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Biodegradation performance of anaerobic sequencing batch biofilm reactor for oil with polychlorinated biphenyls

Heshmatollah Moradpour, Ali Fatehizadeh, Mohammad Mehdi. Amin, Mahnaz Nikaeen, Arash Shafiea[1], Reza Molayi[1], Amin Sabouri[1], Mohammad Ghasemian, Hossien Farokhzaddeh

Environment Research Center, Isfahan University of Medical Sciences (IUMS), and Department of Environmental Health Engineering, School of Health, IUMS, Isfahan, Iran, [1]Isfahan Steel Company, Isfahan, 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 biodegradability of oil containing polychlorinated biphenyls (PCBs) from electrical transformer by the anaerobic sequencing biofilm batch reactor (ASBBR) with was assessed.

Materials and Methods: Two anaerobic sequencing batch biofilm reactor (ASBBR) containing polyurethane foam cubes as inert support was used. The reactors were operated for 310 days at 35 ± 2°C. The reactors with a total volume of 7 L, 5 L effective volume and 3.5 L for gas production, were operated in a cycle per day. The effect of operational parameters including organic loading rate, PCBs loading rate, co-substrate type, initial PCBs and COD concentration was evaluated.

Results: The results point to admirable reactors stability and over 95% efficiency in PCBs removal, with effluent PCBs concentration of lower than 10 mg/L. However, degradation rates increased as the initial concentration of PCBs as increased. The average of COD removal efficiency by two ASBBR reactors was more than 92% that corresponding to > 9 μg/L of effluent COD. In over all operation, average of biogas production in R1 was 5.7 ± 2.2 L/d and maximum produced biogas was 8.02 L/d at 310 day. The kinetic studies revealed that second – order kinetic model described the COD removal by ASBBR reactors from synthetic wastewater better than two other kinetic model.

Conclusion: Therefore, this investigation demonstrated that the ASBBR have good potential for biodegradation of oil containing PCBs, despite variation of influent PCBs and organic loading rate (OLR).

Key words: Anaerobic treatment, biodegradation, polychlorinated biphenyls

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are contaminants of great environmental concern. They are highly persistent and potentially carcinogenic compounds.[1,2] The characteristics of PCBs are including poorly biodegradable, highly hydrophobic and accumulate in sub-surface sediments.[2] PCBs have

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209 possible variants (congeners) including between one to ten chlorine atoms.[3] Commercial PCBs mixtures were distributed around the world by different trade names as Aroclors (United States), Chlophen (Germany) and Kanechlor (Japan). The aroclors have a distinct difference in respect of the chlorination degree of the biphenyl production procedures. The aroclors mixture are denoted by a four-digit number in which the first two places indicate the number of carbon atoms per molecule and the last two digit number indicate the mass percentage of chlorine in the mixture.[4] PCBs were widely used for industrial applications because of their chemical insulating properties, stability and resistance to burning and used in electrical transformers.

Under anaerobic conditions, anaerobic bacteria convert highly chlorinated congeners in to less chlorinated biphenyls by reductive dechlorination, leaving the ring intact.[5,6] Investigations on PCBs have shown that these compounds could be degraded by both anaerobic and aerobic bacteria. Such PCBs congeners are very recalcitrant to aerobic microbial metabolism. Some investigations demonstrated anaerobic microbial reduce dehalogenation of PCBs.[7,8] Thus, PCBs can be transformed in anaerobic bioreactors and microbial process in the surface biofilm that plays special role in their biodegradation. This surface formed at the interface between anaerobic conditions.[9] Biodegradation is in two forms, mineralization and co-metabolism. In mineralization, competent microorganisms were used organic pollutants as a source of carbon and energy resulting in the reduction of pollutants to their constituent elements. Co-metabolism requires a second substance as a source of carbon and energy for microorganisms but the target pollutant is transformed at the same time.[10] PCBs particularly higher chlorinated congeners are highly oxidized compounds and can serve as electron acceptors for energy storage in anaerobic environments under electron acceptor deficit; during this process, they undergo reductive dechlorination.[9,10] The specific methanogenic activity tests (SMA) recognize the PCBs concentration that inhibits biogas production due to microorganism activity in reactors.[7] The anaerobic sequencing batch biofilm reactor (ASBBR) has received much attention in recent years, mainly in industrial wastewater treatment. The hydraulic retention time (HRT) for high-rate anaerobic sequencing batch biofilm reactor is much shorter than the solids retention time (SRT). Therefore, the volume of such reactors is much smaller compared to the volume of low-rate anaerobic systems. They seem stable reactors even under changing operating parameters.[1,11,12] Recent studies have shown that aroclors 1254 was dechlorinated by the microbial granules and reductive dechlorination patterns of PCBs. It has been demonstrated by the sediment cultures and anaerobic PCBs dechlorination removed 32% of the chlorine from aroclor 1242, too.[5,6] Using sludge in anaerobic reactors caused a significant increase in microbial population and dechlorination is augmented.[3,6] This study aimed to dechlorinate the aroclor 1254, 1260 and 1242 PCBs by two anaerobic sequencing batch biofilm reactors with using two auxiliary substrates; Acetic acid and Acetone have been surveyed for determination of dechlorination rate.

