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Comparative Study on the Performance of Anaerobic and Aerobic Biotrickling Filter for Removal of Chloroform

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Abstract

Use of biotrickling filter (BTF) for gas phase treatment of volatile trihalomethanes (THMs) stripped from water treatment plants could be an attractive treatment option. The aim of this study is to use laboratory-scale anaerobic BTF to treat gaseous chloroform (recalcitrant to biological transformation) as a model THM and compare results with aerobic BTF. Additional investigations were conducted to determine the microbial diversity present within the BTFs. Chloroform is a hydrophobic volatile THM known to be difficult to biodegrade. To improve the degradation process, ethanol was used as a cometabolite at a different ratio to chloroform. The experimental plan was designed to operate one BTF under anaerobic condition and the other one under aerobic acidic condition. Higher elimination capacity (EC) of 0.23 ± 0.01 g/[m^3·h] was observed with a removal efficiency of $80.9\% \pm 4\%$ for the aerobic BTF operating at pH 4 for the concentration ratio of 1:40 chloroform to ethanol. For similar ratio, the anaerobic BTF supported lower removal efficiency of $59\% \pm 10\%$ with corresponding lower EC of 0.16 ± 0.01 g/[m^3·h]. Carbon recovery

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acquired for anaerobic and aerobic BTFs was 59% and 63%, respectively. The loading rate for chloroform on both BTFs was 0.27 g/[m^3·h] (per m^3 of filter bed volume). Variations of the microbial community were attributed to degradation of chloroform in each BTF. *Azospira oryzae* and *Azospira restrica* were the dominant bacteria and potential candidates for chloroform degradation for the anaerobic BTF, whereas *Fusarium* sp. and *Fusarium solani* were the dominant fungi and potential candidates for chloroform degradation in the aerobic BTF.

Keywords

Aerobic; anaerobic; biotrickling filter; microbial diversity; trihalomethanes

Introduction

Drinking water disinfection by chlorination is the most important step in water treatment to kill pathogens and reduce waterborne diseases. However, chlorine also reacts with the natural organic matter (NOM) that is present in most surface water and produces many harmful disinfection byproducts (DBPs). Most DBPs are known to be toxic and pose a risk to human health (Gopal *et al.*, 2007). Many DBPs are also bioaccumulative and thus, long-term exposure to low DBPs causes a chronic health risk. The common DBPs from chlorination of water include trihalomethanes (THMs) and haloacetic acids (HAAs) (Krasner *et al.*, 1989; Dalvi *et al.*, 2000). The main THMs include chloroform (CF), dichlorobromomethane (DCBM), dibromochloromethane (DBCM), and bromoform (Lichtfouse, 2005). Various factors affecting the formation of DBPs include the water pH and temperature, the concentration and contact time of chlorine and bromine, and the concentration of NOMs (Pourmoghaddas and Stevens, 1995).

The methods currently used to reduce NOMs and minimize the formation of DBPs include the use of activated carbon filters and conventional water treatment processes, including clarification, coagulation, flocculation, sedimentation, and filtration (Xie, 2005). However, these controlling methods can only remove about 30% of the precursors for THMs (Gh and Gh, 2011). In addition, removing these THMs by physical and/or chemical methods at low concentrations found in drinking water is expensive and may generate a secondary pollutant. The high Henry's law constant of many of the THMs allows alternative approaches for treatment such as gas stripping combined with biological treatment (Staudinger and Roberts, 2001). Thus, the formation of THMs in drinking water has highlighted the need for exploring alternative disinfectants for chlorine and new treatment technologies for removing THMs after they are formed.

In this study, chloroform was taken as a model DBP since it is the most toxic and most abundant of the THMs. Chloroform is a volatile THM and could be removed from contaminated waters to the gaseous phase by air stripping (McGregor *et al.*, 1988; Lichtfouse, 2005; LaKind *et al.*, 2010). Biological treatment techniques for volatile organic compounds (VOCs) removal have several advantages. Compared to the conventional methods, such as incineration, catalytic oxidation, and adsorption, biological treatments could be cost-effective as safer and eco-friendly (Delhoménie *et al.*, 2005). Most of the

research on the biological treatment of chloroform has been limited to batch liquid phase processes at wastewater treatment plants or hazardous waste disposal sites.

Under anaerobic conditions, chloroform could undergo a reductive biotransformation by pure cultures of methanogens (Egli *et al.*, 1987; Yu and Smith, 1997), acetogenic bacteria (Egli *et al.*, 1988), sulfate-reducing bacteria (Egli *et al.*, 1990), and iron-reducing bacteria (Egli *et al.*, 1990; Picardal *et al.*, 1993) producing partial dehalogenation and mineralization (Egli *et al.*, 1988, 1990; Picardal *et al.*, 1993; Yu and Smith, 1997). Thus, biological techniques have resulted in dechlorination of chloroform to dichloromethane, methane (CH_4), and carbon dioxide (CO_2) (Egli *et al.*, 1990; Mikesell and Boyd, 1990; Becker and Freedman, 1994).

Chloroform is a trichlorinated CH_4 compound and is recalcitrant to biological transformation. It can only be transformed or biodegraded in the presence of a cometabolite under anaerobic or aerobic environments (Zitomer and Speece, 1995; Field and Sierra-Alvarez, 2004; Cappelletti *et al.*, 2012). Furthermore, the halogenic nature of chloroform can affect the biodegradation process (Leson and Winer, 1991). To overcome this obstacle, halogenated organic compounds often require the presence of an easily degradable substrate that can increase their biodegradability by cometabolism (Leson and Winer, 1991). Anaerobic dechlorination of chloroform has been observed by different researchers by using methanogenic microbes with electron donating cometabolites in reductive chloroform biotransformation (Bouwer *et al.*, 1981; Krone *et al.*, 1989; Mikesell and Boyd, 1990; Bagley and Gossett, 1995). In addition, chloroform removal ranging between 13% and 43% was obtained in a study of cometabolism of chloroform and other THMs (Wahman *et al.*, 2006).

Although most studies show successful biodegradation of chloroform in the liquid phase, there is a limited amount of reported work on the use of biofiltration for the removal of chloroform from gaseous streams. Biofiltration is one of the proven technologies for removing VOCs from high volume stream as it is environment friendly, cost-effective, and releases fewer byproducts (Yoon *et al.*, 2002). The use of an aerobic biofiltration technique has been reported for the biotreatment of chloroform with other mixtures of different VOCs (Yoon *et al.*, 2002; Balasubramanian *et al.*, 2012). Yoon *et al.* (2002) have shown the degradation potential of nine VOCs, including chloroform, and found the highest removal was for toluene (99%) and the lowest removal was for chloroform (89.4%). Similarly, Balasubramanian *et al.* evaluated the biodegradation of chloroform along with a mixture of VOCs commonly found in pharmaceutical emissions, using a biotrickling filter (BTF). Their study showed that increasing the rate of chloroform loading significantly reduced the degradation efficiency of the reactor for the mixture of VOCs (Balasubramanian *et al.*, 2012). Similarly, in our previous work, an aerobic BTF was used to treat gaseous chloroform in the presence of ethanol as a cometabolite (Palanisamy *et al.*, 2016). However, to the best of our knowledge, no reported work in literature is available for the use of anaerobic BTF in treating chloroform.

