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Biodegradation of tetrachloroethylene-rich synthetic wastewater in anaerobic hybrid reactor

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ABSTRACT

In this study, the anaerobic hybrid reactor (AHR) was used for the treatment of synthetic wastewater containing tetrachloroethylene (PCE) by systematic increment in the influent PCE and chemical oxygen demand (COD) concentrations. According to the acclimatization study, 99.95 ± 0.07% of PCE and 98.26 ± 0.46% of COD removals were obtained at an influent PCE and COD concentrations of 50mg/l and 2,000 mg/l, respectively. The maximum yield coefficient (Y_m) was found to be 0.055 mg biomass/mg COD, while the maximum specific substrate as COD (q_m S) and co-substrate as PCE (q_m CS) utilization rates were found to be 570 mg COD/g volatile suspended solids (VSS)/d and 15.83 mg PCE/g VSS/d, respectively, during the bio-kinetics study. The optimum hydraulic retention time for the maximum PCE removal was observed to be 18 h. The results of this study broadly indicated that the AHR has the high potentiality for the treatment of wastewater containing high concentration of PCE.

Keywords: Anaerobic hybrid reactor; Tetrachloroethylene; Acclimatization; HRT; Bio-kinetics

1. Introduction

The rapidly changing technologies, products, and practices today frequently carry with them an increasing amount of organic and inorganic materials in their wastes. Different types of industries have their own characteristic effluents and contain many hazardous substances. The untreated or partially treated wastes and effluents when discharged into the environment create several types of environmental problems. Chlorinated organic compounds are among the largest industrial chemicals, which have been produced at a rate of millions of tons according to the annual production figures [1]. These compounds are widely used as industrial solvents for painting, degreasing, dry cleaning, etc. In recent years, chlorinated organic compounds, such as tetrachloroethylene (PCE), trichloroethylene (TCE), dichloroethylene (DCE), vinyl chloride (VC), etc., which are carcinogenic, have been detected in ground water and drinking water as a result of long-standing solvent disposal malpractice, improper handling and storage [2,3].

Being cost-effective, the biological degradation of PCE either aerobically or anaerobically has been a major focus of the scientists today. Many researchers found that PCE can successfully be degraded by aerobic treatment process [4,5]. However, the volatilization

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(stripping off) of PCE in aerobic treatment process causing direct air pollution is one of the major constraints associated with the process [5]. In this context, anaerobic process is gaining more attention amongst the researchers and scientists for the treatment of PCE [3].

Treatment efficiency of a bio-reactor lies in the biomass retention capacity and the proper granulation of the biomass inside the reactor. Anaerobic treatment of wastewater using upflow anaerobic sludge blanket (UASB) reactor was considered to be the most effective for the treatment of a wide range of pollutants [6–8]. But the poor granulation, low sludge retention time and in some cases washout of sludge in currently used UASB reactor have been reported by many researchers [9,10]. In order to minimize the limitations of the UASB reactors, hybrid configuration of bioreactors combining the sludge blanket in the lower part of the reactor and an inert filter media in the upper part of the reactor have been proposed by many researchers [10,11]. Such hybrid reactors have been reported to promote the advantages of both the UASB and the upflow filter, while minimizing their limitations. However, the detailed studies on the process performance of the anaerobic hybrid reactor (AHR) for the treatment of varieties of organic compounds which persists most frequently in the industrial wastewaters are still lacking. Till date, no literature is available on the treatment of PCE in AHR. In this regard, this study was undertaken to remove high concentration of PCE from a synthetic wastewater, in AHR.

2. Materials and methodology

2.1. Chemicals

The chlorinated organic compounds used in this study were PCE (Merck India, 98%), TCE (Merck India, 98%), trans-1, 2-DCE (Aldrich Chemical, Wisconsin, USA, 98%), cis-1, 2-DCE (Aldrich Chemical, Wisconsin, USA, 98%), and VC (Sigma Aldrich, India, 98%). Standard gases were 5% (v/v) methane (Span gas India) and 108 ppm ethylene (Span gas India).