MATERIALS AND METHODS

Anaerobic sequencing batch biofilm reactor set up

The experiments were conducted using two laboratory-scales ASBBR glass reactor (25 cm diameter and 32 cm height) with total volume of 7 L, 5 L effective volume and 3.5 L for gas production. The schematic drawing of ASBBR is presented in Figure 1. The reactors maintained at temperature of 35 ± 2°C by a thermostatically adjusted warm water bath. In order to complete mixing, magnetic stirring was applied.

Sludge culture and inert support

The reactors were seeded with comingled sludge consist of anaerobic digested sludge from urban wastewater treatment plant and anaerobic sludge collected from the batch vials in the laboratory for adapting to oil containing PCBs. The material selected for immobilization of the sludge was polyurethane foam (EPU) with cut in 1 cm cubes Figure 2. that fulfilled in to plastic box and then located in ASBBR rectors. These boxes were occupied 2 L of ASBBR.

Substrate features

In this study, oil containing PCB were obtained from the out of service electrical transformers of Isfahan Steel Plant Power unit. The oil was contained two types of PCBs including Aroclor 1242 and 1254 (PCB concentration > 2000 mg) and used as the main substrate. The acetic acid (low cast and available co-substrate) and acetone (effective solvent) were used as substrate aid and solvent solution in the reactor 1 and 2 (R1 and R2), respectively. The nutrients and trace elements with following composition were provided by adding to feed solution: NH_4Cl (1.242 mg/L), KH_2PO_4 (0.1625 mg/L), K_2HPO_4 (0.1445 mg/L), $FeCl_3.6H_2O$ (0.0166 mg/L), $CaCl_2.2H_2O$ (0.04225 mg/L), $MgSO_4.7H_2O$ (0.0348 mg/L), $MnCl_2.4H_2O$ (5.25×10^{-3} mg/L), $CoCl_2.6H_2O$ (1.495×10^{-3} mg/L), $ZnSO_4.7H_2O$ (9.62×10^{-3} mg/L), $Na_2MoO_4.2H_2O$ (10.335×10^{-3} mg/L), $CuCl_2.2H_2O$ (2.6×10^{-3} mg/L) and $NiSO_4.6H_2O$ (1.24×10^{-3} mg/L). The KOH and NaOH (2 M) were used in order to pH adjustment.

Asbbr start-up and operation

The programmable logical controller (PLC) system was used in order to controlling feed substrate, outlet effluent, injection pumps and magnetic stir. The total operational cycle was 24 h and included the following steps: Feeding (1 min), reaction (23.47 h or 1408 min), settling (30 min) and drainage (1 min). The ASBBR was operated without idle phase

between the feed and discharge stage. The reactors mixing were done by the magnetic stir in ON/OFF schedule of 5 min and 25 min, respectively. The reactors were operated for 310 days under different operational condition including organic loading rate (OLR), PCB loading rate (PCBs.LR), initial PCB concentration and operating duration. The variation of operational condition was summarized in Table 1.

The ASBBR start up is vital stage and was conducted using the initial OLR of 0.27 g_{COD}/L.d with 24 h cycle. The COD concentration in feed substrate was varied from 1300 to 25000 mg/L. The OLR and removal efficiency were calculated with the following equations:

$$R = \left[\left(C_{in} - C_e \right) / C_{in} \right] \times 100$$

$$OLR = \left(Q / V_r \right) \times C_{in} \times n \times t_{fill}$$

Where C_{in} and C_e is influent and effluent concentration of constituent (mg/L), Q effluent flow rate (L/d), V_r effective volume of reactor, n number of cycle per day and t_{fill} fill time in ASBBR feeding, respectively.

Sampling and analysis

The pH solution, COD and VSS were analyzed and the test methods were adapted from standard methods for the examination of water and wastewater.[13] PCBs concentrations was determined by gas chromatograph (Agilent) coupled by GC-MS and GC-ECD based on UNEP method (instruction of No. 71 for chlorinated hydrocarbon analysis in aqueous solution). The analytical conditions used were the following:

- Injector temperature: 250°C
- Oven temperature: 70°C for 2 min and increase 3°C/min up to 260°C for 5 min
- Detector temperature: 300°C
- Carrier gas (Nitrogen) flow rate: L mL/min

Biogas production monitoring

In this study, the ability of anaerobic bacteria in biodegradation of oil containing PCBs and biogas production was tested. The biogas production monitoring was accomplished by two methods. In reactor 1, the biogas formation from anaerobic degradation was monitored by means of an ELSTER wet gasmeter, PVC model (Germany). The composition of biogas from anaerobic degradation (reactor 1 and 2) was determined by means of a FIRST CHECK 6000 portable gasmeter.

