer	On	On	Off	Off
Decant valve	Off	Off	Off	On

Performance of ASBR in an Operation Cycle

Profiles of operational parameters in a cycle are presented in Figure 7. The result showed that the COD and EDC removal increased from 19% to 73% and 20% to 75%, respectively, when HRT varied from 0.5 h to 24 h. During this period, the pH of the solution was increased from 7 to 8.92 and followed by gradual decrease to 8.14.

DISCUSSION

ASBR Performance on COD and EDC Removal

During the start-up period, COD concentration in the effluent was kept at 1700 mg/L whereas the OLR was promptly increased from 0.5 to 1 gCOD/L.d. Correspondingly, the COD removal efficiency was more than 80% after 40 days ASBR operation. In this time, the pH value increased gradually from 7 to 8.83. This result revealed that methanogenic bacteria populations are dominated and capable to consume VFA as a substrate source.

According to Figures 3 and 4, there was significant fluctuation on OLR and EDC loading rate in the experiment, and the COD and EDC removal efficiency were varied. In each stage of OLR and EDC loading rate, with increasing SRT, the COD and EDC removal was increased. In first stage of OLR and EDC loading rate, increasing the SRT from 49 to 92 days resulted in the COD and EDC removal efficiency to increase from 31% to 95% and 36% to 95%, respectively. A gradual decrease in COD removal was observed with OLR increases during the experimental phase. The COD removal efficiencies decreased from 95% to 48% for OLR fluctuation from 0.5 to 4 g COD/cycle.

Variations in COD and EDC removal efficiencies at the same volumetric loading rates were due to the variations in specific loading rates.[22] The results clearly show that the presence of bacterial species cause COD and EDC biodegradation. With variation of OLR and EDC loading rate, COD and EDC removal efficiency fluctuated. These changes in COD and EDC removal indicate an increase in biological activity, which would also suggest an increase in pH effluent, as observed in past studies.[12] Theoretically, feeding material to the microbial reactor can be removed by three possible mechanisms: (i) Adsorption onto the sludge, (ii) biological degradation and (iii) stripping into the gas phase.[23-25]

Table 2: Operating parameters of the ASBR reactor

Parameter	Start-up (day)		Operation (day)				
	1-30	30-48	49-92	93-108	109-124	125-140	141-163
Flow (L/d)	0.735	0.735	0.735	0.735	0.735	0.735	0.735
COD_{Inf} (mg/L)	1700	3400	1700	3400	6800	10210	13600
OLR (gCOD/L.d)	0.5	1	0.5	1	2	3	4
F/M (d^{-1})	0.04	0.04	0.04	0.09	0.17	0.31	0.35
EDC_{Inf} (mg/L)	0	0	2000	4000	8000	12000	16000
EDC loading rate (gEDC/L.d)	0	0	0.59	1.18	2.35	3.53	4.71
MLSS (mg/L)	11500	11500	11500	11500	11500	11500	11500

Under anaerobic conditions, most of the wastewater COD is converted to methane emitted into the gas phase. In bio-sludge, the main transformation of EDC was carried out by dichloro elimination reactions, resulting in the production of ethane (65–70%). Another type of reductive dechlorination mechanism, reductive hydrogenolysis has produced only small amounts of ethane (less than 1%). A conversion of EDC into carbon dioxide cannot be excluded.[1,26] EDC changed very little without the addition of electron donor.[1,3] In some previous studies, it has been mentioned that species such as Xanthobacter, Pseudomonas, Desulfobacteriur and Mycobacterium are capable to biodegrade the chlorinated hydrocarbons.[11,27-29]