2.2. Experimental setup

A laboratory-scale model of AHR was designed and fabricated as per the guidelines provided by Lettinga et al. [12]. Fig. 1 shows the schematic view of the AHR. The reactor was made of 6 mm thick transparent acrylic sheet with a capacity of 15.5 l. A hopper (150 mm height) was provided at the bottom of the reactor. The feed (synthetic wastewater) containing PCE was fed to the reactor through the inlet pipe (diameter = 25 mm) at the bottom using a peristaltic pump. A filter media (300 mm height), consisting of 25 mm diameter of PVC pipes, were provided below the outlet. The effluent outlet pipe of 25 mm diameter was provided at the top of the reactor. A gas liquid solid separator device made of square pyramid with bottom dimensions of 80×80 mm and side slopes of 50° was provided at the top to facilitate the separation of gas from the biomass and effluent. Gas outlet pipe of 25 mm diameter was provided at the apex of the reactor for facilitating outlet of biogas produced in the reactor.

2.3. Wastewater and nutrients

The composition of the synthetic wastewater is given in Table 1 [13]. One milliliter of the trace metal solution was added/l of the synthetic feed. Sodium acetate, methanol, and acetone were used as the carbon source for the study.

2.4. Analytical methods

The pH, chemical oxygen demand (COD), and volatile suspended solids (VSS) were analyzed as per the standard methods [14], and the rate of biogas production was measured by water displacement method.

The PCE, TCE, DCE, and VC were analyzed by injecting 100 μl headspace sample to a Gas Chromatograph (GC 2000A) equipped with a 30 m × 0.25 mm capillary column (DB-1) and an electron capture detector (Ni-63). The starting temperature of the oven was kept at 50 °C which reached upto 180 °C at a rate of 10° C/min. Both the injector and detector temperatures were kept at 200 °C. The carrier gas nitrogen was passed at a flow rate of 1 ml/min. Calibration standards were prepared from stock solutions of 100 mg/l of PCE, TCE, DCE, and VC in methanol [15]. Vials of 10 ml capacity with Teflon-faced septas and screw caps were used. The headspace concentration of each sample was calculated using Henry's law relationships [16]. Effluent samples were filtered using 0.45 μm membrane filter and analyzed within 3 h of being collected.

Methane and ethylene were analyzed by a GC equipped with a flame ionization detector using a 2 m × 3 mm ss column packed with N-octane on 80/100 mesh porasil-C. The oven and injector temperatures were set at 40 °C and 100 °C, respectively, and the detector temperature was set at 100 °C. Nitrogen was used as a carrier gas at a flow rate of 20 ml/min.

2.5. Experimental protocol

The anaerobic sludge was brought from the domestic waste water treatment plant. The sludge was

