**Comparative study on the performance of Anaerobic and Aerobic Biotrickling Filter for the Removal of Chloroform**

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**Abstract**

The use of biotrickling filter (BTF) for gas phase treatment of volatile trihalomethanes (THMs) stripped from water treatment plants could be an attractive treatment option. In this study, two independent parallel lab-scale BTFs were run to degrade chloroform as a model THMs. It is a hydrophobic volatile THM known to be difficult to bio degrade. To improve the degradation process, ethanol was used as a co-metabolite 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 removal capacity of 0.23±0.01 g/mair3.hr was observed with a removal efficiency of 80.9 ± 4% for the aerobic BTF operating at pH 4 for the volume ratio of 1:40 chloroform to ethanol. For similar ratio, the anaerobic BTF supported lower removal efficiency of 59±10% with corresponding lower elimination capacity of 0.16±0.01 g/m3.hr. The loading rate for chloroform on both BTFs was 0.27 g/m3.hr. The microbial community analysis suggested that *Fusarium sp.* and *F. solani* were the dominant fungi responsible for higher chloroform degradation. *A. oryzae* and *A. restrica* were the dominant and responsible bacteria species observed in the anaerobic BTF.

**Key Words:** 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 are present in most surface water, and produces many harmful disinfection byproducts (DBP). Most DBP are knownto be toxic and pose a risk to human health (Gopal *et al.*, 2007). Many DBPs are also bio accumulative, 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 DBP include the water pH and temperature, the concentration and contact time of chlorine and bromine, and the concentration of natural organic matters (Pourmoghaddas and Stevens, 1995). The methods currently used to reduce NOMs and to minimize the formation of DBS include the use of activated carbon and conventional water treatment processes including clarification, coagulation, flocculation, sedimentation, and filtration (Xie, 2006). However, these controlling methods can only remove about 30% of the precursors for THMs (Gh and Gh, 2011). The challenge is also, eliminating these THMs by physical and chemical methods at low concentrations found in drinking water is expensive, which may generate secondary pollutants. 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 DBPs since it is the most toxic and most abundant of the THMs. Chloroform is a volatile THMs and could be removed from contaminated waters to the gaseous phase by air stripping (LaKind *et al.*, 2010, Lichtfouse, 2005, McGregor *et al.*, 1988). Biological treatment techniques for volatile organic compounds (VOC) 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 (Yu and Smith, 1997, Egli *et al.*, 1988, Egli *et al.*, 1990, Picardal *et al.*, 1993). Thus, biological techniques have resulted in dechlorination of chloroform to dichloromethane, methane and carbon dioxide (Egli *et al.*, 1990, Mikesell and Boyd, 1990b, Becker and Freedman, 1994). On the other hand, under aerobic degradation co-metabolic process is required (Cappelletti *et al.*, 2012, Frascari *et al.*, 2005). Dey et al. reported that the use of aerobic Bacillus sp. 2479 showed the capacity to degrade chloroform (Dey and Roy, 2011). Rahni et al. also reported that pseudomonas putida bacteria mutants could be immobilized and used to degrade chloroform discharge in the field and also in a reactor type system (Rahni *et al.*, 1986).

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 environmentally friendly, cost effective and releases fewer byproducts (Yoon *et al.*, 2002). The use of 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. 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%) (Yoon *et al.*, 2002). Similarly, Balasubramanian et al. evaluated the biodegradation of chloroform along with a mixture of VOCs commonly found in pharmaceutical emissions, using a biotrickling filter. 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). Even though chloroform is a recalcitrant compound to biological transformation, it can be transformed in the presence of a co-metabolite under aerobic or anaerobic environments (Zitomer and Speece, 1995). Anaerobic dechlorination of chloroform has been observed by different researchers by using methanogenic microbes with electron donating co-metabolites in reductive chloroform biotransformation (Bagley and Gossett, 1995, Bouwer *et al.*, 1981, Krone *et al.*, 1989, Mikesell and Boyd, 1990a). Aerobic co-metabolic chloroform transformation has been studied by different researchers (Cappelletti *et al.*, 2012, Dey and Roy, 2011, Frascari *et al.*, 2005). Additionally, chloroform removal ranging between 13 % and 43 % was obtained in a study of aerobic co-metabolism of chloroform and other THMs conducted with a nitrifying biofilm in a biofiltration system by Wahman et al. (Wahman *et al.*, 2006).