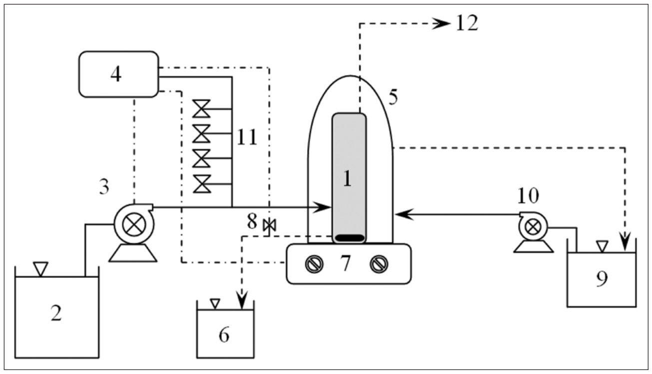

Figure 1: Experimental setup of the ASBR used in this study (1: ASBR, 2: Substrate reservoir, 3: Feed pump, 4: PLC, 5: Bath room, 6: Effluent reservoir, 7: Magnetic stirrer, 8: Electronic valve, 9: Oil reservoir, 10: Oil pump, 11: Solenoid valves and 12: Gas meter)

Profile of VFA Content

VFA is an intermediate and indicator in the anaerobic process. Because the acid-producing bacteria grow more quickly than the acid-consuming bacteria on readily biodegradable organics, it will

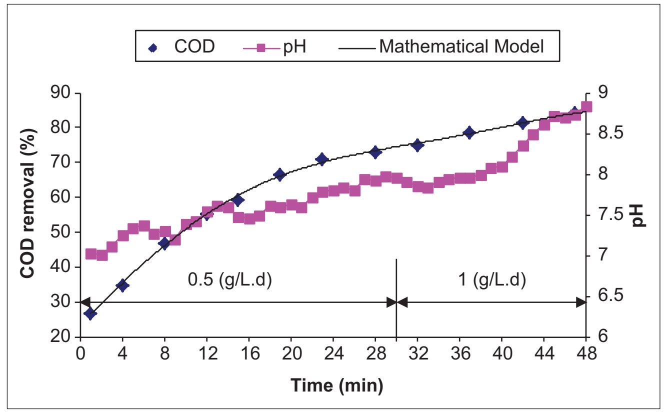

Figure 2: Removal of COD and pH solution variation during acclimation (COD: 1700 mg/L and pH: 7 ± 0.04)

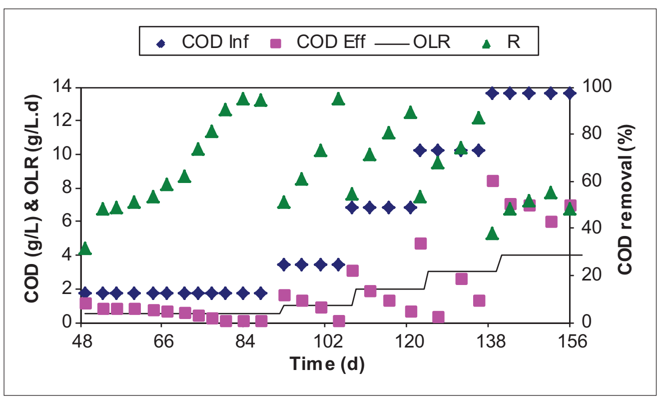

Figure 3: The variation of COD_{Inf}, COD_{Eff} and organic loading rate (OLR) during the experiment

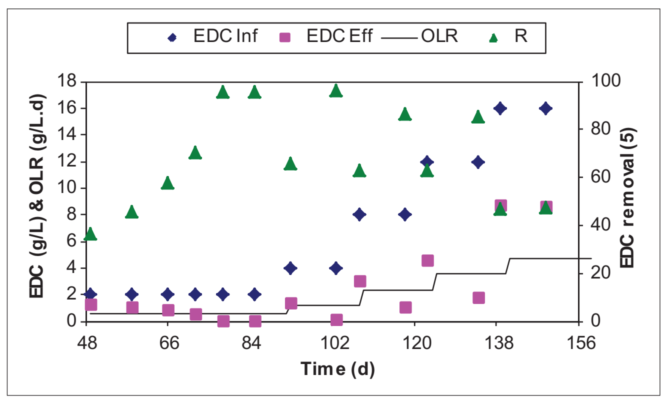

Figure 4: The fluctuation of EDC_{Inf}, EDC_{Eff} and organic loading rate (OLR) during the experiment