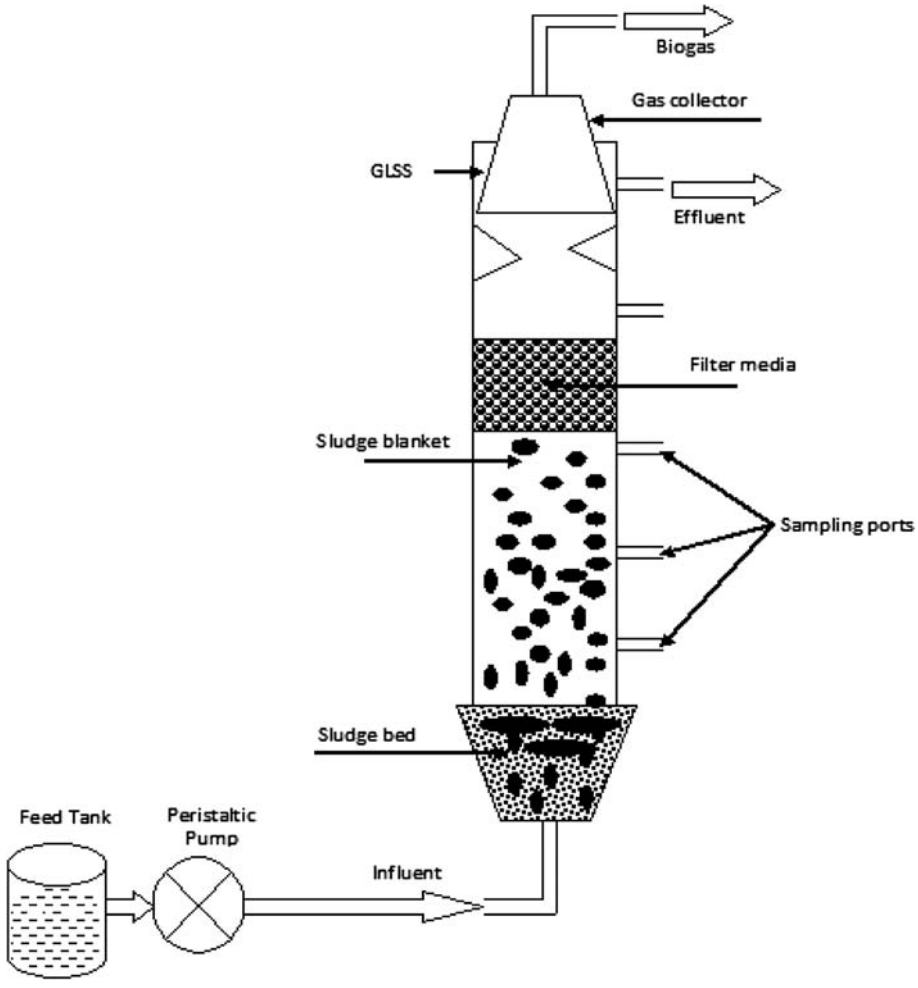

Fig. 1. Schematic diagram of the AHR.

first screened to remove debris using 150 μm sieves. A 5 l of sludge with VSS concentration of 15 g/l was transferred to the AHR. After that, the start-up phase was carried out by feeding the sludge inside the reactor with the synthetic wastewater which contained 1,000 mg/l of COD. The target pollutant (PCE) was not added at this stage.

After the completion of the start-up phase, the acclimatization study was performed. This study was carried out in two phases, namely the first phase of acclimatization and the second phase of acclimatization. During the first phase of the acclimatization, the influent COD concentration (1,000 mg/l) was kept constant and the influent PCE concentration was gradually increased from 5 to 50 mg/l (5, 10, 15, 20, 30, 40, and 50 mg/l) in a step wise manner. In the second phase of the acclimatization, the influent PCE concentration (50 mg/l) was kept constant, while the influent COD concentration was gradually increased from 1,000mg/l to 2,000 mg/l in a step wise manner (1,250, 1,500, 1,750, and 2,000 mg/l) to study the performance of the AHR at different higher organic loading rates (OLR). The hydraulic retention time (HRT) was kept constant at 24 h throughout the start-up and the acclimatization study.

The bio-kinetics study was carried out with the data obtained from the start-up and the acclimatization study. The graphical forms of the Lawrence and McCarty's Model [17] was employed for the determination of the yield coefficient (Y_m), bacterial decay coefficient (K_d), maximum specific substrate (COD), and maximum specific co-substrate (PCE) utilization rates (q_m S and q_m CS as maximum specific substrate and co-substrate utilization rates, respectively) and half velocity constants for substrate (K_s S) and co-substrate (K_s CS).

The effects of different HRTs (48, 36, 24, 18, 12 and 6 h) on PCE biodegradation were studied after the completion of the entire phase of acclimatization. The influent PCE and COD concentrations were

Table 1
Composition of the synthetic wastewater

Sl. no.	Compounds	Concentration (mg/l)
1	Sodium acetate	789–1,578
2	Methanol	222–444
3	Acetone	151–302
4	PCE	5–50
5	K_2HPO_4	8.4–15.8
6	KH_2PO_4	15.2–30
7	$(NH_4)_2SO_4$	20–40
8	NH_4Cl	100–200
9	$NaHCO_3$	1,000–2,000
10	$CaCl_2{\cdot}2H_2O$	294
11	$MgSO_4{\cdot}7H_2O$	5,000
12	$FeCl_2{\cdot}4H_2O$	6,000
13	$CoCl_2{\cdot}4H_2O$	880
14	H_3BO_3	100
15	$ZnSO_4{\cdot}7H_2O$	100
16	$CuSO_4{\cdot}5H_2O$	1,000
17	$NiSO_4{\cdot}8H_2O$	1,000
18	$MnCl_2{\cdot}4H_2O$	5,000
19	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	640

maintained at 50 mg/l and 2,000 mg/l, respectively, during the HRT study. Once the reactor reached to a steady-state condition, the observed variations were recorded before changing the HRT. The observations were recorded over a period of 10 days at each HRT, during the steady-state condition.