Inhibition rate of oil contain polychlorinated biphenyls

In order to determine the inhibition rates of oil contain PCBs, the specific methanogenic activity (SMA) test in batch system was performed Figure 3. The SMA test was conducted at 35°C using 6 vials with 120 mL volume. The vials were filled by 15% (v/v) of sludge, or contaminated soil with PCBs, mixture of contaminated soil and sludge, 75 mL of substrate and 30 mL head space of vial for biogas collection. In order to complete mixing in to vials, the magnetic stir was applied. Methane production was monitored via displacement of liquid by KOH as CO2 absorbent. When an alkaline solution was used as the displacement liquid, the CO2 was scrubbed from the biogas and methane collected. The gas production was measured over time and incubation time was typically 10-12 days.

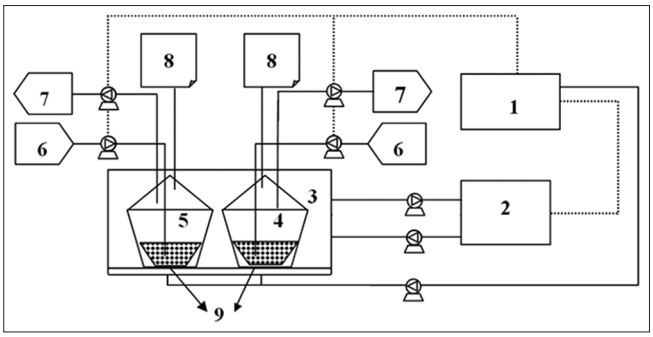

Figure 1: Schematic diagram of the APSBBR system (1: PLC, 2: Warm water reservoir, 3: Warm water bath, 4: Reactor 1, 5: Reactor 2, 6: Inlet reservoir, 7: Outlet tank, 8: Gas chamber, 9: Magnetic stir

Figure 2: Photograph of the polyurethane foam (EPU)

Table 1: Anaerobic sequencing batch biofilm reactor operating conditions

Period (d)	PCBs.LR (mgPCBs/L.d)	PCB concentration (µg/L)	Co-substrate		Nutrient and microelement (mg/L)
			Acetic acid (g/L)	Acetone (g/L)	
1-60	0.008	40	1.05	0.79	20
60-90	0.04	200	1.05	0.79	20
91-150	0.08	400	1.05	0.79	25
151-240	0.12	600	1.05	0.79	25
241-300	0.16	800	3.15	1.58	25
301-310	0.2	1000	3.15	1.58	50

RESULT

Polychlorinated biphenyls biodegradation

The results of PCBs removal and PCBs loading rate (PCBs. LR) during 310 day operation of ASBBR reactors (R1 and R2) was exposed in Figures 4 and 5. As seen, the PCBs was introduced to each reactors at three distinct OLR stage (I: influent PCBs concentration of 200 μg/L and PCBs.LR of 40 μg_{SPCB}/L.d; II: influent PCBs concentration of 400 μg/L and PCBs.LR of 80 μg_{SPCB}/L.d; III: influent PCBs concentration of 600 μg/L and PCBs.LR of 120 μg_{SPCB}/L.d). For any PCBs.LR, the removal efficiency of PCBs from oil containing PCBs by ASBBR reactors was > 95%. Figure 6 indicates the GC-ECD peaks for PCBs of reactors influent and effluent.

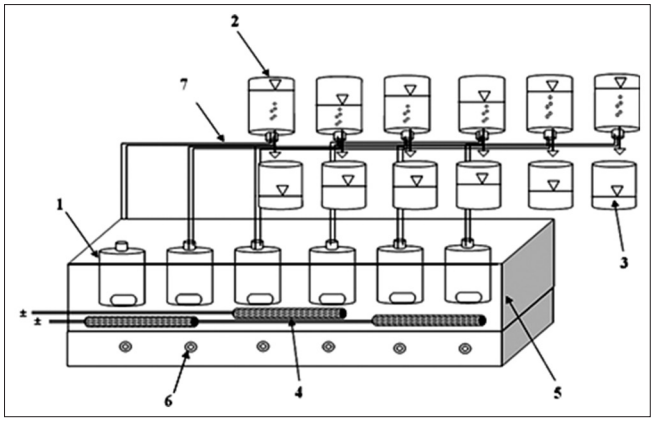

Figure 3: SMA test set up (1: Test vial, 2: KOH vial, 3: Collection vial, 4: Heater, 5: Warm water bath, 6: Magnetic stir, 7: Connection pipe)

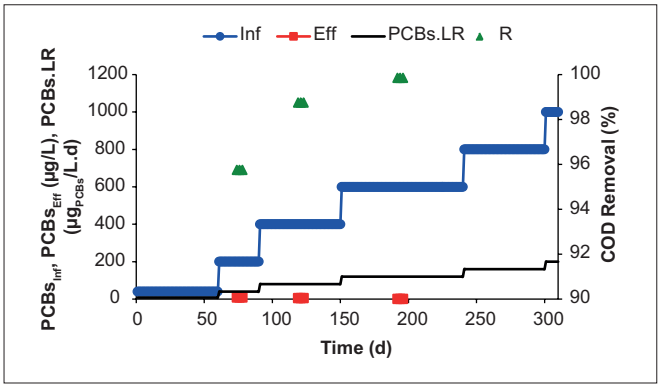

Figure 4: Profile of PCB_{Inf}, PCB_{Eff}, PCB removal and PCBs.LR during the experiment (R1)