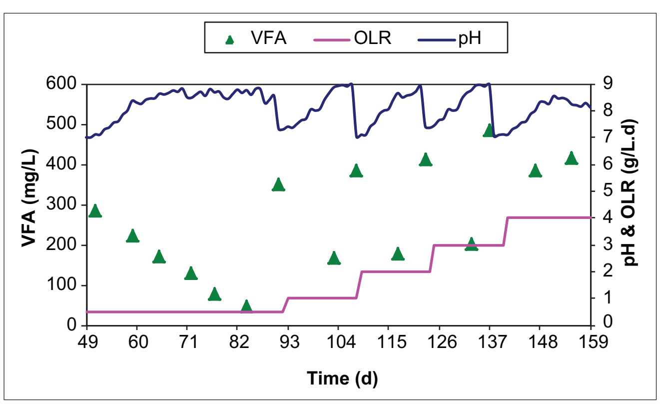

Figure 5: The variation of pH solution and volatile fatty acid (VFA) in ASBR operation

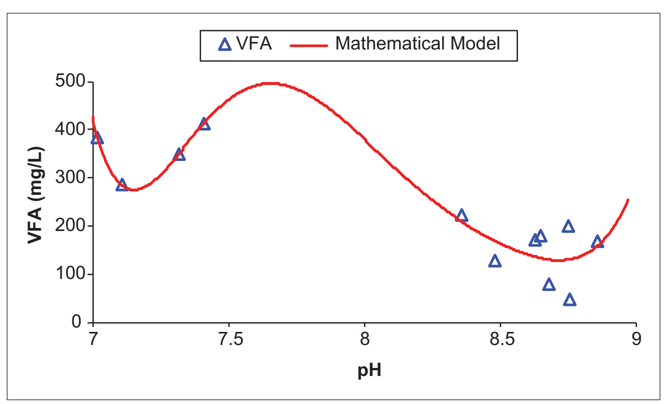

Figure 6: Variation of volatile fatty acid (VFA) content as function of pH solution for all organic loading rates (OLR)

provoke accumulation of these acids, mainly at the beginning of a cycle, which may lead to pH reduction. Previous studies have shown that acetic, propionic, valeric, caproic, formic and butyric acids were the major constituents of VFA.[30,31]

Therefore, it is critical to balance the VFA production and utilization in order to keep a low VFA concentration in a cycle.[13] VFAs are not directly used by methanogens, but serve as substrates for proton-reducing syntrophic bacteria.[31]

From Figure 5, VFAs were mostly consumed in the ASBR system. At each stage of OLR, with increasing of SRT, the VFA content was decreased. In first stage of OLR, VFA concentration was measured 48 mg/L instead of 286 mg/L and at end of period.