The main goal of this study is to examine gas phase chloroform removal by using anaerobic BTF in the presence of ethanol as a cometabolite. In addition, a comparison was conducted

on the performances of this current anaerobic and previously studied aerobic BTF. The study also investigated the microbial ecology within both BTFs to get a deep insight of the factors affecting BTFs.

Materials and Methods

Materials

Chloroform with 99.8% purity was obtained from Fisher Scientific (Pittsburgh, PA) and ethanol with 99.5% purity was obtained from Sigma-Aldrich (St. Louis, MO). Chloroform is highly hydrophobic with a Henry's law constant, K_H, of 3.5×10^{-3} [atm·m^3]/mol at 25°C, and the K_H value of the hydrophilic ethanol is 5.1×10^{-6} [atm·m^3]/mol at 25°C (Butler *et al.*, 1935; Chen *et al.*, 2012). The measuring sensors for pH, dissolved oxygen (DO), and ammonia were acquired from Accumate Instruments. Genomic DNA extractions of bacterial and fungi strains were performed using the Mo Bio PowerSoil DNA (M Bio Lab, Inc., Carlsbad, CA) Kit, which was done by Molecular Research LP (MR DNA, Shallowater, TX).

Biotrickling filter

In this work, an anaerobic BTF is evaluated for degrading chloroform. The results were used to compare the performance to a previously studied aerobic BTF. The loading rate of chloroform for both BTFs was kept at 0.27 g/[m^3·h] (i.e., per m^3 of filter volume) throughout the experiment. Ethanol (hydrophilic VOC) was introduced as a gaseous cometabolite at different loading rates for both BTFs. Table 1 shows all the operational parameters for the anaerobic BTF. Figure 1 also shows the schematic diagram of each BTF. Each BTF column consists of seven cylindrical glass sections with an internal diameter of 7.6 cm and a total length of 130 cm, and is packed with pelletized diatomaceous earth biological support media to a depth of about 60 cm (Celite® 6 mm R-635 Bio-Catalyst Carrier; Celite Corp., Lompoc, CA).

Both BTFs operated in a cocurrent mode with both gas and liquid flow downward to acclimatize and enhance the growth of biomass. In this anaerobic BTF system, nitrogen was used as a carrier gas with a flowrate of 0.5 L/min, which provides a corresponding empty bed residence time (EBRT) of 5.44 min. The initial chloroform concentration was 5 ppmv. Methanogenic microorganisms were used to inoculate the filter bed. Initially, these bacteria were obtained from a nutrient-enriched solution kept under a blanket of nitrogen gas that was acclimated in our laboratory to chloroform in a 4 L amber batch reactor for 2 months. The chloroform feed was stepwise increased from 5 to 50 ppmv within the 2-month period. This inoculum was mixed in the ratio of 1:1 with another methanogenic bacteria acquired from another bioreactor that was treating food waste before seeding the BTF. The origin of these methanogenic bacteria was from an anaerobic digester at a local wastewater treatment plant.

The buffered nutrient solution containing ammonia as electron donor was supplied at an average rate of 2.0 L/day. The growth media for anaerobic BTF were prepared with medium concentrations of 996 mg/L NH_4Cl, 414 mg/L KH_2PO_4, 390 mg/L $MgCl_2 \cdot 6H_2O$, 280 mg/L

$CaCl_2 \cdot 2H_2O$, 2 mg/L $FeCl_2 \cdot 4H_2O$, 4.79 mg/L $CuSO_4 \cdot 5H_2O$, 6.53 mg/L $MnSO_4 \cdot H_2O$, 5.24 mg/L $ZnCl_2$, 4.58 mg/L $CoCl_2 \cdot 6H_2O$, 0.32 mg/L $B(OH)_3$, 4.79 mg/L $NiCl_2 \cdot 6H_2O$, 0.12 mg/L 4-aminobenzoic acid (99%), 0.048 mg/L biotin, 0.0024 mg/L cyanocobalamin, 0.05 mg/L, folic acid dihydrate (99%), 0.12 mg/L nicotinic acid (98%), 0.12 mg/L pantothenic acid Ca-salt hydrate (98%), 0.24 mg/L pyridoxine hydrochloride (98%), 0.12 mg/L riboflavin (98%), 0.12 mg/L thiamine hydrochloride (99%), and 0.12 mg/L thioctic acid (98%). The composition of the nutrient solution was used according to the ones provided in literature (Zitomer and Speece, 1995; Gupta *et al.*, 1996; Wu *et al.*, 2015). One molar $NaHCO_3$ was used as a buffer to maintain the pH at 7.

The temperature was kept at 35°C in a temperature-controlled room to maintain favorable methanogen growth, whereas in the aerobic system, air was used as a carrier gas with a flowrate of 0.5 L/min at a corresponding EBRT of 5.44 min. In this case, the buffered nutrient solution containing nitrate was supplied at an average rate of 2.0 L/day. The nutrients were supplied at an acidic pH of 4 by the addition of sodium formate buffer to encourage the growth of fungi colonies. The buffered solution contains all necessary macronutrients, micronutrients, and buffers, as described by Sorial *et al.* (1995). The temperature of the aerobic BTF was maintained at 35°C, similar to the anaerobic BTF. Liquid chloroform and ethanol were injected through separate syringe pumps in series and vaporized into the nitrogen or air stream.

Strategies of biomass control

Aerobic BTF operation was tested for different biomass control technologies, namely stagnation and backwashing. The stagnation nonuse period was observed during 2 consecutive days per week. During the stagnation period, the BTF did not get any nutrients, VOCs, or air, whereas backwashing involves flushing the media bed with 18 L of buffered nutrient solution, inducing medium fluidization at ~50% bed expansion when the system is offline. Following this, the recirculating nutrient solution will be stopped, the biofilter is drained, and then another 18 L of the nutrients will be supplied for a final rinse. More details on biomass control technologies can be found in Hassan and Sorial (2009). However, for the case of anaerobic system, there was no need to use any kind of biomass controlling technique since there was no related biomass growth problem.