A mass balance of PCE biodegradation was carried out at different HRTs. Using influent PCE concentration as a parameter, a mass balance considering the relative distributions of PCE, TCE, DCE, VC, and ethylene in liquid and gas phases were worked out.

The entire experimentation was done at 27 ± 0.5°C. The results of all the parameters reported in the text are the average data obtained during the steady-state condition.

3. Results and discussion

3.1. Start-up of the AHR

During the start-up period, it was observed that initially the COD reduction in the AHR was found to be 52.55 ± 0.51%, which increased gradually with time and reached to 98.88 ± 0.42% after the completion of the start-up phase. The biogas production also increased gradually and found to be 7.0 ± 0.58 l/d which contained 67.58 ± 0.55% of methane in its composition. The performance of the reactor in terms of percentage COD reduction and biogas production during the start-up period is shown in Fig. 2. It can be seen from the figure that after 51 days of operation, the AHR showed maximum COD reduction (>98%) and biogas production (>6.8 l/d) in a steady manner. Thus, 51 days of operation was considered for the completion of start-up of the AHR in this study. Many researchers [18–20] reported about the start-up periods of 2–3 months to one year (or even more) for different anaerobic reactors.

3.2. Acclimatization

Acclimatization of the seed sludge helps in the genetic changes in microorganism and induction of biodegradation capability in microorganisms [21]. The performance of the AHR during the first and second phase of acclimatization is discussed below.

3.2.1. First phase of acclimatization

It was observed that during each step increment in the influent PCE concentration, the percentage PCE and COD reductions dropped down due to the inhibitory effect of PCE, and then gradually reached to a steady-state condition within 7–15 days of operation. After being acclimatized to each PCE load, the reactor was further run for another 10 days at that same PCE load to obtain the average data of the AHR's performance during the steady-state condition. Thus, the reactor was operated for 181 days and during that period, the reactor was found to be well acclimatized with the influent PCE and COD loads. The PCE and COD reductions were found to be 99.9 ± 0.11% and 97.64 ± 0.52%, respectively, during the steady-state condition, after the completion of the first phase of acclimatization. The biogas production at this stage

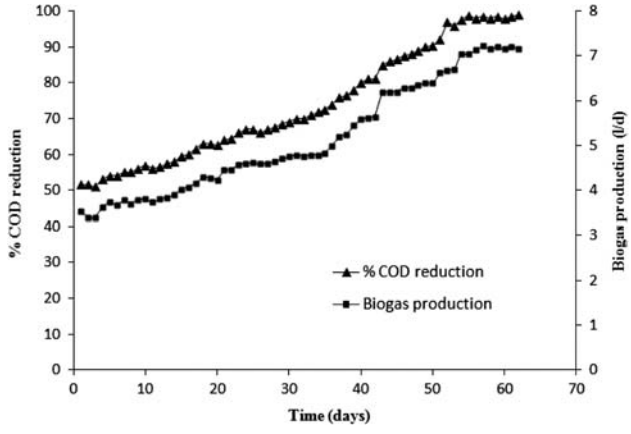

Fig. 2. Percentage COD reduction and biogas production in the AHR during the start-up phase.

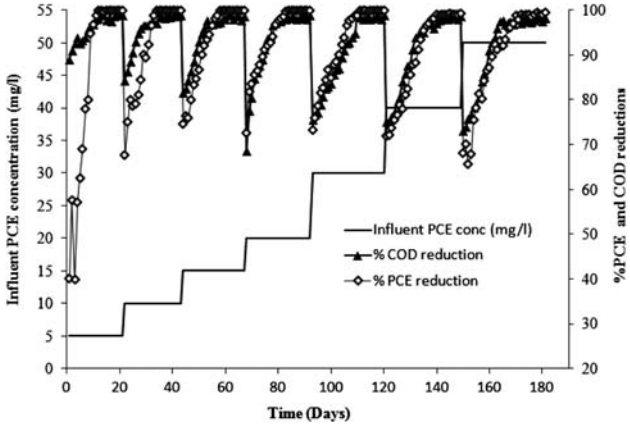

Fig. 3. Percentage PCE and COD reductions in the AHR during the first phase of acclimatization.

was found to be 7.54 ± 0.28 l/d which contained 65.2 ± 0.54% of methane in its composition. Fig. 3 depicts the performance of the reactor during the first phase of acclimatization.