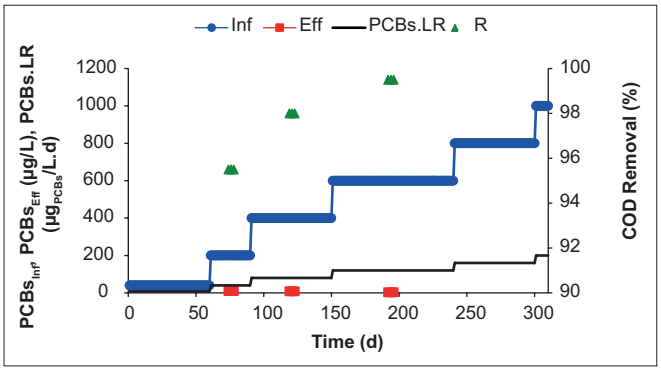

Figure 5: Profile of PCB_{Inf}, PCB_{Eff}, PCB removal and PCBs.LR during the experiment (R2)

COD removal

The experiment lasted for 310 days. The time courses of COD in influent and effluent and organic loading rate (OLR) for each reactor are shown in Figures 7 and 8. In this stage, the ASBBR reactors were operated at six different OLR. In R1, the operation time of each condition was including of 60 days for OLR (I), 30 days for OLR (II), 60 days for OLR (III), 104 days for OLR (IV), 46 days for OLR (V) and 10 days for OLR (VI). The average values of COD removal of 73.4 ± 19.5, 92.1 ± 0.8, 84.2 ± 3.3, 64.3 ± 8.4, 79.1 ± 2.6 and 58.1 ±

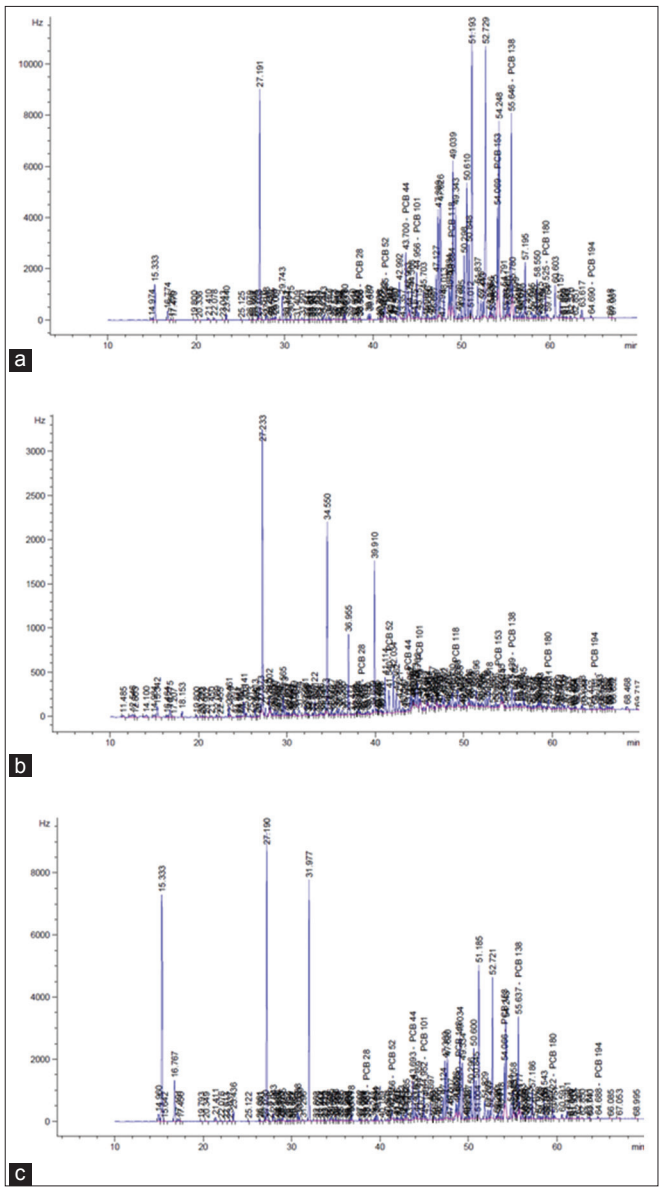

Figure 6: GC-ECD peaks for PCBs: (a) Influent (b) R1 effluent and (c) R2 effluent

0.1 was achieved for OLR I, II, III, IV, V and VI, respectively.

In R2, the operation time of each condition consisted of 60 days for OLR (I), 42 days for OLR (II), 48 days for OLR (III), 90 days for OLR (IV), 60 days for OLR (V) and 10 days for OLR (VI). The average values of COD removal of 66.4 ± 17.9, 82.6 ± 2.5, 90.3 ± 3.3, 60.3 ± 6.3, 77.4 ± 3.3 and 46.6 ± 0.1 was accomplished for OLR I, II, III, IV, V and VI, respectively.