Sampling and analysis

Gas and liquid samples were collected daily from the BTF systems 5 days per week for the measurement of composition of feed and effluent gas/liquid streams. Liquid samples were collected for the measurement of the influent and effluent liquid pH, ammonia, and organic matter. The gas flow pressure drop across the bed and operating temperature were taken on daily basis. DO for the anaerobic BTF was taken every day to check for any leak by using Accumate DO probe. Gas phase samples for anaerobic BTF were taken online from different points along the BTF column using an electrically controlled low-bleed eight-port Valco valve and analyzed by gas chromatograph.

The samples were analyzed for chloroform, ethanol, or CH_4 as a byproduct. They were injected into gas chromatography (GC)–HP, Column: HP, 608, 30 m × 530 μm film

thickness, injection splitless through 5 mL sample loop equipped with a flame ionization detector (FID). The GC oven was programmed isothermal at 60°C (2 min) ramped to 90°C at a rate of 10°C/min. The carrier gas (He) flow rate was set at 3.5 mL/min at a constant flow rate. The FID was used with N_2 make-up gas at a flow rate of 30 mL/min, a fuel gas flow (H_2) of 40 mL/min, and airflow of 400 mL/min. Retention time for chloroform was 3.8 min under the above conditions used. For determining levels of reaction products, such as CO_2, samples were also taken automatically by GC HP-thermal conductivity detector (TCD) from each sampling port in the BTF. The GC oven was programmed isothermal at 60°C (1 min), ramped to 115°C at 25°C/min. The carrier gas (He) flow rate was set at 3.5 mL/min; the TCD was used with helium make-up gas at a flow rate of 5 mL/min.

Liquid samples were collected from the effluent stream of BTF once a week. The samples were filtered through a 0.45 μm membrane filter (Whatman Co.) and analyzed for influent and effluent concentrations of ammonia, nitrate, dissolved total carbon (TC), dissolved inorganic carbon (IC), and volatile suspended solids. The concentration of ammonia and nitrate was determined using ammonia and nitrate electrode sensors. Dissolved TC and dissolved IC content of the liquid samples were determined with a Shimadzu total organic carbon analyzer model TOC-L (Shimadzu Corp., Tokyo, Japan). The volatile suspended solids analysis was conducted according to Standard Method 2540G (APHA, 2005).

It should be noted that before samples are analyzed in the GC/FID, GC/TCD, electrode instruments, or TC/IC, the instruments are checked for meeting an instrument stability calibration criterion. This criterion is determined by using six concentration levels for target analytes. The response factor (RF) for each standard concentration level is then determined. The instrument stability for initial calibration is acceptable when the RF for each concentration level of the standard solutions is below 10% from the overall mean value for the six standard solutions.

Microbial community molecular analysis

Biofilm samples were collected from anaerobic and aerobic BTF within the media as shown in Fig. 1. The samples were taken from port 2 (first port from the top within the media) at the end of each phase before proceeding to the next phase. To get the microbial analysis result, samples from biofilter were collected at the end of each experimental phase (Zehraoui *et al.*, 2014; Zhai *et al.*, 2017). The samples consisted of about five media pellets covered with biomass suspended in liquid. All the samples collected were stored in a −20°C freezer before sending them to a molecular research laboratory (Molecular Research LP). In this microbial analysis study, bacteria and fungi were chosen for anaerobic and aerobic BTF, respectively. The main reason for bacteria used in the anaerobic BTF is that fungi could not grow under an anaerobic environment at a neutral condition.

Some researchers confirmed the strong correlation of bacterial community growth with pH, while decrease in pH favorably increased fungal growth (Bárcenas-Moreno *et al.*, 2011; Zehraoui *et al.*, 2014). The DNA of microbial mass in the samples was extracted using Mo Bio PowerSoil DNA (M Bio Lab, Inc.) following the manufacturer's instruction that includes cell breakage steps followed by the addition of detergents and high salt buffers, and enzymatic digestion with lysozyme and proteases. For ion torrent sequencing, the 16S

ribosomal RNA (rRNA) gene V4 variable region polymerase chain reaction (PCR) primers 515/806 were used in a single-step 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen), under the following conditions: 94°C for 3 min, followed by 28 cycles (5 cycle used on PCR products) of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed.

Sequencing was carried out at Molecular Research LP (www.mrdnalab.com) on an Ion Torrent Personal Genome machine (PGM) following the manufacturer's guidelines. Sequence data were processed using a proprietary analysis pipeline. Sequences were first depleted of barcodes and primers, and those under 150 bp or with ambiguous base calls, or with homopolymer runs exceeding 6 bp were removed. Operational taxonomic units (OTUs), which were defined by clustering at 3% divergence (97% similarity) (Dowd *et al.*, 2008; Edgar, 2010; Capone *et al.*, 2011; Eren *et al.*, 2011; Swanson *et al.*, 2011), were generated after denoising sequences and removing chimeras. The last OTUs were taxonomically classified using BLASTn against a database derived from RDPII (http://rdp.cme.msu.edu) and NCBI (www.ncbi.nlm.nih.gov) (DeSantis *et al.*, 2006).

Experimental Results

Anaerobic BTF performance—In this study, the effects of a cometabolite at different loading rates on the performance of anaerobic BTF were evaluated. The cometabolite was allowed to mix with chloroform in the mixing chamber to achieve higher removal efficiency by providing an additional electron donor to the microorganisms. Ethanol was used as a cometabolite since it readily mixes with chloroform and water. It is worth noting that the removal efficiency of ethanol was always above 98% for the given loading rate conditions studied for both BTFs. Therefore, the emphasis is placed on the performance of the BTF for chloroform degradation. The details of operation for anaerobic BTF is given in Table 1, where at every phase of operation, the corresponding influent concentration, loading rate, and days of operation are provided. Table 1 also summarizes the results of the BTF, including average removal efficiency and its standard deviation, and the elimination capacities of each phase of operation.

Figure 2 presents examples of a statistical summary of the removal efficiency as a box plot at different loading rates. The lower boundary of the box denotes the lower quartile, a line within the box marks the median, and the boundary of the box furthest from zero indicates the upper quartile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. In phase I, the BTF started up with a chloroform influent concentration of 5 ppmv and ethanol concentration of 25 ppmv providing a corresponding chloroform loading rate of 0.27 g/[m^3·h]. The BTF was run for 44 days under the conditions of phase I, and the average removal efficiency for this phase was 49% ± 9%, which provided an average elimination capacity (EC) of 0.13 ± 0.02 g/[m^3·h] (Table 1).