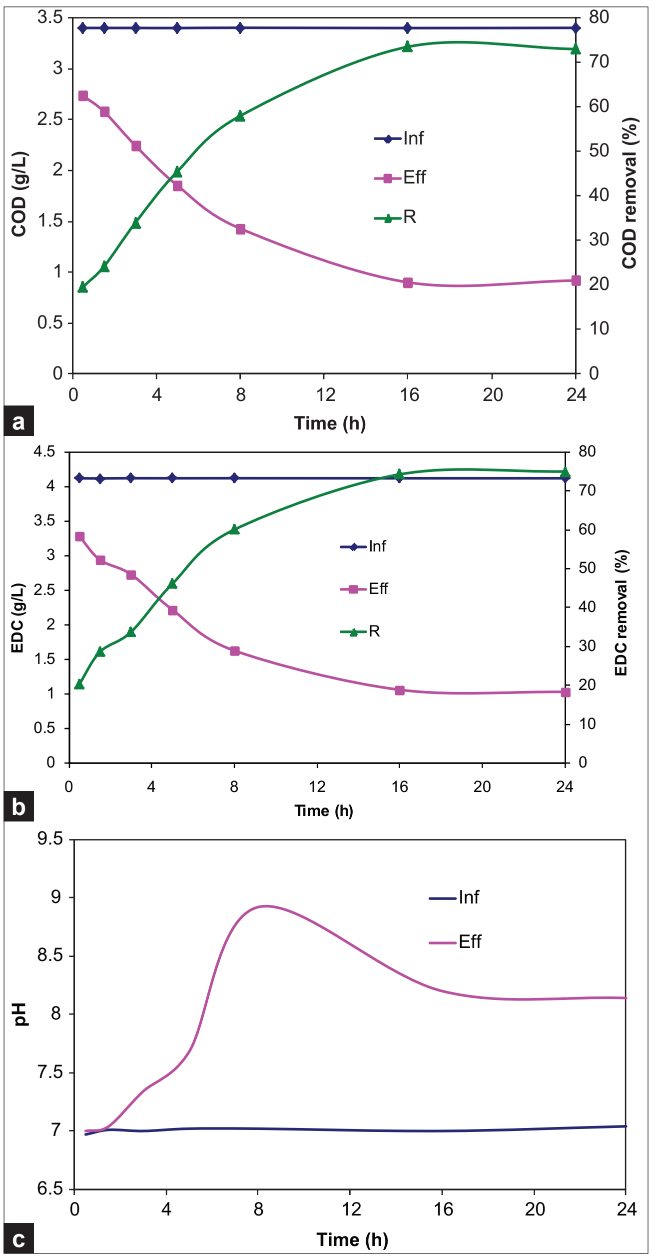

Figure 7: Performance of ASBR on (a) COD removal, (b) EDC removal and (c) pH variation in a cycle of operation

Temperature significantly affected the VFA degradation with higher VFA degradation occurring at a lower reaction temperature. However, this does not seem to result in significantly higher production of methane. This obvious imbalance may be attributed to less conversion efficiency of VFAs to methane at the lower temperature than at the higher temperature.[32]

The Variation of pH Solution in ASBR

The amounts of pH solution reflected the acid concentration results, which accumulated acid the most rapidly, reaching the lowest pH during the beginning of OLR changing [Figure 5].

Anaerobic reactions are highly pH dependent. The optimal pH range for methane producing bacteria is 6.8–7.2, while acid-forming bacteria can stand under more acidic pH values.[17] In initial anaerobic digestion, with increasing the amount of VFA, the pH was declined. This fact is related to accumulation of VFA, at beginning, methanogenic bacteria are unable to consume the high amount of VFA produced by acid production bacteria.[9]

After transition to methanogenic conditions, pH values increased and total alkalinity concentrations tended to decrease because methanogens utilized the available VFA as substrate.[30] On the other hand, the bicarbonate alkalinity (BA) generation and the low values of VFA in the previous periods were considered indicators of the balance between acidogenesis and methanogenesis.[10]

Adequate alkalinity, or buffer capacity, is necessary to maintain a stable pH in the anaerobic reactor for optimal biological activity. Tchobanoglous, *et al.* mentioned that an alkalinity level ranging from 1000 to 5000 mg/L as $CaCO_3$ is necessary.[33] This link between VFA removal and pH increase was observed by Van Gulck, *et al.* and Lozecznik, *et al.*[12,34]

Kinetic Study

Variations in composition and concentration of materials in the reactor are the main factors in the purification of water and wastewater. To completely describe a reactor system and its design, reaction rates that occur in the reactor must be specified because these rates directly affect the reactor size. Therefore, the study of reaction kinetics to predict pollutant removal rates is very important in designing and modeling the treatment process.[33]

Determination of the kinetics of the ASBR process on COD and EDC removal reactions is necessary to estimate an optimum time required for the removal reaction.