Biogas production and pH variation

Biogas production was measured daily. The fluctuations of biogas production during the experiment from each ASBBR reactors are presented in Figure 9. The result revalued that with increasing COD removal and OLR, the biogas generation ascended. In over all operation, average of biogas production in R1 was 5.7 ± 2.2 L.d.

Figures 10 and 11 illustrates the biogas composition of ASBBR reactors over whole of operation.

The obtained results from the pH variation of two ASBBR reactors in relation to the organic loading rates are depicted in Figure 12. As seen, with rising of OLR, the pH solution quickly dropped owing to accumulation of fatty acids and the gradually mounted. Additionally, the effluent pH remained at suitable levels for anaerobic processes, ranging from 6.9 to 8.5 throughout the experimental period.

Performance of anaerobic sequencing batch biofilm reactor in a cycle

The profile of COD and pH variation during a cycle of ASBBR reactors operation are shown in Figure 13. The result showed that the COD removal via R1 and R2 increased from 11% to 83% and 8% to 2%, respectively when HRT varied from 0.5 h to 24 h.

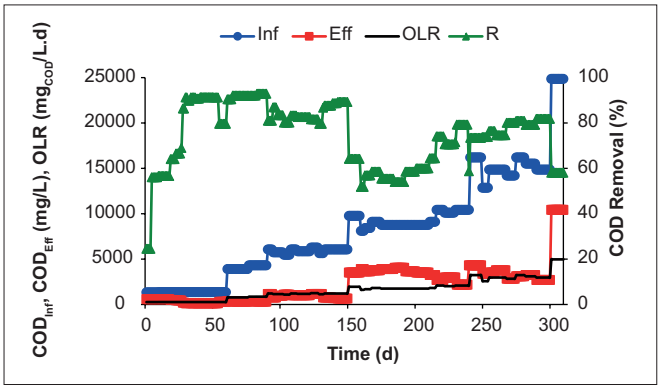

Figure 7: Variation of COD_{Inf}, COD_{Eff}, COD removal and OLR during the experiment (R1)

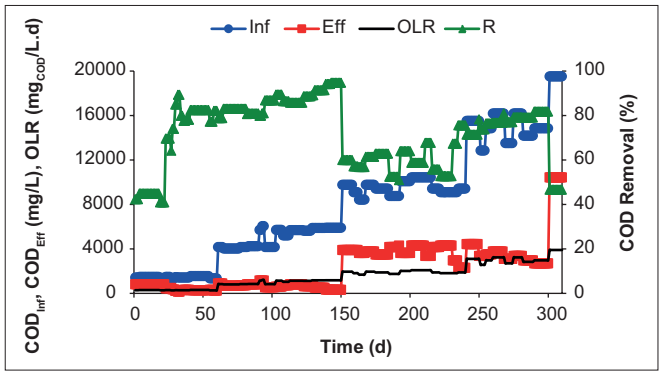

Figure 8: Variation of COD_{Inf}, COD_{Eff}, COD removal and OLR during the experiment (R2)

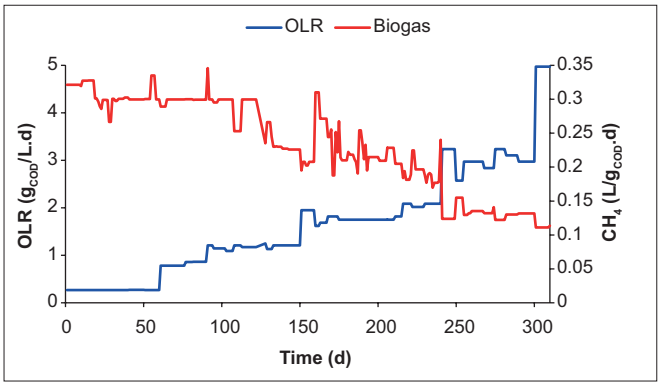

Figure 9: Variation of biogas production with OLR during the experiment (Acetic acid co-substrate (R1))

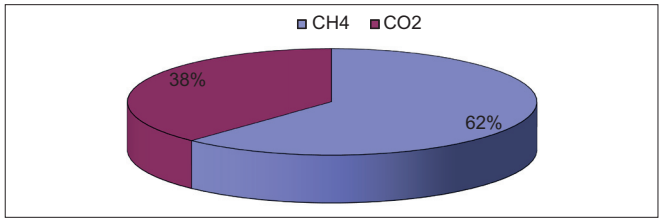

Figure 10: Biogas composition (Reactor 1)

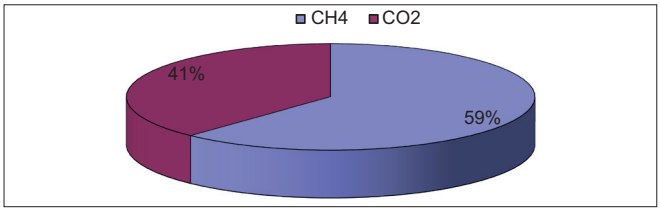

Figure 11: Biogas Composition (Reactor 2)