On day 45, the influent concentration of ethanol was further increased to 50 ppmv with a corresponding ethanol–chloroform ratio of 1:10. In phase II, the removal efficiency slightly increased to 52% ± 7% with an EC of 0.14 ± 0.01 g/[m^3·h]. After the system was left to run for 33 days (during phase II), the ethanol concentration was increased to 100 ppmv in phase III. At this level, the system ran for 41 days and the removal efficiency with a corresponding

EC was 56% ± 7% and 0.15 ± 0.02 g/[m^3·h], respectively. On day 118, the ratio of chloroform to ethanol was further increased to 1:40. During phase (IV, the removal efficiency was at 59% ± 10%, which provided a higher EC of 0.16 ± 0.01 g/[m^3·h] compared to the previous phases.

Aerobic BTF performance—The result for aerobic BTF was reported in our previous study (Palanisamy *et al.,* 2016). The details of operation for aerobic BTF is given in Table 1, where at every phase of operation, the corresponding influent concentration, loading rate, and days of operation are provided. Table 1 also summarizes the results of the BTF, including average removal efficiency with its standard deviation and the EC. During phase I, the removal efficiency of chloroform was 69.9% ± 9% with a corresponding EC of 0.21 ± 0.01 g/[m^3·h]. In phase II, the removal efficiency of chloroform was 71.6% ± 5% with an EC of 0.22 ± 0.01 g/[m^3·h]. In phase III, the removal efficiency of chloroform increased to 75.1% ± 9%, providing an EC of 0.22 ± 0.01 g/[m^3·h]. Finally, in phase IV, the removal efficiency of chloroform increased to 80.9% with a standard deviation of 4%. The corresponding EC for this phase was 0.23 ± 0.01 g/[m^3·h].

Discussion of the Results

Performance comparison for anaerobic and aerobic BTFs

Use of a cometabolite improved chloroform degradation for both BTFs. It has been observed that for both BTFs, the performance increased with an increase in the cometabolite concentration. Few studies have been conducted for the use of a cometabolite for chloroform degradation. The study conducted by Gupta *et al.* (1996) investigated the use of acetic acid as a cometabolite in anaerobic chloroform biotransformation in the liquid phase, which resulted in higher removal efficiency. Similarly, aerobic chloroform biodegradation has been observed during the oxidation of other cometabolites. Chloroform cooxidation with a formate or CH_4, with a butane oxidizing and nitrifying bacterium has been reported (Field and Sierra-Alvarez, 2004). In this study, chloroform displayed significant biodegradation rates when using ethanol as a cosubstrate at the ratio of 1:40 (phase IV). A similar conclusion was reported in our previous study in a fungal-based system (Palanisamy *et al.,*2016). In this work, fungi utilization greatly enhanced the performance of the aerobic BTF compared to the anaerobic one. The highest removal efficiency reported under an acidic aerobic condition significantly reached 80.9% ± 4% (Table 1). Interestingly, the highest EC was obtained during phase IV of the aerobic BTF (Table 1). It is postulated that the use of fungi in the aerobic system helped in enhancing the EC of chloroform. This enhanced performance could be due to the resilience of fungi to acid and dry conditions compared to bacteria, which is a helpful property when operating biofilters. Moreover, it is hypothesized that the aerial mycelia of fungi, which are in direct contact with the gas, can take up hydrophobic compounds faster than flat aqueous bacterial biofilm surfaces.

Although the aerobic condition showed an enhanced performance for the degradation of chloroform, the significance of the anaerobic degradation is the renewable energy source. The anaerobic process produces CH_4-rich biogas suitable for energy production, helping to replace fossil fuels. The ratio of CH_4 to CO_2 ranged from 1.77 to 2.05 (Table 1) for this

system. These values also correlated with the corresponding removal efficiency values. As the removal efficiency increased, the ratio also increased.

Kinetics of chloroform removal in BTFs

Removal performances as a function of depth within each BTF were measured weekly. For aerobic BTF, it was conducted 1 day following stagnation at the sampling ports located along the depth. At the same time, a similar measurement was taken for the anaerobic BTF. The samples were taken along the BTFs from ports that are located at 7.6, 23, 38, 53, and 60 cm down from the top of the packed bed. The kinetic analysis was conducted using the data from sampling ports within the media as there is a possibility of biodegradation on the top portion of the BTF above the media, or at the bottom disengagement chamber used for separation of liquid and gas effluents. The BTF is assumed to function as a plug flow reactor, and the removal kinetics was based on the pseudo-first-order reaction as a function of the depth of each BTF. At least three sampling data sets from each port were taken for every phase.

The sampling data for every phase were fitted to a linear model with the independent variable, time (seconds), and the dependent variable, $\log_e(C/C_0)$, where C is the effluent concentration and C_0 is the influent concentration. The kinetics reaction rate constants were obtained from the slopes of the regression lines. Figure 3 provides the results where the error bars represent the standard deviation from at least three data sets. Figure 3 clearly shows the advantage of fungi utilization in the BTF, which is indicated by a higher reaction rate constant compared to the anaerobic BTF at the same influent concentration. Chloroform reaction rate constant increased as the influent cometabolite loading increased. The reaction rate constant values for the four phases of the anaerobic BTF ranged from 0.001 to 0.0014/s. On the other hand, the reaction rate constant for the aerobic BTF ranged from 0.0011 to 0.0018/s. The highest reaction rate constant was observed in phase IV of each BTF. In the case of the anaerobic BTF, it correlates with the increase of ethanol loading rate. It is worth to note that increasing ethanol loading rates favored the growth of microbial population, which resulted in an increase in the biocatalyst, and thus improving the rates of biodegradation. During a similar ratio of chloroform to ethanol, the reaction rate constant for anaerobic BTF was always less compared with aerobic BTF, which correlates well with the removal efficiencies reported in Table 1.

Carbon mass balance

Cumulative CO_2 equivalent of chloroform in the influent was compared to the same equivalent in the effluent for both BTFs. The influent cumulative CO_2 consists of influent gaseous concentration and influent aqueous inorganic and organic carbon. The effluent CO_2 equivalent includes the effluent aqueous inorganic and organic carbon, effluent volatile suspended solids (VSS), gaseous CO_2 and CH_4 (only for anaerobic BTF), and effluent chloroform and ethanol concentrations. Figure 4 presents the cumulative influent and effluent for anaerobic BTF as an example. The CO_2 equivalence of all the carbon components was calculated in moles and a cumulative input and output CO_2 equivalence of carbon was plotted on sequential time (Fig. 4). The difference between the influent and effluent carbon on average was 41% with a standard deviation of 8.8%. A difference of 27%

with standard deviations of 3.1% was obtained for aerobic BTF. The carbon recovery for the anaerobic BTF was 59% and the recovery for the aerobic BTF for the four phases was 63% (Palanisamy *et al.*, 2016). The loss of influent and effluent carbon was produced as biomass within the BTF. This hypothesis is justified by comparing the loss of carbon to the amount of biomass accumulated within the bed. The cellular composition for typical heterogeneous anaerobic microorganisms is represented as $C_{4.9}H_{9.4}NO_{2.9}$ and the aerobic filamentous fungi is also presented by $C_9H_{15}O_5N$ (Rittmann and McCarty, 2001). These compositions were used as the basis for relating the ammonia and nitrate consumed in building up new biomass to estimate the amount of biomass retained within each BTF. A *t*-test was performed to compare the results of the carbon consumed and the biomass produced. The anaerobic test results ranged from 7.32×10^{-8} to 4.52×10^{-6} with p-value <0.05 indicating that the difference between the carbon retained and the biomass produced was statistically significant, therefore, confirming that the loss of carbon within the BTF was utilized for biomass growth.

