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Biodegradation performance of sequencing batch biofilm reactor for Polychlorinated biphenyls in transformer oil

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Key words: chlorobenzoic compound, Polychlorinated biphenyls (PCBs), sequencing batch biofilm reactor (SBBR), congeners.

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Abstract

In order that the biodegradability of oil contain PCBs (aroclor 1242 and 1254) from electrical transformer, two pilot - scale sequencing batch biofilm reactor were used with polyurethane foam as an inert and growth media. The reactors were operated for 238 days. During the operation, the effects of main operation parameters such as pH, organic loading rate, PCBs loading rate, co-substrate type and initial COD concentration were investigated. It was observed that microorganisms were able to utilize and degrade the oil containing PCBs. Optical density results showed that the increase of neutral pH resulted in the increase in cell number and optical density. The reactors showed PCBs removal efficiency more than 99%, with effluent PCBs concentration of lower than 3µg/l. COD removal efficiency and surface loading rate increased as the initial concentration of PCBs increased. The average of COD reduction by SBBRs was more than 85% corresponding to COD <100 mg/l in reactors effluent. The average of SLR in two reactors was more than 4 corresponding to PCBs removal efficiency which was more than 99% in two reactors. GC-MS chromatography analyses detected the chlorobenzoic compounds in reactors effluent as the byproduct of PCBs biodegradation. The results of 16s rDNA sequence analysis showed that *Rodococcus*, *Pseudoxanthomonas*, *Agromycess* and *Pseudomonas* were predominant bacteria in reactors. The study showed that SBBRs have a good potential for biodegradation of oil contain PCBs. Also, acetic acid could be recommended as a good co-substrate and alternative solvent because it is inexpensive, abundant and safe for environment.

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Introduction

Polychlorinated biphenyls (PCBs) are xenobiotic chemicals that have been used in a wide range of industrial applications including oil transformer, dielectric in capacitors and lubricants for turbines and pumps. Worldwide, about 0.4×10^6 tones of PCBs have discharged to the environment (D'Angelo and Nunez, 2010). PCBs persist in the environment (Torella *et al.*, 2006) for a long time; therefore, one of the major concerns about PCBs is their bioaccumulation and biomagnifications in the food chain (Dadong *et al.,* 2010). They have also strong adsorption of organic matter that leads to the accumulation in soil and aquatic sediments. Many studies show that marine sediments may be the ultimate fate of PCBs accumulation (Fava *et al.*, 2003, Kuo *et al.*, 1999, Wiegel and Wu, 2000). These compounds enter food chain and accumulate in fatty tissues of human, fish, animal and birds (Atlas and Philp, 2005, Mondello, 2002). Despite their ban in the USA and Europe in the mid-1970s, some of them were still used in the 21st century in developing countries (Barrett *et al.*, 2005). The most common effects of PCBs on human beings are acute and chronic ones, including acne, rashes, liver and reproduction system disorders and cancer (Abraham *et al.*, 2002, ATSDR, 2001).

There are various methods for PCBs congeners removal from aqueous solutions. Application of physical and chemical methods such as adsorption in solid phase and advanced oxidation may be very expensive. Furthermore, these methods can release undesirable compounds to the environment during the PCBs reduction (Mondello, 2002). Microbial-mediated degradation is considered as one of the most efficient processes in removal of PCBs pollution from contaminated environments (Sakai *et al.*, 2002, Seto *et al.,* 1995). This method is inexpensive and safe from environmental health point of view (Mondello, 2002). However, in order to accelerate PCBs biodegradation, environmental parameters such as temperature, oxygen and nutrients must be controlled (Mondello, 2002).

Co- metabolism is one of the biodegradation methods, and microorganisms use second substrate and organic pollutant as sources of carbon and energy at the same time, while in mineralization method, microorganisms use only organic pollutant as a source of carbon and energy and transform to their constituents. Studies have shown that some microorganisms are able to degrade PCBs compounds under aerobic conditions (Mondello, 2002). A common pathway for biphenyl degradation by aerobic bacteria includes metabolites of these compounds followed by chlorobenzoic acid production. Chlorobenzoic acid can be easily mineralized in environment (D'Angelo and Nunez, 2010, Tarakovsky *et al.*, 2001). The combination of PCR amplification of 16S rDNA genes by denaturing gradient gel electro phoresis (DGGE) analysis has provided a useful means to directly characterize PCBs biodegrading bacterial populations directly(Casserly and Erijman, 2003, Liu *et al.*, 2002). Recent studies have shown that fixed bioreactors expanded by inert media provide improved production for biofilm by limiting microbial inhabitation from hazardous contaminants, whereas increasing removal efficiencies, especially during treatment startup (Gopinath *et al.*, 2013).