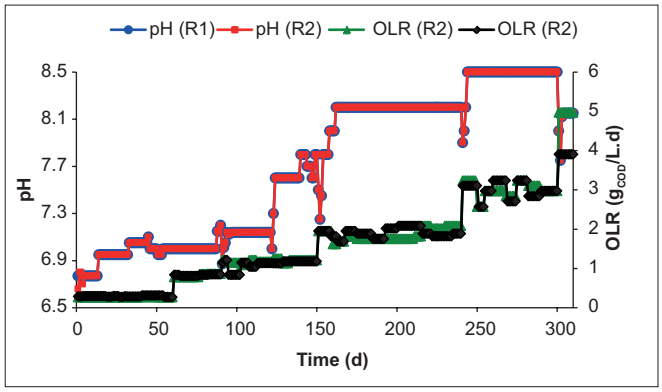

Figure 12: Profiles of pH variation during ASBBR operation

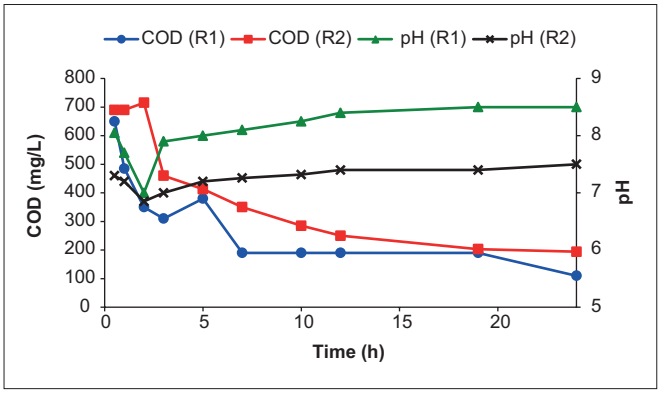

Figure 13: COD and pH fluctuation in a cycle operation

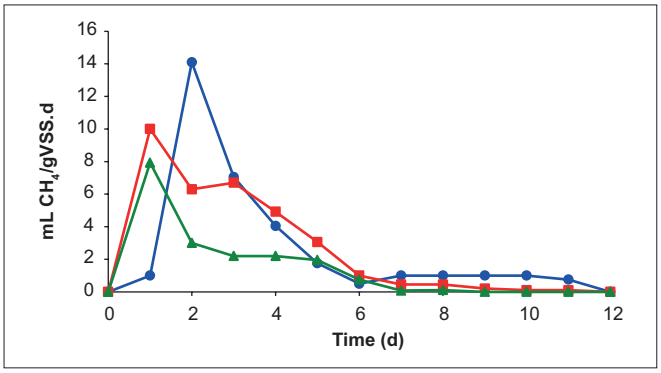

Figure 15: Methanogenic activity results at different condition: PCBs concentration: (•) Acetic acid: 0.1 mL, PCBs: 3 μg/L, soil: 1 g, (■) Acetic acid: 0.1 mL, PCBs: 6 μg/L, soil: 1 g, (▲) Acetic acid: 0.1 mL, PCBs: 9 μg/L, soil: 1 g

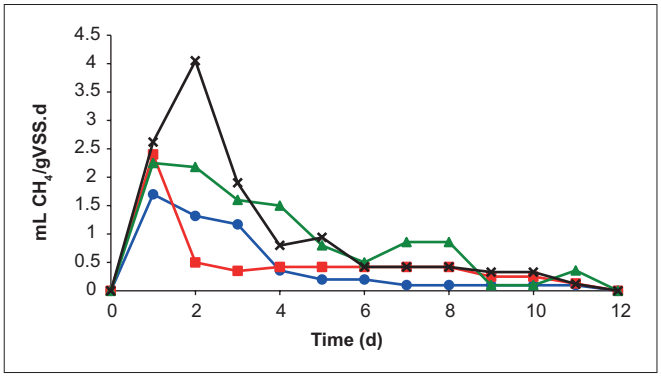

Figure 14: Methanogenic activity results at different condition: (•) Acetic acid: 1 mL, PCBs: 0 μg/L, soil: 0 g, (■) Acetic acid: 1 mL, PCBs: 6 μg/L, soil: 1 g, (▲) Acetic acid: 1 mL, PCBs: 6 μg/L, soil: 0 g, (×) Acetic acid: 1 mL, PCBs: 0 μg/L, soil: 1 g

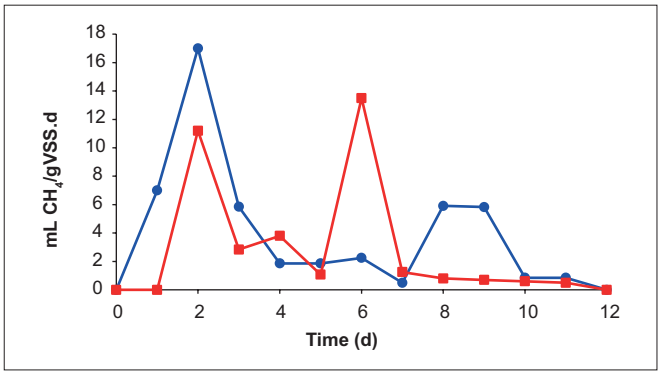

Figure 16: Methanogenic activity results at different condition: PCBs concentration: (•) Acetic acid: 0.1 mL, PCBs: 30 μg/L, soil: 1 g, (■) Acetic acid: 1 mL, PCBs: 45 μg/L, soil: 0 g

Specific methanogenic activity test

In Figures 14-16, the biogas generation during SMA test with different condition (initial co-substrate concentration, initial PCBs concentration and soil additive) are displayed.