It is worthwhile to note that the main carbon contributors to the carbon balance for both BTFs are the gas phase concentrations of the influent and effluent chloroform and ethanol concentration, and effluent gaseous CO_2. CH_4 is another effluent gas for the anaerobic BTF. The amount of carbon in the liquid phase obtained from the volatile suspended solids, influent and effluent organic could be considered negligible since their sum did not exceed 5% of the total carbon in the system.

Microbial ecological analyses and correlation

Bacterial and fungi structures of anaerobic and aerobic BTFs were studied by using Ion Torrent PGM system. Samples for the microbial analysis were collected from each BTF after reacclimation to the different phase when 99% of the original performance was attained. To get a high diversity of microbes, inoculums usually come from digested activated sludge or previously cultivated microflora (Wagner *et al.*, 2002). For the anaerobic biofilter, initially, microbes were acclimated for chloroform-based culture by using methanogenic bacteria from food waste. Figure 5 shows the relative abundance and the diversity of the anaerobic microbial community observed for phases I to III of the anaerobic BTF. Due to the erratic performance of the anaerobic BTF after day 143, no microbial samples were taken in the last phase (phase IV). The microbial analysis is based on 97% identity of 16S rRNA gene sequences in class level.

Figure 5 provides the results of analysis for the samples collected from port 2 (Fig. 1) of each phase. During phase I, the most dominant species were *Azospira restrica* and *Azospira oryzae* (46% and 21%) followed by *Geobacter* spp. (16%) and *Aminivibrio pyruvatiphilus* (6%). However, during phase II, the amounts of *A. restrica* and *A. oryzae* reduced to 18% and 37%, respectively. The retrieved amount of *Geobacter* spp. also reduced to 2%. The amount of *A. pyruvatiphilus* also decreased to less than 1%, while *Azonexus fungiphilus* (15%) showed a significant relative abundance than in phase I. The amount of *Clostridium* spp. was also higher in phase II, 7% compared to 2% in phase I. In phase III, *A. restrica*, *A. oryzae*, *A. fungiphilus,* and *Anaerobaculum mobile* were the dominant species with the relative abundance of 47%, 29%, 6%, and 4%, respectively. With the addition of ethanol in

the anaerobic BTF system, the growth of *A. restrica* and *A. oryzae* was greatly enhanced. Furthermore, the addition of more ethanol in phase II has affected the growth of chloroform degrading species like *A. restrica*, *A. oryzae*, and *Geobacter* spp., which were the dominant species during phase I. This effect was clearly noticed when the chloroform feed stream was supplemented with more cometabolites in the BTFs during phase II, where the concentration of *A. fungiphilus* and *A. mobile* increased significantly from 1% each to 6% and 15%, respectively. Moreover, during phase III with higher cometabolite concentration (100 ppmv), it can be noticed that the growth of *A. restrica and A. oryzae* increased more than the other dominant species.

In general, the relative abundance of *A. oryzae* increased with the degradation of chloroform, which correlates to the corresponding removal efficiency and EC. It is therefore speculated that *A. oryzae* could be the primary bacteria for the degradation of chloroform under anaerobic conditions. *A. oryzae* and *A. restrica* were the main species in all the three phases. The prevalence of these species has also been reported previously from various microbial utilization and studies related to anaerobic biodegradation, *A. oryzae* (Hutchison *et al.*, 2013). Similarly, Bae *et al.* (2007) studied the species of *A. restrica* and found out that it is a nitrogen-fixing bacterium.

In the case of aerobic BTF, *Fusarium* sp. and *Fusarium solani* were the major species detected for the four phases. Figure 6 provides the fungi community diversity observed over the four phases of aerobic BTF for samples collected from the top port of the biofilter. Figure 6 suggests the significant phase-dependent changes in the detected fungi communities of the BTF. Phase I fed with chloroform and 5 ppmv of ethanol, the most dominant species were *Fusarium* sp., *Aspergillus* sp., and *Ascotricha* sp. with relative abundance of 64%, 15%, and 11%, respectively. The availability of *F. solani* was 4%. However, in phase II, when the BTF was fed with more ethanol (50 ppmv), the dominant species were *Fusarium* sp. with 95% followed by *F. solani* and *F. Nectria haematococca* with 2% each. In this phase, the amount of *Aspergillus* sp. and *Ascotricha* sp. reduced to less than 0.3%, which supported more growth to *Fusarium* sp. Another very important observation is that the amount of *Fusarium* sp. increased more than 30% from the previous phase (phase I). This could be due to the increase in ethanol concentration, which favors more carbon source for the microbes.

During phase III, again *Fusarium* sp. was dominant by 86% and followed by *F. solani* at 10%. As reported in our previous work (Palanisamy *et al.*, 2016), in this phase, the system was left to run for more than 100 days and could be the main reason for the increase and dominancy of *Fusarium* sp. and *F. solani* species over other fungi species within the aerobic BTF. It is also very important to note that, when ethanol concentration increased to 100 ppmv (ratio of 1:20), the percentage of *F. solani* also increased more than 8% from the previous phase. In addition, a new kind of fungi species called *Cylindrocarpon* sp. (1%) was detected in this phase. During phase IV, the aerobic BTF was mainly dominated by *Fusarium* sp. (59%) and *F. solani* (36%). It is interesting to note that *F. solani* increased significantly in this phase compared to the previous phase. It could be attributed to the increase of ethanol concentration to 200 ppmv. Similarly, *Cylindrocarpon* sp. increased to 4% during this phase. Finally, it can be concluded that the abundance of fungi population might explain the high

removal efficiency of chloroform in the acidic aerobic BTF. Especially, *Fusarium* sp. and *F. solani* were the most dominant and abundant fungi species in this aerobic BTF. Other studies reported that *F. solani* was used to biodegrade n-hexane (Arriaga and Revah, 2005; Hernández-Meléndez *et al.*, 2008). Sagar and Singh (2011) conducted a study on the biodegradation of lindane pesticide by *Fusarium* sp. and demonstrated that *F. solani* biodegraded lindane up to 59.4%.