Biofilters were proven to be a good option for hydrophilic compounds; however, its effective performance is hindered when the compound to be treated is less soluble. Van Groenestijn et al. discussed that replacing the working consortium in a biofilter from Bacteria to fungi is advantageous (Van Groenestijn *et al.*, 2001). Fungi are more resistant to acidification and drying out. Fungi are tolerant to pH fluctuations, unlike bacteria which requires neutral pH for sustenance (Kennes and Veiga, 2004). The aerial mycelia of fungi form a larger surface area in the gas phase than bacterial biofilms, which may facilitate the uptake of hydrophobic volatile compounds overtaking the rate limiting step by accelerating the mass transfer of hydrophobic compounds from the air to the biofilm (Rene *et al.*, 2010a, Hassan and Sorial, 2010). Fungi were utilized in the operation of BTFs and proven to be a better option for insoluble compounds like n-hexane (Hassan and Sorial, 2010) and alkyl benzenes (Kennes and Veiga, 2004). On the other hand, acidic biofiltration of chloroform in the presence of fungi, to the best of our knowledge, is rarely reported in literature except for our previous work in treating chloroform with ethanol used as a co-metabolite (Palanisamy *et al.*, 2016).

In this study, two independent parallel BTFs were run continuously to degrade chloroform; one designated as Anaerobic BTF and the other one as Aerobic BTF. The loading rate of chloroform was kept at 0.27 g/mair3 h throughout this experiment. Ethanol (hydrophilic VOC) was introduced as gaseous co-metabolite at different loading rates on both BTFs. The study will also investigate the microbial ecology within both BTFs to get a deep insight of the factors affecting their performances.

 **2. Materials and methods**

*2.1 Materials*

Chloroform with 99.8% purity was obtained from Fisher Scientific (Pittsburgh, PA, USA) and Ethanol with 99.5% purity obtained from Sigma Aldrich (St. Louis, MO, USA). Chloroform is highly hydrophobic with a Henry’s law constant, KH of 3.5×10−3 atm.m3/ mol at 25 oC, and the KH value of the hydrophilic ethanol is 5.1×10−6 atm.m3/ mol at 25 °C. The measuring sensors for pH, nitrate, 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).

*2.2 Biotrickling Filter (BTF)*

Two BTFs run under anaerobic and aerobic conditions independently were used for this comparative study. Fig. 1 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 co-current mode with both gas and liquid flow downwards to acclimatize and enhance the growth of biomass. In the 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. Initially methanogenic microorganisms were used to inoculate the filter bed. The buffered nutrient solution containing ammonia as electron donor was supplied at an average rate of 2.0 L/ day, the composition of the nutrient solution was used according to the ones provided by literature (Gupta *et al.*, 1996), (Atikovic *et al.*, 2008), (Zitomer and Speece, 1995). One Molar NaHCO3 was used as a buffer to maintain the pH at 7. The temperature was kept at 350C to maintain favorable methanogens growth. Whereas, in the aerobic system, air was used as a carrier gas with a flowrate of 0.5 L/ min at 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, is described by Sorial et al. (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 via separate syringe pumps in series and vaporized into the nitrogen or air stream.

*2.3. Strategies of biomass control*

The aerobic BTF operation was tested for different biomass control technologies namely starvation, stagnation and backwashing. Starvation and stagnation non-use periods were observed during two consecutive days per week. During the starvation period, the BTF only received the nutrients, devoid of any supply of VOCs and air. Under stagnation, 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 approximately 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 (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.