Among reactors that are used for biological treatment, sequencing batch reactors is performed commonly for industrial wastewater because of high operation flexibility, simplicity and maintenance of optimal conditions (Movahedian *et al.*, 2008, Rittmann and McCarty, 2001). Given the importance of PCBs removal, this study was carried out to biotransform the aroclor 1242 and 1254 PCBs by two sequencing batch biofilm reactors using two co-substrate; Acetone and Acetic acid for determination of biodegradation rate have been investigated.

Materials and methods

Sequencing batch biofilm reactors setup

The experiment was conducted using two laboratory-scale SBBR glass reactor (35cm diameter and 34 cm height) which had an empty volume of 14 L and effective volume of 10 L (useful working volume of 71

percent). The schematic of SBBRs is shown in Fig. 1. The reactors had hydraulic retention time of 10 day and infinite solid retention time that were maintained at temperature of 20 – 25 ºC by a thermostatically adjusted water bath. An air blower was used to provide aeration with a ratio of 23 L air per L of solution in each reactor every day. The substance selected for immobilization of the sludge was polyurethane foam cubes (1 × 1 × 1 cm) that was fixed inside the plastic box and was located in SBBR reactors. In order to minimize risk assessment, granular activated carbon was used on the top of each reactor. Dissolved oxygen was monitored daily and its concentration was 1.5-2 mg/l during operation.

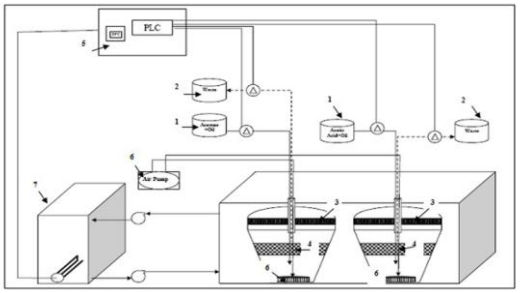

Fig. 1. Schematic diagram of the SBBR reactors (1-influent tank, 2-outlet tank, 3-granule activated, 4-polyurethane foam media, 5-PLC, 6-air blower, 7-warm water reservoir).

SBBRs start up and operation

The programmable logical controller (PLC) was used in order to control operational cycles. The total operational cycles were 24 h by following the given steps: filling (1min), reaction (1378min), settling (60min), and draw (1min) (Bedard *et al.*, 1986). Fig. 2 shows the diagram of SBBRs working cycles. The reactors were operated for 238 days with different conditions including organic loading rate, PCBs loading rate and PCBs initial concentration.

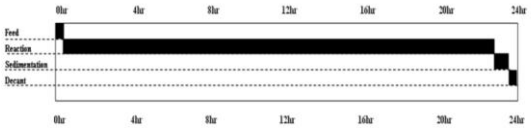

Fig. 2. Diagram of working cycles of the SBBR systems.

SBBRs startup was done using initial organic loading rate of 40mg$_{COD}$/l in 24 h cycle. OLR was calculated in accordance with the following equation:

$$OLR = (Q/V_r) \times C_{in} \times n \times t_{fill} \quad \text{(Equation.1)}$$

Where C_{in} is influent as mg/l, Q effluent flow rate as l/d, V_r working volume of each reactor, n number of cycle per day and t_{fill} fill time in feeding cycle, respectively.

Substrate characteristics

In this study, oil contained two types of PCBs: aroclor 1242 and 1254 with concentration of more than 2000 mg that was used as the main substrate and was obtained from the electrical transformers which were out of service in Isfahan steel company. The acetone and acetic acid were used as co-substrate and oil solvent solution in SBBR1 and SBBR2, respectively. The nutrient and microelement compositions were provided by adding so as to feed solution as follows : NH_4Cl (267.5 mg/l), KH_2PO_4 (7 mg/l), K_2HPO_4 (17.7 mg/l), $FeCl_3$ (0.71mg/l), $CaCl_2.2H_2O$ (1.44 mg/l), HBO_4 (2 mg/l), $MgSO_4.7H_2O$ (1.49 mg/l), $MnCl_2.2H_2O$ (0.238 mg/l), $CoCl_2.6H_2O$ (0.0644 mg/l), $NiSO_4.6H_2O$ (0.05341mg/l), $ZnSO_4.7H_2O$ (0.0414mg/l), $Na_2MOO_4.2H_2O$ (0.057 mg/l), $CuCl_2.2H_2O$ (0.0112mg/l) (Amin, 2004). The KOH and NaOH (2 M) were used for pH adjustment.