Conclusion

In this study, we examined the removal of gas phase chloroform under two environmental conditions (anaerobic and aerobic), and in the presence of ethanol as a cometabolite. Investigations of the biological community structure within the BTFs were also conducted. The use of aerobic fungi BTF under an acidic condition successfully enhanced the biodegradation process of chloroform. The BTF provided a more stable performance by having a smaller standard deviation in the removal efficiency compared to the anaerobic BTF. Hence, acidic aerobic BTF had achieved significant improvement in the removal of chloroform. Operation at an acidic pH enhanced greatly the performance, providing removal efficiency around the 80.9% level. Using fungi culture led to higher loading rates that could not be achieved by an anaerobic microbial culture.

The result obtained from microbial analysis showed that the most dominant fungi, which promote higher removal efficiency, were *Fusarium* sp. and *F. solani*. *A. oryzae* and *A. restrica* were the responsible bacteria community species responsible for anaerobic BTF. This study proves the effectiveness of the use of BTF in postaeration processes installed at different points in the water distribution system for the removal of DBPs. The added stability in performance could put more trust in the cost-effectiveness of biological treatment of hydrophobic compounds.

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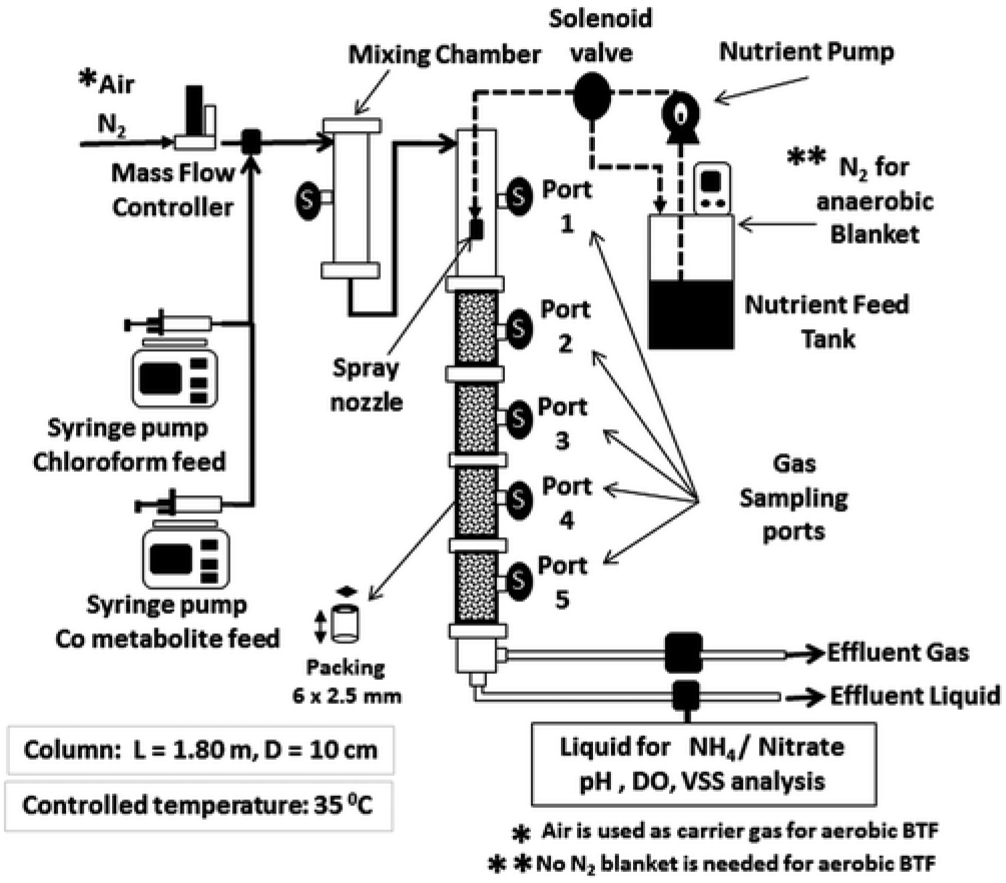

FIG. 1.
Schematic diagram of anaerobic and aerobic BTFs. BTFs, biotrickling filters.

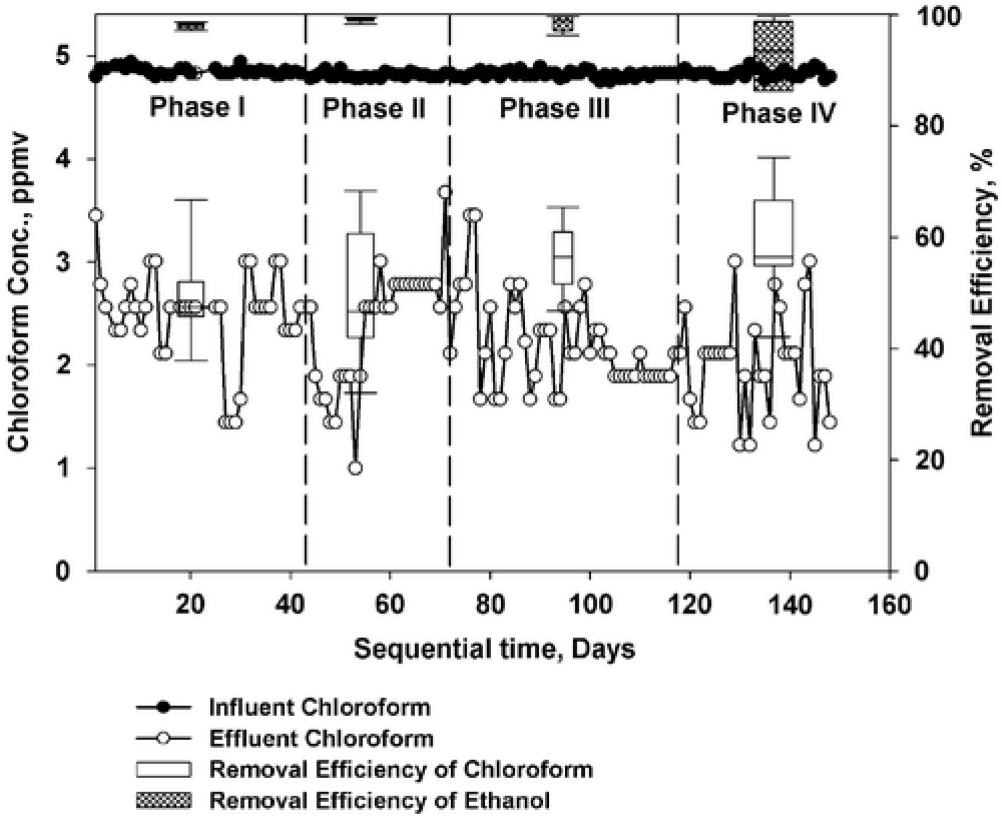

FIG. 2.