A kinetic analysis was conducted by fitting the time-course performance data with zero, first, and pseudo-second-order kinetic equations as shown in Table 3 and Figure 8, where r_c is the rate of conversion, k_0, k_1, and k_2 are reaction rate coefficients, t is time, and C_0 and C are the initial and final concentration of the constituent in the liquid, respectively.

Table 3: Equations and linear forms and results of kinetics model

Kinetic model	Equation	Linear form	Parameter	COD	EDC
Zero-order	$r_c = \frac{dC}{dt} = k_0$	$C - C_0 = -k_0 t$	k_0	0.092	0.112
			R^2	0.78	0.77
First-order	$r_c = \frac{dC}{dt} = k_1 C$	$\ln\frac{C}{C_0} = -k_1 t$	k_1	0.054	0.057
			R^2	0.89	0.87
Second-order	$r_c = \frac{dC}{dt} = k_2 C^2$	$\frac{1}{C} - \frac{1}{C_0} = k_2 t$	k_2	0.036	0.034
			R^2	0.92	0.94

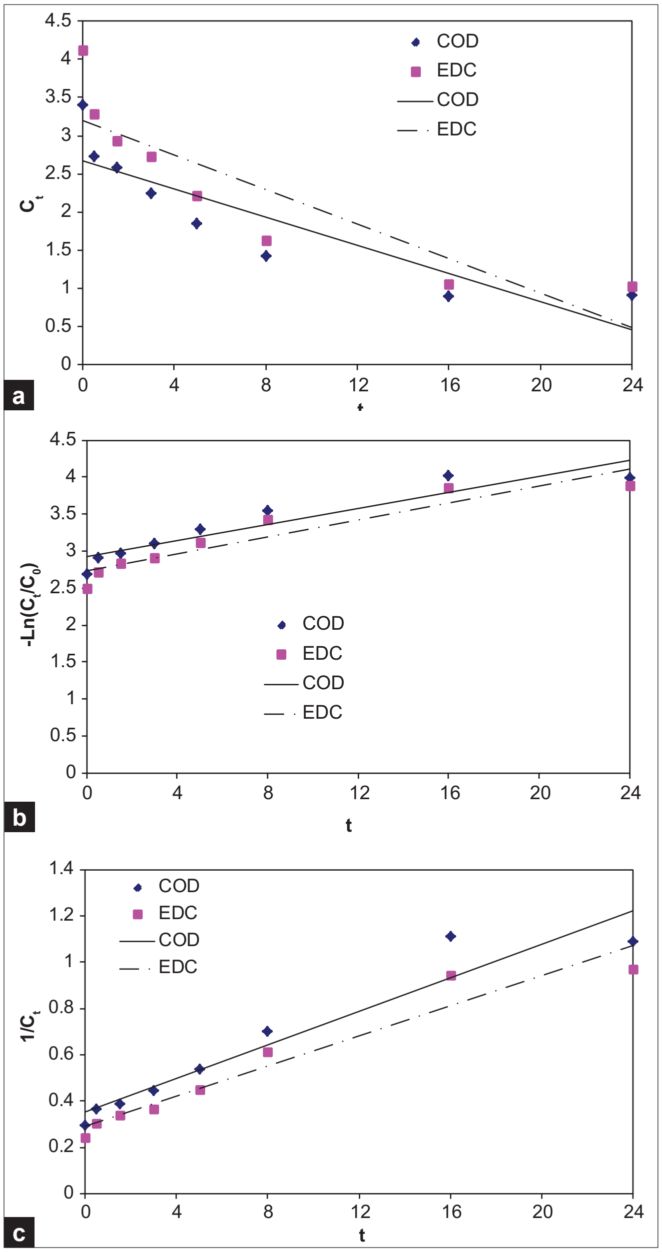

Figure 8: Kinetics study of EDC and COD removal in a cycle of ASBR operation (a) Zero order, (b) First order and (c) Second order

The data were properly correlated (with a higher R^2 than the other models) with the second-order kinetic model revealing that the second-order model can successfully simulate the COD and EDC removal in the ASBR ($R^2 > 0.92$). Second-order reaction showed that the reaction progressed at a rate proportional to the second power of the reactants initial concentrations. According to Table 3, the second-order kinetic constant (k_2) related to the COD removal was higher than that for the EDC removal (0.036 versus 0.034). It means that, at the same time, ASBR was capable to reduce COD more than EDC.