3.2.2. Second phase of acclimatization

After the completion of the first phase of acclimatization, the second phase of acclimatization study was carried out. It was observed that, during stepwise increment in the influent COD concentration, initially the PCE and COD reductions decreased slightly, and within 7–10 days of operation the steady removals of PCE and COD were achieved. The performance of the reactor during the second phase of acclimatization has been shown in Fig. 4. Compared with the first phase of acclimatization, the AHR showed slight better performance in terms of PCE and COD reductions (99.95 ± 0.07% and 98.26 ± 0.46% of PCE and COD reductions, respectively) during the steady-state condition, after the completion of this phase of acclimatization. The above observation proved the ability of the AHR to be operated at high OLR. The improvement in the performance of the AHR at this phase of acclimatization might be attributed to the optimum sludge loading rate (SLR) (0.11 kg COD/kg VSS/d) and the proper mixing inside the reactor due to high rate of biogas production (14.63 ± 0.56 l/d which contained 66 ± 0.74% of methane in its composition). Prakash and Gupta observed an optimum SLR of 0.1 kg COD/kg VSS/d for anaerobic PCE degradation in a UASB reactor [13]. Sponza reported 92% of COD removal and 88% of PCE removal efficiencies of a UASB reactor when the reactor was operated at 30 mg/l/d and 10.5 g/l/d of PCE and COD loading rates, respectively [22]. The high level of PCE and COD removals obtained in this study might be due to the dual advantages of the AHR which facilitated the suspended as well as the attached growth of the micro organisms inside the reactor.

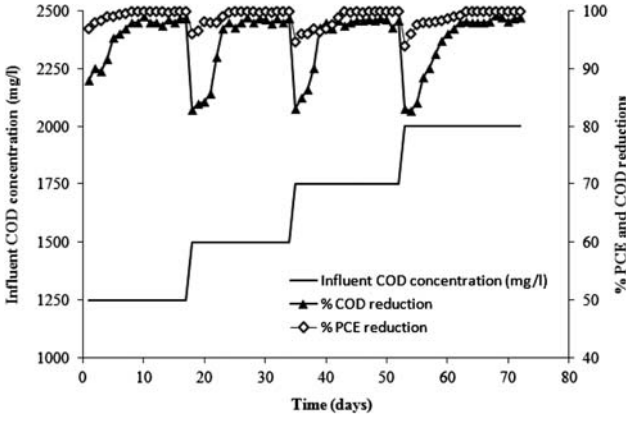

Fig. 4. Percentage PCE and COD reductions in the AHR during the second phase of acclimatization.

3.3. Bio-kinetics study

Determination of the kinetic coefficient is essential to examine the performance of any biological reactor. An understanding of the bio-kinetics parameters for any biological system is important for the prediction of the performance of the system in respect to the biomass growth as well as substrate or co-substrate utilization.

The bio-kinetics studies revealed a substantial biomass yield inside the reactor ($Y_m = 0.055$ mg biomass/mg COD) and a lower decay co-efficient ($K_d = 0.001$/d) which suggested a healthy growth of the microorganisms inside the AHR. Gupta et al. reported the Y_m and K_d values of 0.0532 mg biomass/mg COD and 0.0041/d, respectively, of an AHR treating distillery spent wash [10]. The q_m S and q_m CS were found to be 570 mg COD/g VSS/d and 15.83 mg PCE/g VSS/d, respectively, whereas the K_s S, and K_s CS were found to be 276.18 mg COD/l and 0.188 mg PCE/l, respectively. Table 2 summarizes the data obtained during the bio-kinetics study. It can be seen from the Table 2 that the values of the q_m S and K_s S are much higher than the values of q_m CS and K_s CS, which is a clear indication of the relative affinity of the micro organisms for the simple easily biodegradable substrate than the bio resistant PCE. Carter and Jewell reported the q_m and K_s values of 5.33 mg PCE/g VSS/d and 0.009 mg PCE/l, respectively in an anaerobic attached-film expanded-bed process [23]. Sponza reported the q_m and K_s values of 2.38 mg