*2.4 Sampling and Analysis*

Gas and Liquid samples were collected daily from both BTF systems five 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, nitrate, and organic matter. The gas flow pressure-drop across the bed, and operating temperature were taken on daily basis. Dissolved oxygen for the anaerobic BTF was taken every day in order to check for any leak by using Accurate DO probe. Gas phase samples for anaerobic BTF were taken on-line from different points along the BTF column using an electrically controlled low-bleed eight-port Valco valve and analyzed by gas chromatograph. Whereas the gas samples for aerobic system were taken manually using airtight syringes. The samples were analyzed for chloroform, ethanol, and by-products for methane and CO2. They were injected into GC – HP, Column: HP, 608, 30 m X 530 μm film thickness, injection splitless through 5ml sample loop equipped with a flame ionization detector (FID). The GC oven was programmed isothermal at 60 °C (2min) ramped to 90 °C at a rate of 10 oC/ min. The carrier gas (He) flow rate was set at 3.5 mL/ min at constant flow rate. The FID was used with N2 make-up gas at a flow rate of 30 mL/ min, a fuel gas flow (H2) 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 carbon dioxide (CO2), samples were also taken automatically by GC HP- TCD from each sampling port in the BTF. The GC oven was programmed isothermal at 60°C (1min), 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. A detailed description of the analytical method is provided by Cai et al (Cai *et al.*, 2005).

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, dissolved inorganic carbon, and volatile suspended solids. The concentration of ammonia was determined using an ammonia electrode sensor. Dissolved total carbon and dissolved inorganic carbon 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 (Association, 2005).

*2.5 Microbial Community Molecular Analysis*

 Biofilm samples were collected from anaerobic and aerobic BTF within the media as shown in figure 1. The samples were taken when the biofilters were running at the stated different ethanol loading rates. The microbial samples were taken from port 2 (first port from the top within the media) of each BTF and placed in sampling tubes. 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 prior sending them to molecular research laboratory (Molecular Research LP Shallowater, TX). The DNA of microbial mass in the samples was extracted using Mo Bio PowerSoil DNA (M Bio Lab, Inc., Carlsbad, CA) following 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 rRNA gene V4 variable region PCR primers 515/806 were used in a single-step 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 28 cycles (5 cycle used on PCR products) of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. Sequencing was carried out at Molecular Research LP (www.mrdnalab.com, Shallowater, TX, USA) 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 150bp or with ambiguous base calls or with homopolymer runs exceeding 6bp 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).

**3. Experimental Results**

*3.1. Anaerobic Biotrickling Filter Performance*

In this study, the effects of co-metabolite at different loading rates on the performance of anaerobic BTF, was evaluated. The co-metabolite was allowed to mix with chloroform in the mixing chamber to achieve higher removal efficiency by providing additional electron donor to the micro-organisms. Ethanol was used as a co-metabolite since it readily mixes with chloroform and water, and is non-toxic to microbial community. 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. The table also summarizes the results of the BTF including average removal efficiency and its standard deviation and the elimination capacities of each phase of operation. Fig. 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 chloroform influent concentration of 5 ppmv and ethanol concentration of 25 ppmv providing a corresponding chloroform loading rate of 0.27 g/m3.hr. 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/m3.hr (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/m3.hr. After the system 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/m3.hr 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 elimination capacity of 0.16 ± 0.01 g / m3.hr as compared to the other previous phases.

*3.1. Aerobic Biotrickling Filter 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. The table also summarizes the results of the BTF including average removal efficiency with its standard deviation and the elimination capacity. During Phase I, with the ratio of chloroform to ethanol of 1:5 the removal efficiency of chloroform was achieved at 69.9±9% with a corresponding EC of 0.21 ± 0.01 g/ m3.hr. On phase II, for the ratio of 1:10, the removal efficiency of chloroform was 71.6 ± 5% with EC of 0.22 ± 0.01 g/ m3.hr. The ratio at Phase III was 1:20 with ethanol loading rate of 2.30 g/ m3.hr while maintaining the same loading rate for chloroform, the removal efficiency of chloroform increased to 75.1 ± 6.7 providing EC of 0.22±0.01 g/ m3.hr. The loading rate of ethanol was increased to 4.59 g/ m3.hr in phase IV and was run for 35 days. The removal efficiency of chloroform increased to 80.9% with a standard deviation of 4%. For the last two phases namely phase III and IV, the biomass control strategy of stagnation was used in order to control the high pressure drop across the system and to control excessive biomass growth around the nutrients spray nozzle. This strategy also minimized the periodic need for backwashing the filter bed usually performed to avoid short-circuiting within the biofilter. The corresponding EC for this phase was 0.23 ± 0.01 g/ m3.hr.