The results of this study clearly showed that the ASBR was able to achieve high removal efficiencies related to organic matter and EDC. The authors suggest that the ASBR technology as a simple, efficient and reliable method performs a good potential and could be an alternative method for the treatment of wastewater containing EDC.

ACKNOWLEDGMENTS

The authors wish to thank to Research Center of Bandar Imam Petrochemical Industries for financial support of this research project.

REFERENCES

1. Gupta SK, Mali SC. Reductive dechlorination of 1,2-dichloroethane using anaerobic sequencing batch reactor (ASBR). Water Sci Technol 2008;57:225-9.
2. Mc Cann J, Simmon V, Streitwieser D, Ames BN. Mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane Zethylene dichloride., chloroethanol Zethylene chlorohydrin., vinyl chloride, and cyclophosphamide. Proc Natl Acad Sci U S A 1975;72:3190-3.
3. Wildeman SD, Nollet H, Langenhove HV, Verstraete W. Reductive biodegradation of 1,2-dichloroethane by methanogenic granular sludge in lab-scale UASB reactors. Adv Environ Res 2001;6:17-27.
4. Bowler RM, Gysens S, Hartney C. Neuropsychological Effects of Ethylene Dichloride Exposure. Neurotoxicology 2003;24:553-62.
5. Wohlt JE, Frobish RA, Davis CL, Bryant MP, Mackie RI. Thermophilic methane production from dairy cattle waste. Biol Wastes 1990;32:193-207.
6. Zhang RH, Tao J, Dugba PN. Evaluation of two-stage anaerobic sequencing batch reactor systems for animalwastewater treatment. Ame Soc Agri Eng 2000;43:1795-801.
7. Lo KV, Liao PH. High-rate anaerobic digestion of screened dairy manure. J Agric Eng Res 1985;32:349-58.
8. Pfeffer JT, Leiter M, Worland JR. Population dynamics in anaerobic digestion. J Water Pollut Control Fed 1967;39:1305-22.
9. Ruiz C, Torrijos M, Sousbie P, Martinez J, Moletta R. The anaeribic SBR process: Basic principal for design and aytomation. Water Sci Technol