Table 2
Summarization of the data of the bio-kinetics coefficients

Bio-kinetics coefficients	Values
Y_m (mg biomass/mg COD)	0.055
K_d (per day)	0.001
q_m S (mg COD/g VSS/d)	570
q_m CS (mg PCE/g VSS/d)	15.83
K_s S (mg COD/l)	276.18
K_s CS (mg PCE/l)	0.188

COD/mg TSS/d and 108 mg COD/l, respectively for anaerobic PCE biotransformation together with glucose as carbon and energy source [22]. The results of the bio-kinetics study of this research paper are well comparable with the previous findings mentioned above.

3.4. Performance of the AHR treating PCE at different HRTs

Performance of the AHR at various HRTs dictated that the biodegradation of PCE varied marginally from 48 to 18 h and found to be maximum (99.97 ± 0.11%) at 18 h of HRT. However, when the HRT was further reduced from 18 to 6 h, the rate of PCE biodegradation decreased, showing a stress to the PCE degrading bacteria due to many times increase in substrate and co-substrate loading rates, and found to be minimum at 6 h of HRT (98.64 ± 0.28%). Almost similar observations were obtained in the COD reduction profile of the reactor. The COD reduction was found to be 98.0 ± 0.36% during the steady-state condition at 48 h of HRT, which reached to a maximum of 98.87 ± 0.4% at 18 h of HRT and further subsequently reduced to 92.25 ± 0.61% at 6 h of HRT. Thus 18 h of HRT was considered to be the optimum HRT for the maximum PCE and COD reductions. The biogas production during the steady-state condition at this optimum HRT (18 h) was found to be 20.15 ± 0.72 l/d, which contained 66.8 ± 0.87% of methane in its composition. The high rates of biogas productions obtained in this study can be supported by the previous researches, where high rates of biogas productions have been reported at different high OLRs [8,10,24]. The data obtained during the steady-state condition at different HRTs are summarized in Table 3. Hwu and Lu reported that the PCE (loading rate of 3 mg PCE/l/d) removal increased from 51 ± 5% to 87 ± 3% when HRT was increased from 1day to 4 days [25]. Basu and Gupta reported an optimum HRT of 24 h for the treatment of 1,1,2,2-tetrachloroethane (>98% removal) in a UASB reactor [26]. The reactor's performance of the present study at different HRTs proved that the AHR can effectively remove high PCE and organic loads (0.066 kg PCE/m^3/d and 2.66 kg COD/m^3/d, respectively) within a shorter period of time (optimum HRT = 18 h).

3.5. Mass balance for PCE biodegradation at different HRTs

A theoretical mass balance of PCE biodegradation, based on the average data obtained during the steady-state condition at different HRTs, was carried out. PCE fed to the reactor could theoretically be removed by adsorption in the sludge, biological degradation or stripping into the gas phase. Since the sludge was saturated, the removal of PCE by adsorption was neglected. Hence, the biological degradation and stripping of PCE and its intermediates were considered for preparing the mass balance of PCE. The stripping of PCE and its intermediates into the produced gas might be due to the high rate of biogas production inside the reactor and the volatile nature of the compounds. Since the PCE fed to the AHR was in liquid form, the stripped fractions of PCE and its intermediates in the produced gas were converted to their corresponding hypothetical liquid phase concentrations using Henry's law, for the preparation of the mass balance of PCE [13,16]. During the biodegradation of PCE, the dehalogination by-products such as TCE, cis-DCE, VC and ethylene were detected either in the effluent, or in the biogas, or in both at the same time at different concentrations throughout the study. DCE has two isomers that is, cis-DCE and trans-DCE, out of which trans-DCE isomer was not detected in the effluent and biogas throughout the study. (Method detection limit for trans-DCE was 0.02 mg/l.) Most of the previous studies reported about the dechlorination of PCE and TCE to VC, or ethylene through cis-DCE [2,13,23]. The transformation of PCE occurred on a one to one molar basis. Hence, one mole of TCE, cis-DCE, VC, and ethylene produced in the reactor corresponds to an equal mole of PCE.