Performance of anaerobic BTF in four phases. Phase I: 1:5, phase II: 1:10, phase III: 1:20, and phase IV: 1:40 chloroform to ethanol. The *box* and *whiskers* plot show the median removal efficiencies and quartiles of $CHCl_3$ and ethanol for each operation phase.

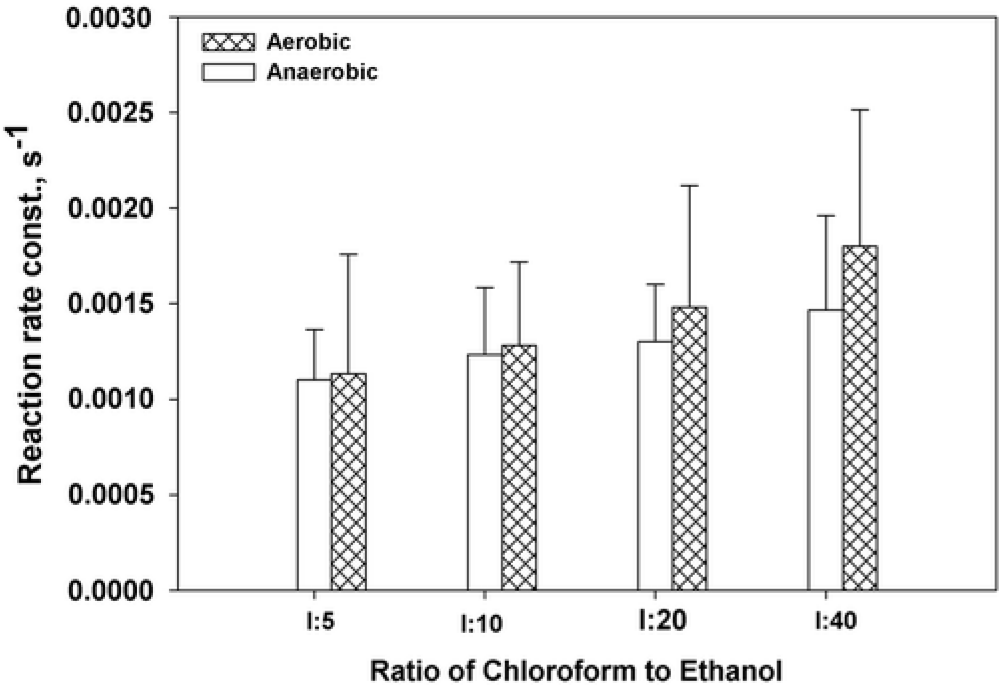

FIG. 3.

Reaction rate constants for chloroform for both anaerobic and aerobic BTFs in the four phases. Phase I: 1:5, phase II: 1:10, phase III: 1:20, and phase IV: 1:40 chloroform to ethanol.

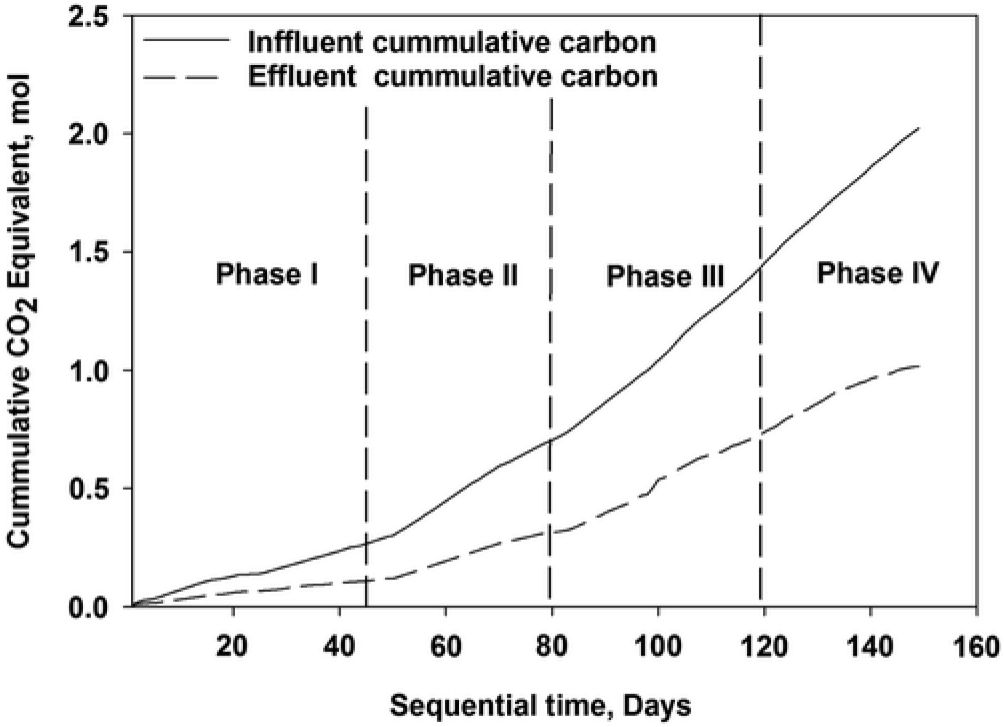

FIG. 4.

Carbon mass balance: cumulative carbon input and output as CO_2 equivalent in mole for anaerobic BTF. CO_2, carbon dioxide.