Sampling and analysis

pH solution and COD were analyzed as adapted from Standard Methods for water and wastewater examination (APHA, 2012). Dissolved oxygen was also measured by DO meter every day. PCBs concentration was determined by gas chromatograph (Agilent 6890N) equipped by GC-MS and GC-ECD based on UNEP method (instruction of NO.71 for chlorinated hydrocarbons analysis in aqueous solution). The analytical conditions used for GC-ECD were as follows:

- Column type: HP-5 (dimensions 30m × 0.32 mm and thickness of 0.25 µm)
- Carrier gas: helium
- Injector temperature: 250°C

- Detector temperature: 300˚C
- Oven temperature: 70˚C (2 min holding time) and increase up to 260˚C (5 min holding time)

The analytical conditions used for GC- MS were as follows:
- Column type: capillary column (dimensions 30m × 0.32 mm and thickness of 0.25 µm)
- Carrier gas: helium
- Oven temperature: 90˚C (2 min holding time) and increase up to 200˚C (2˚C per min) and then increased up to 290˚C (3˚C per min).

Microorganisms acclimation

Batch scale test was conducted at 25 - 30˚C using 6 Erlen meyer flasks with 250 ml volume. The Erlen meyer flasks were filled with 10% (v/v) of sludge (VSS = 3000mg/l), contaminated soil with PCBs, mixture of contaminated soil and sludge (3 erlen meyer with acetone and 3 erlen meyer with acetic acid co – substrate), 150 ml of substrate. Flasks were covered and shaked with shaker – incubator at 50 – 80 RPM. DO and pH was daily measured. Due to microorganism growth survey, optical density was measured with spectrophotometer at wavelength of 600nm. With the appearance of yellow color related to biphenyl metabolism, adaptation time was successfully completed. Seeding of the reactors was performed by microorganisms which have already been acclimatized to PCBs in batch scale experiment.

Molecular identification of aerobic PCBs degrading microorganisms

Biofilm samples were collected at the end of experiment by scraping biomass from the bio-carriers and vortexing in 5 ml of sterile water for 5 minutes to homogenize the bacteria. Aliquots of biofilm samples were then spread on agar plates and incubated at 30 ºC After 2-7 days of incubation; all bacterial colonies were characterized based on the colony and cell morphology. Each distinct colony was suspended in 100 µl of deionized water and genomic DNA was extracted by boiling for 15 minutes and centrifuging at 13000 rpm for 5 minutes. The supernatant was used for PCR amplification of 16s rDNA with Eubac27F and 1492R1 primers, which amplifies a ~1420 bp fragment of 16s rDNA (movahedian, 2009,Begonja Kolar *et al.*,2007).

The PCR amplification was conducted in a final volume of 50 µl containing 2 µl of template DNA, 0.2 µM of each primer, 0.2 mM of each dNTPs, 5 µl of 10x PCR buffer and 1.5 units of Taq DNA polymerase. The DNA sequencing of the amplified gene was performed, and DNA sequences-analysis was undertaken by BLAST algorithms and databases from the National Center for Biotechnology (www.ncbi.nlm.nih.gov).

Results

PCBs biodegradation

The results of PCBs concentration and biodegradation are shown in figs. 3 and 4.

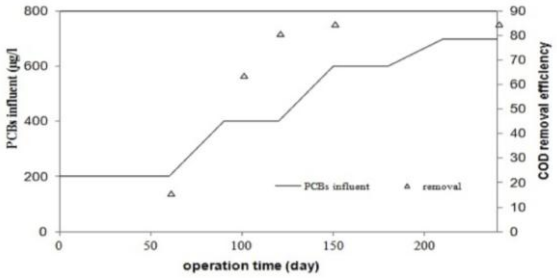

Fig. 3. Profile of PCBs influent and COD removal during the operation (SBBR1).