The mass balance of PCE revealed that out of the total influent PCE fed to the reactor at an optimum 18 h of HRT, approximately, 0.22% and 0.6% of PCE and TCE, respectively, were found to be accumulated in the effluent and biogas while, 0.72%, 1%, and 90.75% of cis-DCE, VC and ethylene, respectively, were accumulated in the biogas (Fig. 5). Remaining 6.71% was un-accounted. The cis-DCE and VC accumulation were not detected in the effluent during the 48 to 18 h of HRTs (Method detection limits for cis-DCE and VC were 0.02 mg/l and 0.04 mg/l, respectively), but were found to be accumulated in the

Table 3
Summarization of the data of the AHR treating PCE at different HRTs

HRTs (h)	Inf. PCE loading rate (kg PCE/ m^3/d)	Organic loading rate (kg COD/m^3/d)	COD reduction (%)	PCE reduction (%)	Biogas production (l/d)	Methane (%)
48	0.025	1	98.0 ± 0.36	99.92 ± 0.09	7.52 ± 0.61	65.2 ± 0.75
36	0.033	1.33	98.21 ± 0.51	99.93 ± 0.15	10.12 ± 0. 54	65.6 ± 1.11
24	0.05	2	98.26 ± 0.46	99.95 ± 0.07	14.63 ± 0.56	66 ± 0.74
18	0.066	2.66	98.87 ± 0.4	99.97 ± 0.11	20.15 ± 0.72	66.8 ± 0.87
12	0.1	4	95.25 ± 0.55	99.18 ± 0.17	29.15 ± 0.63	65 ± 0.81
6	0.2	8	92.25 ± 0.61	98.64 ± 0.28	56.86 ± 0.71	64 ± 0.73

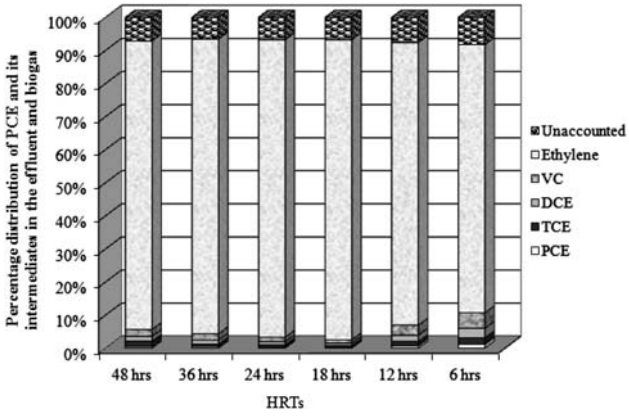

Fig. 5. Mass balance of PCE at different HRTs.

produced biogas due to stripping. The accumulation of PCE, TCE, cis-DCE and VC concentrations in the effluent and biogas were increased upon further decrease in the HRTs from 18 to 6 h. The major end product of the PCE dehalogination was found to be ethylene (>80%) at every HRT which is a non-chlorinated product. In the previous studies, mass balance for PCE demonstrated almost 95–98% conversion of PCE and intermediates in an anaerobic fixed bed column [27] and 98.9% conversion of PCE and intermediates in an expanded-bed granular activated carbon anaerobic reactor [28]. However, in this case the percentage conversion of PCE into ethylene was greater than reported in the previous studies [13,23,27,28].

4. Conclusions

From this study it can be concluded that the AHR showed an overall higher level of performance in terms of PCE and COD reductions. The PCE and COD reductions were found to be 99.95 ± 0.07% and 98.26 ± 0.46% respectively, during the steady-state condition after the completion of the entire phase of acclimatization. The biogas produced during the biodegradation process was found to be very rich in methane which can effectively be used as an economic bio-fuel if properly processed. The bio-kinetics study revealed higher rates of substrate and co-substrate utilization rates (The q_m S and q_m CS was found to be 570 mg COD/g VSS/d and 15.83 mg PCE/g VSS/d, respectively). The optimum HRT for the highest PCE and COD reductions was found to be 18 h in which, 99.97 ± 0.11% of PCE and 98.87 ± 0.4% of COD reductions took place in the AHR. More importantly, the dehalogination intermediates of PCE such as TCE, cis-DCE and VC were found to be very less in concentration in the effluent and biogas at different HRTs. In this study, the AHR was found to be very effective for the treatment of high concentration of PCE within a short period of time.

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