2001;43:201-8.
10. Sarti A, Zaiat M. Anaerobic treatment of sulfate-rich wastewater in an anaerobic sequential batch reactor (AnSBR) using butanol as the carbon source. J Environ Manage 2011;92:1537-41.
11. Shokrollahzadeh S, Azizmohseni F, Golmohammad F, Shokouhi H, Khademhaghighat F. Biodegradation potential and bacterial diversity of a petrochemical wastewater treatment plant in Iran. Bioresour Technol 2008;99:6127-33.
12. Lozecznik S, Sparling R, Oleszkiewicz JA, Clark S, Van Gulck JF. Leachate treatment before injection into a bioreactor landfill: Clogging potential reduction and benefits of using methanogenesis. Waste Manag 2010;30:2030-6.
13. Xiangwen S, Dangcong P, Zhaohua T, Xinghua J. Treatment of brewery wastewater using anaerobic sequencing batch reactor (ASBR). Bioresour Technol 2008;99:3182-6.
14. Dugba PN, Zhang R. Treatment of dairy wastewater with two stage anaerobic sequencing batch reactor systems: Thermophic versus mesophilic operations. Bioresour Technol 1999;68:225-33.
15. Wun-Jern N. A Sequencing Batch Anaerobic Reactor for Treating Piggery Wastewater. Biol Wastes 1989;28:39-51.
16. Kayranli B, Ugurlu A. Effects of temperature and biomass concentration on the performance of anaerobic sequencing batch reactor treating low strength wastewater. Desalination 2011;278:77-83.
17. Sarti A, Garcia ML, Zaiat M, Foresti E. Domestic sewage treatment in a pilotscale anaerobic sequencing batch biofilm reactor. Resour Conserv Recycl 2007;51:237-47.
18. Shizas I, Bagley DM. Improving anaerobic sequencing batch reactor performance by modifying operationalparameters. Water Res 2002;36:363-7.
19. Ndon UJ, Dague RR. Effects of temperature and hydraulic retention time on anaerobic sequencing batch reactor treatment of low-strength wastewater. Water Res 1997;31:2455-66.
20. Ghasemian M, Amin MM, Morgenroth E, Jaafarzadeh N. Anaerobic biodegradation of methyl tert-butyl ether and tert-butyl alcohol in petrochemical wastewater. Environ Technol 2012:1-7.
21. APHA. Standard Methods for the Examination of Water and Wastewater. 21th ed. Washington, DC: American Public Health Association; 2005.
22. Timur H, Oeztur I. Anaerobic sequencing batch reactor treatment of landfill leachate. Water Res 1999;33:3225-30.
23. Van Eekert MH, Schröder TJ, van Rhee A, Stams AJ, Schraa G, Field JA. Constitutive dechlorination of chlorinated ethenes by a methanol degrading methanogenic consortium. Bioresour Technol 2001;77:163-70.
24. Hwu CS, Lu CJ. Continuous Dechlorination of Tetrachloroethene in an Upflow Anaerobic Sludge Blanket Reactor. Biotechnol Lett 2008;30:1589-93.
25. Amin MM, Faraji M, Momenbeik F, Hasanzadeh A, Sadani M. Performance evaluation of an Anaerobic Migrating Blanket Reactor in the biodegradation of perchloroethylene from industrial wastewaters. Int J Env Health Eng 2012;1:1
26. Bouwer EJ, Mc Carty PL. Transformations of 1-carbon and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl Environ Microbiol 1983;45:1286-94.
27. Hashimoto A, Iwasaki K, Nakasugi N, Nakajami M, Yagi O. Degradation of trichloroethylene and related compounds by Mycobacterium sp. isolated from soil. Clean Production Process 2000;2:167-73.
28. Baptista R II, Peeva LG, Zhou NY, Leak DJ, Mantalaris A, Livingston AG. Stability and performance of Xanthobacter autotrophicus GJ10 during 1,2-dichloroethane biodegradation. App Environ Microbiol 2006;72(6):4411-7.
29. Egli C, Scholtz R, Cook AM, Leisinger T. Anaerobic dechlorination of tetrachloromethane and 1,2-dichloroethane to degradable products by pure cultures of Desulfobacteriurn sp. and Methanobacterium sp. FEMS Microbiol Lett 1987;43:257-61.
30. Cheong DY, Hansen CL. Effect of feeding strategy on the stability of anaerobic sequencing batch reactor responses to organic loading conditions. Bioresour Technol 2008;99:5058-68.
31. Amin MM, Zilles J, Greiner J, Charbonneau S, Raskin L, Morgenroth E. Influence of the antibiotic erythromycin on anaerobic treatment of a pharmaceutical wastewater. Environ Sci Technol 2006;40:3971-7.
32. Ndegwa PM, Hamilton DW, Lalman JA, Cumba HJ. Effects of cycle-frequency and temperature on the performance of anaerobic sequencing batch reactors (ASBRs) treating swine waste. Bioresour Technol 2008;99:1972-80.
33. Tchobanoglous G, Burton F, Stensel H. Wastewater Engineering: Treatment, Disposal and Reuse 4 th Ed. New York: Mc Graw-Hill; 2003.
34. Van Gulck JF, Rowe RK, Rittmann BE, Cooke AJ. Biogeochemical calcium precipitation in landfill leachate collection systems. Biodegradation 2003;14:331-46.

Source of Support: Bandar Imam Petrochemical Industries, Khouzestan, Iran, **Conflict of Interest:** None declared.