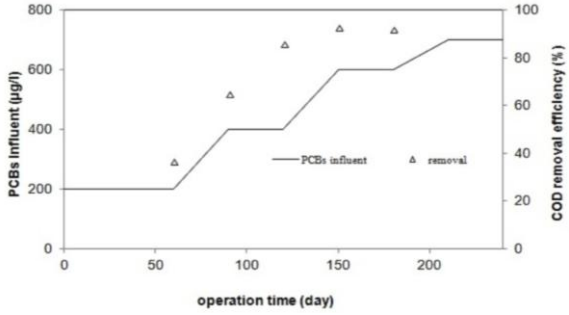

Fig. 4. Profile of PCBs influent and COD removal during the operation (SBBR2).

As seen, PCBs influence to each SBBR was achieved in four stages corresponding to 4 OLR stage (PCBs influent of 200µg/l and loading rate of 0.02 μg_{PCBs}/l,

PCBs influent of 400µg/l and loading rate of 0.04 μg_{PCBs}/l, PCBs influent of 600µg/l and loading rate of 0.06 μg_{PCBs}/l and finally PCBs influent of 700µg/l and loading rate of 0.07 μg_{PCBs}/l). It can be learned from fig.s 3 and 4 that COD removal efficiency was less than 50% during startup period of reactors. However, with the passage of time, for PCBs concentration more than 400µg/l the removal efficiency of PCBs by SBBRs was >85%. Of course its ratio was constant in PCBs concentration more than 700µg/l in two bioreactors. PCBs reduction was more than 99% (in PCBs concentration ≥ 400µg/l) corresponding to PCBs effluent which was less than 3µg/l in two bioreactors. Two reactors had maximum COD removal efficiency in PCBs concentration 600 µg/l. However, with the increase of PCBs concentration more than 600 µg/l COD removal efficiency decreased and was 83% and 90% in SBBR1 and SBBR2, respectively.

pH changes and bacteria growth

The experiments lasted about 240 days. Fig.s 5 shows pH and optical density changes during bioreactors operation time. It can be seen from fig. 5 that during the startup period of reactors, average pH was between the amounts of 5.5 – 6. Optical density shows the turbidity related to bacteria growth which was less than 3 in primary operation period. Over time, pH was increased gradually to 8 – 8.5 in overall time of the operation. Also, the growth profile of bacteria was increased to 4.5 -4.8 in the reactors. This can be ideal for the increase of the COD removal efficiency more than 85% in two reactors.

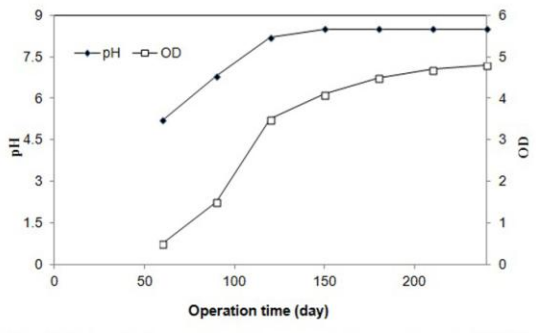

Fig. 5. Growth profile of aerobic bacteria versus pH values during the SBBR.

Organic Loading Rate variances and Surface Loading Rate

In this study, PCBs (aroclor 1242 and 1254) were dissolved in the oil. Therefore, increasing of PCBs concentration was resulted in organic loading rate with some variations increased, too. Fig. 6 shows the variances of SLR values versus the increase of OLR in each bioreactor. During 240 days of operation experiment, average values of influent OLR to each reactor varied between 40 mg $_{COD}$/l.day - 800 mg $_{COD}$/l.day. Average of COD removal efficiency 40.2± 15 to 95.3± 5 percent was accomplished for mentioned OLR.

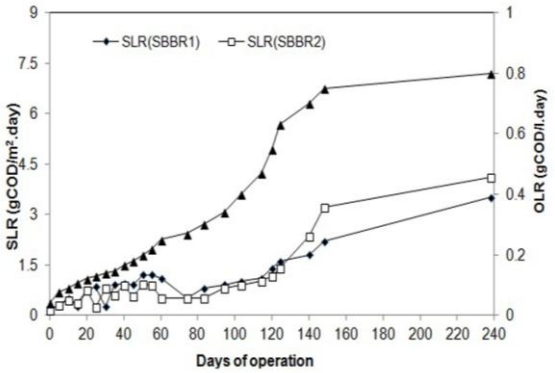

Fig. 6. Variation of OLR and surface loading rate values of each bioreactor during the experiment.