**4. Discussion of the results**

*4.1. Performance comparison for Anaerobic and Aerobic BTFs*

The use of co-metabolite improved chloroform degradation for both BTFs. It has been observed that for both BTFs the performance increased with an increase in the co-metabolite concentration. Few studies have been conducted for the use of co-metabolite for chloroform degradation. The study conducted by Gupta et al. investigated the use of acetic acid as a co-metabolite in anaerobic chloroform biotransformation in the liquid phase which resulted in higher removal efficiency (Gupta *et al.*, 1996). Similarly, aerobic chloroform biodegradation has been observed during the oxidation of other co metabolites. Chloroform co-oxidation with formate or methane, with butane oxidizing, and nitrifying bacterium has been reported (Field and Sierra-Alvarez, 2004). In our current study, chloroform displayed significant biodegradation rates when using ethanol as a co-substrate in a fungal-based system. In this work, fungi utilization greatly enhanced the performance of the aerobic BTF as compared to the anaerobic one. Several researches have indicated that the switch of microorganisms to fungi has shown greater performance. Van Groenestijn et al. investigated the use of fungi based biofilter for the removal of toluene (Rene *et al.*, 2010a). As a result, they stated that 80 – 125 g/ m3.hr toluene could be eliminated. Similarly, styrene was treated successfully with fungi at a maximum elimination capacity of 67 g/m3. h (Rene *et al.*, 2010a). The same research group reported that elimination capacities could reach up to 336 g/(m3 h) by using a newly isolated fungus Sporothrix variecibatus (Rene *et al.*, 2010b). In a biofilter treating xylene, fungi were isolated and were identified as the active species in the biofiltration process (Prachuabmo and Panich, 2010). The percent removal efficiency in our current study under acidic aerobic conditions increased significantly and reached to 80.9 ± 3.2 %(see Table 1).  The enhanced performance of the aerobic BTF could be also due to the uniformity of biomass along the biofilter bed which is achieved by the application of biomass control strategy. In our current study, the highest elimination capacity 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 elimination capacity of chloroform. This enhanced performance could be due to the resilience of fungi to acid and dry conditions as 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 enhanced performance for degradation of chloroform, the significance of the anaerobic degradation is the renewable energy source. The anaerobic process produces methane rich biogas suitable for energy production helping to replace fossil fuels. The methane productions for the anaerobic system varied between 0.20 to 0.82 mg/ day during the four phases of the study.

*4.2. Chloroform Kinetics for the Different Phases*

 The removal performance as a function of depth within each BTF were measured weekly. For aerobic BTF, it was conducted one day following stagnation at the sampling ports located along the depth. At the same time, similar measurement was taken for the anaerobic BTF. The samples were taken along the BTFs from ports that are located at 7.6 cm, 23 cm, 38 cm, 53 cm 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 chloroform concentrations in these samples along with the influent stream concentration were used to develop the transformation kinetics as a pseudo first order reaction rate based on a plug flow reactor model. The kinetics reaction rate constants were obtained from the slopes of the regression lines, from the following equation:

$ln\left.\left( \frac{C}{Co}\right.\right)=kt^{}$ …………………………… (1)

where *t* is time, *C* and *Co* are the effluent and the influent concentration, respectively, and k reaction rate constant (min-1). Fig. 3 clearly shows the advantage of fungi utilization in the BTF which is indicated by a higher reaction rate constant as compared to the anaerobic BTF at the same influent concentration. Chloroform reaction rate constant increased as the influent co-metabolite 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 for both BTFs and 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 similar ratio of chloroform to ethanol the reaction rate constant for anaerobic BTF was always less than that of aerobic BTF which correlates well with the removal efficiencies reported in Table 1.