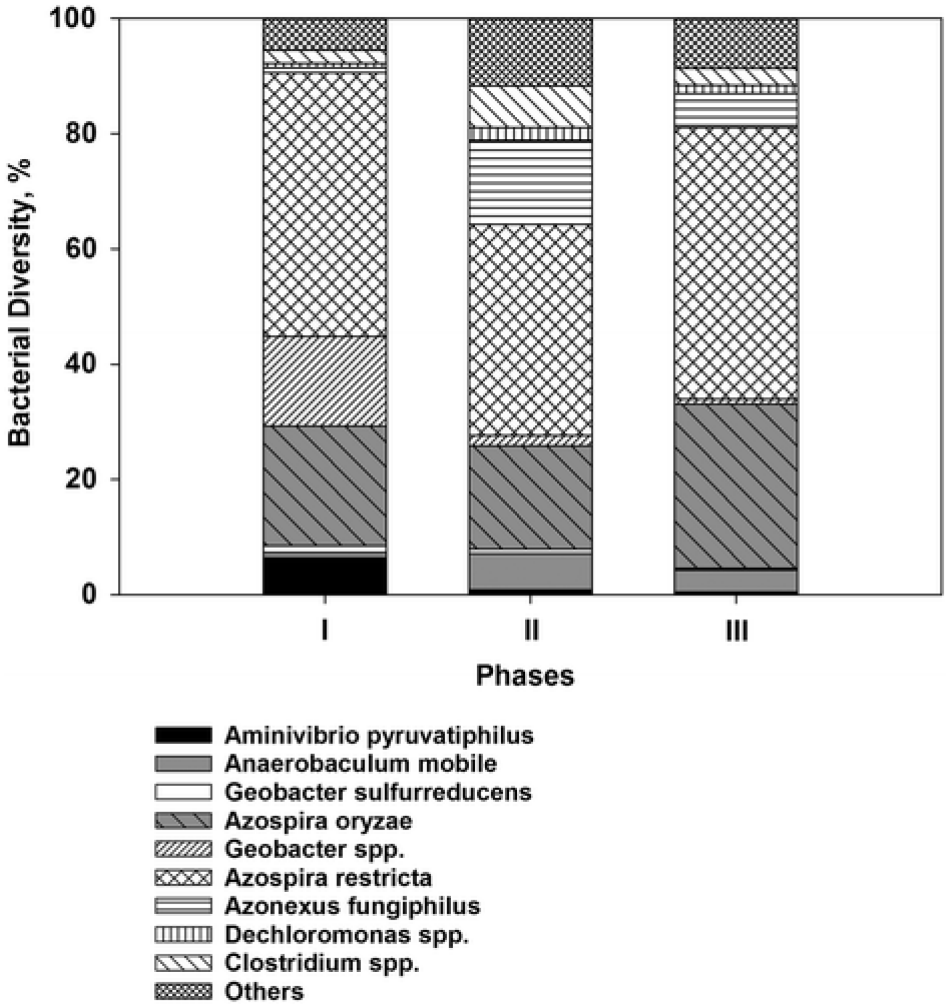

FIG. 5.

Bacterial community diversity for three phases of anaerobic BTF for samples collected at top port of the biofilter. Phase I: 1:5, phase II: 1:10, and phase III: 1:20 chloroform to ethanol.

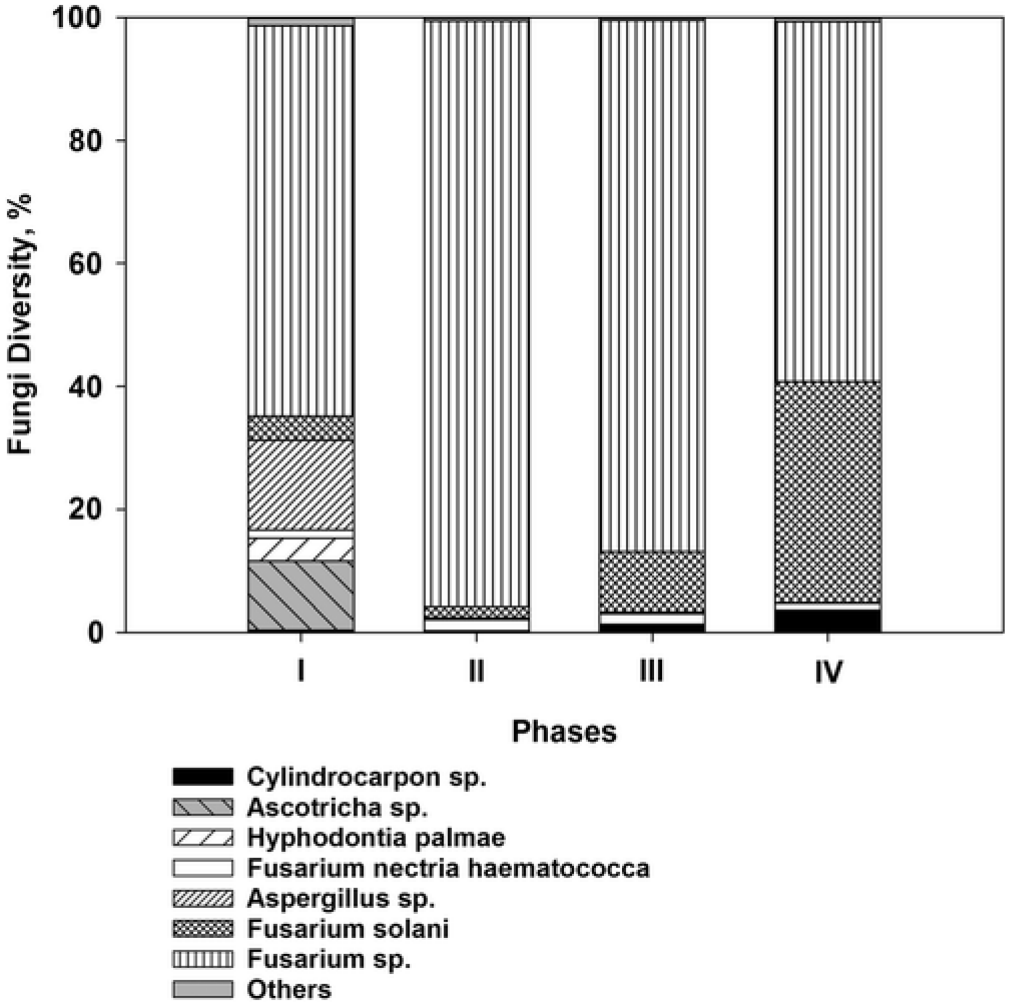

FIG. 6.

Fungi community diversity for the four phases of aerobic BTF for samples collected at the top port of the biofilter. Phase I: 1:5, phase II: 1:10, phase III: 1:20, and phase IV: 1:40 chloroform to ethanol.

Table 1.

Operating Conditions for Anaerobic and Aerobic Biotrickling Filters Degrading Chloroform at a Loading Rate of 0.27 g/m^{3}h

		Phases			
Operating condition	**BTF type**	**I**	**II**	**III**	**IV**
Influent ethanol concentration, ppmv	Anaerobic	25	50	100	200
	Aerobic				
Operation time, days	Anaerobic	44	33	41	35
	Aerobic	30	29	122	33
Average chloroform removal efficiency (%)	Anaerobic	49 ± 9	52 ± 7	56 ± 7	59 ± 10
	Aerobic	69.9 ± 9	71.6 ± 5	75.1 ± 9	80.9 ± 4
Elimination capacity (g/[m^3·h])	Anaerobic	0.13 ± 0.02	0.14 ± 0.01	0.15 ± 0.02	0.16 ± 0.01
	Aerobic	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.23 ± 0.01
Ratio: methane/carbon dioxide	Anaerobic	1.77	1.95	2.01	2.05

BTF, biotrickling filter.