DISCUSSION

Performance of ASBBR on PCBs and COD removal

In this study, efficiency of ASBBR reactor in different steps and characteristics of reactors relating to increasing PCBs. LR and co-substrate was determined based on PCBs removal [Figures 4 and 5]. It can be seen that the two reactors worked very well. The PCBs removal was kept in above than 95% even PCBs.LR was increased promptly from 8 to 200 μg_{SPCB}/L.d. Correspondingly, in reactor 1 and 2, the effluent PCBs concentration was less than 8.5 and 9 μg/L, respectively. The PCBs removal was increasing by growing initial PCBs concentration and PCBs.LR. When PCBs and acetic acid was applied as sole and substrate aid, PCBs removal was higher than to acetone as co-substrate. It was may be due to effective function of acetic acid in acclimation of anaerobic microorganisms to biological degradation of oil containing PCBs and better solubility in feed substrate. Pereira and Zaiat reported that formaldehyde degradation rates increased from 204.9 to 698.3 mg/L h as the initial concentration of formaldehyde was increased from around 100 to around 1100 mg/L.[14] This result is in good agreement with the findings of present study.

The association between PCBs initial concentration and PCBs removal efficiency was analyzed with pair *t*-test was statistically significant (P value < 0.001). Also results of the analysis of the variance (one way ANOVA) are shown that association between different PCBs initial concentration is statistically insignificant (P value > 0.001).

The PCBs are encounter to both aerobic and anaerobic metabolism by microorganisms.[10,15,16] The microorganisms can modify organic pollutants such as PCBs in such a way that have low negative effects on their life. The microorganisms participate in the biodegradation by producing enzymes, which modify the organic pollutant into simpler compounds. Biodegradation is of two forms, mineralization and co-metabolism.[10] The anaerobic transformation of chlorinated organic compounds involves reductive dehalogenation where the halogenated organic compound serves as the electron acceptor; the halogen substituent is replaced with hydrogen as following:[17]

$$R-Cl+2e^{-}+H^{+}\rightarrow R-H+Cl^{-}$$

The electron acceptors are generally the limiting factors for metabolism in anaerobic environments. Therefore, any microorganism that could use PCBs as terminal electron acceptors would be at a selective advantage.[18] The previous study showed that several anaerobic bacteria are involving in dechlorinating such as *Desulfomonile tiedjei*, *Dehalococcoides ethenogenes*, *Dehalobacter restrictus*, *Dehalospirillum multivorans*, *Desulfitobacterium*, *Desulforomonas chloroethenica* and the facultative anaerobes *Enterobacter strain MS-1* and *Enterobacter agglomerans*.[7,19]

Some studies on sediments containing PCBs in Hudson River showed that after 20 weeks incubation these sediments under anaerobic conditions led to significant changes in number of chlorine atoms of PCBs compounds. Using anaerobic-aerobic reactors for removing PCBs from wastewater showed that after 3 months, the number of chlorines of arochlor 1260 was extensively changed.[1,11,12]

The COD removal by two reactors was investigated. In reactor 1 and 2, five distinct phases are indicated with initial COD concentration in the influent corresponding to OLR. In each stage of increasing OLR, the COD removal promptly decreased and COD removal improved as the microbial retention time became longer. In all OLR stages, the COD removal efficiency was more than 40% that relating to effluent COD concentration of 10.4 and 10.1 g/L in R1 and 2. After 140 days operation, the reactors condition reached to steady state that corresponding to 80% in COD removal. In 300 day, by increasing OLR from 3 to 5 g_{COD}/L.d, the COD removal efficiency by R1 and R2 was descended to 58% and 46%, respectively.

In present study, hydraulic retention time (HRT) for ASBBR reactor was 5 days that depending on wastewater characteristics and environmental conditions. The HRT should be long enough to make the anaerobic microorganism's metabolism possible.