*4.3 Carbon Mass Balance*

The cumulative CO2 equivalent of chloroform in the influent was compared to the same equivalent in the effluent for both BTFs. The influent cumulative CO2 consists of influent gaseous concentration and influent aqueous inorganic and organic carbon. The effluent CO2 equivalent includes the effluent aqueous inorganic and organic carbon, effluent VSS, gaseous CO2 and CH4 (only for anaerobic BTF) and effluent chloroform and ethanol concentrations. Fig. 4 presents the cumulative influent and effluent for anaerobic BTF as an example. The CO2 equivalence of all the carbon components was calculated in moles and a cumulative input and output CO2 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 C4.9H9.4 NO2.9 and the aerobic filamentous fungi is also presented by C9H15O5N (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 the 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 x10-8 to 4.52x10-6 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 of for both BTFs are the gas phase concentrations of the inﬂuent and effluent chloroform and ethanol concentration, and effluent gaseous carbon dioxide. Methane is another effluent gas for the anaerobic BTF. The share of carbon in the liquid phase due to the amount calculated from volatile suspended solids, inﬂuent and effluent organic carbon in the aqueous phase could be considered negligible since the total aqueous carbon did not exceed 5 % of the total carbon in the system.

*4.4 Microbial ecological analyses and correlation*

The 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 re-acclimation 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 micro flora (Wagner *et al.*, 2002). For the anaerobic biofilter, initially, microbes were acclimated for chloroform based culture by using methanogenic bacteria from food waste. Fig. 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 on the last phase (phase IV). The microbial analysis is based on 97% identity of 16S rRNA gene sequences in class level. The figure (Fig. 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 *A.* *restrica* and *A. 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*,. *Azonexus 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* were greatly enhanced. Furthermore, the addition of more ethanol on 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 co-metabolite in the BTFs during Phase II, where the concentration of *Azonexus fungiphilus* and *Anaerobaculum mobile* increased significantly from 1% each to 6 and 15% respectively. Moreover, during phase III with higher co-metabolite 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. studied the species of *A. restrica* and found out that it’s a nitrogen-fixing bacteria (Bae *et al.*, 2007).

In the case of aerobic BTF, *Fusarium sp* and *F. solani* were the major species detected for the four phases. Fig. 6 provides the fungi community diversity observed over the four phases of aerobic BTF for samples collected from the top port of the biofilter. The figure suggests that significant changes for the different phases of operation 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 abundancy of 64%, 15% and 11% respectively. The availability *F. Solani* was 4%. However, on 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*. Other 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 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 important to note that, when ethanol concentration increased to 100 ppmv, 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 on 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 *Cylindrocarpon sp.* increased to 4% with the increase of ethanol concentration to 200 ppmv. 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 *Fusarium solani* used to biodegrade n-hexane (Hernández‐Meléndez *et al.*, 2008, Arriaga and Revah, 2005). Sagar and Singh conducted a study on the biodegradation of lindane pesticide by *Fusarium sp*.. and demonstrated that *F. solani*  biodegraded lindane up to 59.4% (Sagar and Singh, 2011) .

**5. 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 co-metabolite. Investigations of the biological community structure within the BTFs were also conducted. The use of aerobic fungi BTF under acidic condition successfully enhanced the biodegradation process of chloroform. The BTF provided more stable performance by having smaller standard deviation in the removal efficiency as compared to the anaerobic BTF. Hence, acidic aerobic BTF had achieved significant improvement in the removal of chloroform. Operation at acidic pH enhanced greatly the performance providing a removal efficiency around the 80.9 % level. Using fungi culture led to higher loading rates that could not be achieved by 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. The current study proves the effectiveness of the use of BTF in post aeration 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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Figure 1 Schematic diagram of Anaerobic and Aerobic Biotrickling Filters (BTFs)

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Figure 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.

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

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