The same flow and the same amount of PCBs were treated by two bioreactors. When organic loading rate to each bioreactor was increased, loading rate to surface media of bioreactors was increased and sped up more than 3. This may be a reason that microorganisms on media surface have more bioavailability for the main substrate and with the increase of OLR, PCBs removal efficiency increased too.

Identification of isolated colonies

The colonies harvested from the biomass of bioreactors were identified. The PCR product with the universal primers for 16SrDNA were sequenced, showing >98% similarities of aerobic PCBs degrading bacteria including: *Rodococcus*, *Pseudoxanthomonas*, *Agromycess* and *Pseudomonas*.

PCBs degradation products

PCBs metabolites were measured by gas chromatography – mass spectrometry (GC-MS). Chlorinated benzoic acid compound corresponding to PCBs biodegradation was detected by GC-MS. All PCBs components were tested as well as biphenyls transfer of yellow meta-cleavage compounds. Of course, in effluent of SBBR bioreactors, a small amount of aroclor 1254 was detected indicating the complete lack of aroclor 1254 degradations by SBBR bioreactors.

Discussion

In this study, efficiencies of SBBR reactors in different stages including OLR, PCBs concentration and co-substrate type were investigated. Acclimation of natural microorganisms of activated sludge and then using them in pilot scale is very important that was achieved in this study, while many studies have used specific species of bacteria for PCBs degradation (unfeasible and un-economic method for everywhere). When biphenyl is degraded by bacteria, meta- ring cleavage is released and yellow color is created in environment that is obvious in aqueous solution. This has been observed in most of the bacteria, specifically *Pseudomona*s (Borja *et al.*, 2005, Mondello, 2002). During the operation of pilot, yellow color in effluent of each bioreactor was appeared. In this survey, yellow color is appeared due to biodegradation of biphenyl, as observed by Wagner (1998) (Wagner-Dober *et al.*, 1998).

PCBs were dissolved in out-service oil electrical transformers and we aimed to biodegrade oil and PCBs at the same time, while oil is known a hazardous liquid for environment and its removal is necessary. So, in this study, two substrates were used as oil solvent and co-substrate. It can be learnt from fig. 3 and 4 that two reactors worked very well. PCBs removal was more than 99% with PCBs loading rate 0.04 – 0.07 μg_{PCBs}/l.day that was corresponding to PCBs effluent concentration which was lower than 3 µg/l in bioreactors, respectively. In general, when acetic acid was used as co – substrate in SBBR2, variances related to SBBR2 start-up was lower than those related to acetone SBBR1. Furthermore, COD removal in SBBR2 was more than SBBR1 which sped up 95% corresponding to LR of 0.07 μg_{PCBs}/l.day. It might be related to the important role of acetic acid in acclimation of aerobic microorganisms so it that can biodegrade oil containing PCBs. Zhao *et al*, 2006 evaluated PAHs biodegradation and found that activated sludge process was able to remove COD more than 80% (Zhao *et al.*, 2006).

The predominant bacteria forms were rode-shaped and cocci. These organisms have been related to PCBs degradation. The growth of the selected bacteria measured by optical density and pH of the bioreactors are shown in Fig. 5. The results showed that the ability of the organisms to utilize PCBs as carbon source in neutral pH was more in lower pH. However, Okerentugba studied crude oil biodegradation by microbial consortium and found that cell number (OD) increased when pH of solution decreased corresponding to microorganisms biodegradation metabolites related to acid production (Okerentugba *et al.*,2003).

SLR variances (Fig. 6) associated with OLR increase shows when OLR increases, surface loading rate to media unit of each bioreactor increases as well. Therefore, bacteria have more access to PCBs and utilization of PCBs and co-substrate as carbon and energy source resulted in their growth with biphenyl degradation.

In this experiment, colonies isolation was carried out by nutrient agar plate, the presence of bacteria species including Pseudomonas, *Rodococcus*, *Pseudoxanthomonas*, Agromycess was confirmed with similarity of more than 98%. The results of 16S rDNA sequence analysis of predominant bacteria by Chang showed the presence of these bacteria as PCBs degrading bacteria in aerobic biodegradation (Anthony, 2000, Cheng *et al.*, 2007, Fedi *et al.*, 2001).