Biogas generation

The biogas produced during the anaerobic degradation is a valuable resource of energy. The quality and quantity of the biogas have special importance. The results of biogas generation were showed that biogas production quantity changed by fluctuation of OLR [Figure 9]. The results showed by increasing OLR and COD removal, the biogas generation augmented. The maximum, average and minimum of biogas production in R1 was about 0.34, 0.23 and 0.11 mL/g_{COD}.d, respectively. The typical amount of biogas production in anaerobic wastewater treatment was 0.3 to 0.5 mL/g. This result is in good agreement with the findings of Amin *et al.* and Erses *et al.*[20,21]

When anaerobic system selected to wastewater treatment, high amount of the wastewater COD is converted to methane and released into the gas phase. For each mole of methane produced (25.3 l at 35°C under standard pressure), 2 mole of oxygen equivalent COD are destroyed (64 g). Thus, 1 g of COD utilization at 35°C (at standard pressure) is equivalent to 0.395 l of methane production, ignoring biomass growth.[22,23]

The analysis of biogas composition showed that the off gas from R1 and R2 was containing 62% and 59% methane and 38% and 41% carbon dioxide, respectively. From previous study, a typical biogas are contains about 65-70% methane and 30-35% carbon dioxide (%v). Cheong and Hansen reported that the methane content of the biogas was 52-86% for the COD of 15000 mg/L in the feed wastewater and higher methane content ratio of biogas occurred at a shorter HRT.[23]

The results 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 12].

Initially, pH values of both reactors were the same (pH > 6.2). The ASBBR reactors started off in acidic conditions in the beginning of the experiment. The pH solution was ascended over operation that implied the two reactors worked very well and produced acid by acidogenic bacteria was consumed by methanogenic bacteria. The minimum and maximum of pH solution in R1 and R2 was 6.5 and 8.5, respectively. 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.[24] This observation is also confirmed with other studies in literature.[20,25]

Performance of ASBBR in a cycle

As seen in Figure 13, with increasing operation time, COD removal efficiency and pH solution was improved. At this time, COD concentration in R1 and R2 was declined from 650 to 110 mg/L and 690 to 190 mg/L that relating to COD removal efficiency of 83% and 72%, respectively. Determination of the kinetics of the ASBBR process on COD removal reaction is needed to estimating the time required for COD removal. A kinetic analysis was conducted by fitting the performance data over a cycle operation with zero, first, and pseudo-second order kinetic equations as shown in Table 2.

Where r_c is the rate of conversion, k_0, k_1, and k_2 are reaction rate coefficients, R^2 is coefficient of determination, t is time, and C_0 and C are the initial and final concentration of the constituent in the liquid, respectively.

According to Table 2, kinetic studies revealed that second – order kinetic model described the COD removal by ASBBR reactors from synthetic wastewater better than two other kinetic model ($R^2 > 0.9$). These results implied the COD removal with ASBBR was done at a rate proportional to the second power of initial COD concentration. The rate constant of COD removal by R1 and R2 was 0.0003 and 0.0002, respectively.

Table 2: Equations and linear forms and results of kinetics model

Kinetic model	Equation	Linear form	Parameter	R1	R2
Zero-order	$r_c = \frac{dC}{dt} = k_o$	$C - C_o = -k_o t$	k_0	19.49	21.92
			R^2	0.65	0.78
First-order	$r_c = \frac{dC}{dt} = k_1 C$	$In\frac{C}{C_o} = k_1 t$	k_1	0.068	0.058
			R^2	0.82	0.89
Second-order	$r_c = \frac{dC}{dt} = k_2 C^2$	$\frac{1}{C} - \frac{1}{C_o} = k_2 t$	k_2	0.0003	0.0002
			R^2	0.93	0.97

Specific methanogenic activity

To investigation of the PCBs concentration on the Specific methanogenic activity, a series of SMA test were conducted. According to Figure 14, at low concentration of PCBs, the bacteria quickly consumed substrate and produced biogas. With Introducing soil into test vials, the biogas generation has no significant variation. As seen in Figure 16, when PCBs concentration was increased to 30 μg/L, the substrate biodegradation was delayed for 1 d. This situation is due to increasing require time for microorganisms adaptation and inhibition effect of oil containing PCBs. The result of SMA test demonstrated that highest COD removal was obtained at low concentration of oil containing PCBs and acetic acid and equal ratio of sole to co-substrate. In this research, the evident inhibitory effect of PCBs with using the acetic acid as substrate aid in the SMA test was monitored at the PCBs concentration of 6 μg/L [Figure 15].

According to obtained results can be concluded that:

- It can be seen that the two reactors worked very well and PCBs removal was kept in above than 95% even PCBs. LR was increased promptly from 8 to 200 μg_{PCB}/L.d.
- In two ASBBR reactors, the optimum PCBs removal efficiency was obtained at 120 μg_{PCB}/L.d that corresponding to PCBs removal efficiency > 99%.
- When PCBs and acetic acid was applied as sole and substrate aid, PCBs removal was higher than to acetone as co-substrate.
- The average of COD removal efficiency by two ASBBR reactors was more than 92% that corresponding to < 10 g/L of effluent COD.
- In R1 and R2, the optimum COD removal efficiency was obtained at 0.82 and 1.185 g_{COD}/L.d that relation to COD removal of 92% and 89%, respectively.
- In R1, the maximum produced biogas was 8.02 L/d at 310 day.
- The kinetic studies revealed that second – order kinetic model described the COD removal by ASBBR reactors from synthetic wastewater better than two other kinetic model.
- The rate constant of COD removal by R1 and R^2 was 0.0003 and 0.0002, respectively.
- The evident inhibitory effect of PCBs with using the acetic acid as substrate aid in the SMA test was monitored at the PCBs concentration of 6 μg/L.

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