GC-MS analysis was another result of PCBs biodegradation in this study that the consequence of

meta-cleavage compounds includes chlorobenzoic compounds. Tarakovsky (2001) reported that PCBs compounds biodegradation was performed when analyses of outlet samples contained benzoic acid compounds (Tarakovsky *et al.*, 2001). Of course, the small amount of aroclor 1254 detected by GC_MS was associated with non-ability of aerobic processes for higher compounds of PCBs. So, some PCBs congeners may not be decomposed by a single aerobic process. This kind of approach can be used to construct hybrid processes of anaerobic – aerobic to success breakdown all of the PCBs congeners. Other studies have shown that aerobic processes are able to biodegrade lighter PCBs compounds more than higher compounds. Biodegradation of higher PCBs congeners (arochlor 1254) by aerobic processes are more time consuming. Borja et.al (2004) found that lighter PCBs compounds biodegrade by performance of aerobic process (Borja *et al.*, 2005).

The microorganisms participate in the biodegradation by producing anzymes, which transfer the organic pollutant to simpler compounds (Borja *et al.*, 2005).

The COD removal by SBBRs was studied. COD concentration was identified through OLR. In each stage of experiment, increasing OLR resulted in COD removal efficiency improved as microbial activities and population were more. After 90 days of bioreactors operation, COD removal efficiency in two reactors was more than 60% corresponding to effluent COD concentration of < 100 mg/l in two bioreactors. After 110 and 150 days of reactor 1 and reactor 2 operations, the condition reached a steady state that COD removal efficiency was > 85%. However, with the increase PCBs concentration to more than 600 µg/l COD removal efficiency decreased to 83% and 90% in SBBR1 and SBBR2 respectively.

Initially pH values of two reactors were about 5.5 – 6 in the start-up experiment. After the operation, the pH of solution was increased that implied to reactors were functioning well and the condition for microorganism activities was suitable. Maximum pH value in SBBRs was 8 – 8.5.

As seen in Fig. 3 and 4, with the passage of time, COD removal efficiency was in stable condition in two bioreactors and decreased from 750 to 112 mg/l in SBBR1 and 800 to 80 mg/l in SBBR2 which was related to COD removal efficiency of 85% and 90% respectively.

Table 1. Equations and results of kinetics model.

Kinetic model	Equation	Linear form	Constant	SBBR1	SBBR2
Zero-order	$r_c = \frac{dC}{dt} = k_0$	$C - C_0 = -k_0 t$	k_0	21.9	20.1
			R^2	0.62	0.74
First-order	$r_c = \frac{dC}{dt} = k_1 C$	$\ln \frac{C}{C_0} = -k_1 t$	k_1	0.08	0.06
			R^2	0.76	0.88
Second-order	$r_c = \frac{dC}{dt} = k_2 C^2$	$\frac{1}{C} - \frac{1}{C_0} = k_2 t$	k_2	0.0003	0.00015
			R^2	0.92	0.95

The results of the analysis of the variance(one way ANOVA) are shown the assosiation between different PCBs loading rate is insignificant, but the relation between PCBs influent and removal efficiencies with t-test were statistically significant (P value<0.001).

In many studies, information kinetics is very important because it can characterize the concentration of the pollutant in any time and estimate the time required for the organic removal pollutants (Martin,1999). In this study, kinetic data was performed based on a cycle operation of the

reactors in stable conditions (Table. 1). It can be learned from Table 1 that in stable condition of reactors operation, second order kinetic model was defined for COD removal from synthetic industrial wastewater with co-substrate by SBBR reactors (R_2> 0.9).

These results also suggested that by providing stable condition in the reactors, COD removal efficiency is proportional to the second order, whereas the rate constant of COD removed by SBBR1 and SBBR2 was 0.0003 and 0.00015, respectively.

Conclusion

In this study, PCBs aerobic biodegradation was successfully performed using two SBBR bioreactors. However, this study has shown that PCBs treatment by a biofilm sequencing batch reactor is capable of removing oil, resulting in the reduction of residual COD. In comparison with acetone, acetic acid is inexpensive, abundant and safe for environment, moreover, it could be used well as co-substrate by bacteria. So, in this study we recommended it as a solvent for oil containing PCBs compounds. PCBs biodegradation rate could be optimized further by using constructed hybrid anaerobic-aerobic processes.

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