EPA Public Access

Author manuscript

Water Air Soil Pollut. Author manuscript; available in PMC 2018 November 01.

About author manuscripts

Submit a manuscript

Published in final edited form as:

Water Air Soil Pollut. 2017 November 1; 228: 1–437. doi:10.1007/s11270-017-3616-x.

Performance of Anaerobic Biotrickling Filter and its Microbial Diversity for the Removal of Stripped Disinfection Byproducts

Bineyam Mezgebe,

PhD Candidate, Department of Biomedical, Chemical, and Environmental Engineering, College of Engineering and Applied Science, University of Cincinnati, 701 Engineering Research Center, 2901 Woodside Drive, P.O. Box 210012, Cincinnati, OH 45221-0012, USA

George A. Sorial*,

Professor of Environmental Engineering & Department Head, Department of Biomedical, Chemical, and Environmental Engineering, College of Engineering and Applied Science, University of Cincinnati, 701 Engineering Research Center, 2901 Woodside Drive, P.O. Box 210012, Cincinnati, OH 45221-0012, USA

E. Sahle-Demessie,

Senior Scientist, US Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Cincinnati, OH 45268, USA

Ashraf Aly Hassan, and

Research Assistant Professor, Department of Civil Engineering, College of Engineering, University of Nebraska–Lincoln, P.O. Box 886105, Lincoln, NE 68588-6105

Jingrang Lu

US Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH, USA

Abstract

The objective of this research was to evaluate the biodegradation of chloroform by using biotrickling filter (BTF) and determining the dominant bacteria responsible for the degradation. The research was conducted in three phases under anaerobic condition, namely, in the presence of co-metabolite (Phase I), in the presence of co-metabolite and surfactant (Phase II) and in the presence of surfactant but no co-metabolite (Phase III). The results showed that the presence of ethanol as a co-metabolite provided 49% removal efficiency. The equivalent elimination capacity (EC) was 0.13 g/(m³.hr). The addition of Tomadol 25 - 7 as a surfactant in the nutrient solution increased the removal efficiency of chloroform to 64% with corresponding EC of 0.17 g/(m³.hr). This research also investigated the overall microbial ecology of the BTF utilizing culture-independent gene sequencing alignment of the 16S rRNA allowing identification of isolated species. Taxonomical composition revealed the abundance of deltaproteobacteria and

* Author to whom all correspondence should be addressed, george.sorial@uc.edu, Tel: +1 (513) 556-2987.

Disclaimer

The views expressed in this article are those of the authors and do not reflect the official policy or position of the United States Environmental Protection Agency. Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation. This manuscript has been subjected to the Agency's review and has been approved for publication.

deltaproteobacteria with species level of 97%. *A. oryzae* (formally dechlorosoma suillum), *A. restricta* and *Geobacter* spp. together with other similar groups were the most valuable bacteria for the degradation of chloroform.

Keywords

Anaerobic dehalogenation; Biotrickling Filter; Disinfection byproducts; Microbial diversity; Trihalomethanes

1. Introduction

Disinfection byproducts (DBPs) are formed as the results of the reaction of free chlorine and a series of complex organic precursors (Li and Blatchley 2007). Many DBPs are carcinogens or have been known to cause other health risks (Durmishi et al. 2015). The precursors forming DBPs range from natural occurring humic and fulvic material to anthropological contaminants that persist in treated water (Richardson et al. 2010). The highest concentrations of DBPs detected in drinking water constitute trihalomethanes (THMs) where chloroform is the major component (Durmishi et al. 2015). The International Agency for Research on Cancer (IARC) has determined that chloroform is possibly carcinogenic to humans (ATSDA 2015). Under the Safe Drinking Water Act (SDWA), the United States Environmental Protection Agency (USEPA) establishes a Maximum Contaminant Level (MCL) of 70 ppb for chloroform (USEPA 2016). Several physical and chemical removal methods such as adsorption, advanced oxidation (Shemer and Narkis 2005), and air stripping (Clark et al. 1994) are used to treat chloroform. Chloroform is a volatile organic compound (VOC) that readily evaporates to the atmosphere from treated water surfaces through air stripping (Houel et al. 1979). Hence, the need for innovative control technology is becoming more enviable. In this research, biological treatment was used to treat the off-gas chloroform by bio-trickling filters (BTF).

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 (McCulloch 2003). Yoon and Park (2002) studied the effect of gas residence time on the aerobic biodegradation of nine VOCs including chloroform. In their study they observed that chloroform showed lower removal efficiency compared to other compounds for longer empty bed residence time of 3 minutes (Yoon and Park 2002). However, 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). Highly chlorinated atoms are good electron targets for anaerobes since chlorine atoms block the activity of oxygenase. Thus, biological techniques have resulted in either partial dechlorination of chloroform to dichloromethane (Egli et al. 1990; Novak et al. 1998) or complete mineralization to methane and carbon dioxide (Egli et al. 1990; Mikesell and Boyd 1990; Becker and Freedman 1994). Although most studies show successful biodegradation of chloroform in the liquid phase,

there has been limited 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. (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. (2012) evaluated the biodegradation of chloroform along with a mixture of VOCs commonly found in pharmaceutical emissions, using a biotrickling filter (Balasubramanian et al. 2012). Their study showed that increasing the rate of chloroform loading significantly reduced the degradation efficiency of the reactor for the mixture of VOCs. The increase in chloroform loading rate from 14.22 g/(m³.hr) to 55.83 g/(m³.hr) and further to 107.85 g/(m³.hr) affected the efficiency of both chloroform as well as the other VOCs (Balasubramanian et al. 2012). However, there has been a limited research work reported on the use of anaerobic BTF for the removal of chloroform from the gas phase at low concentration. Multiple investigations have been performed on co-metabolism, specifically for the treatment of trichloroethylene (TCE) contaminated air using BTF. Chlorinated compounds can be degraded by using a more easily biodegradable non-chlorinated hydrocarbon serving as a co-metabolite (Balasubramanian et al. 2012). Biofilters using methane, butane, propane, propylene, phenol, toluene, or ammonia as a co-metabolite have shown success in the treatment of trichloroethylene (TCE) (Mahon 2013). Mahon et al. (2013) observed a 25% destruction of TCE by feeding both the propane and the TCE into the biofilter (Mahon 2013).

Chloroform is a halogenated compound and recalcitrant to biodegrade. As halogenated organics, its halogen bond can significantly affect the biofiltration process (Leson and Winer 1991). Hence, the biodegradation of higher order chlorinated volatile compounds like chloroform occur mostly under co-metabolic conditions in the presence of primary substrates such as methane, propane, phenol and toluene (Kim et al. 1997; Cappelletti et al. 2012).

A nonionic surfactant can be introduced in the biofiltration system as means for enhancing solubility. Hence, surfactant could increase the apparent solubility of chloroform by micellar formation, which commences at the critical micelle concentration and the apparent solubility is proportional to surfactant concentration (Edwards et al. 1991). The use of surfactants in enhancing the bioavailability of hydrophobic compounds by facilitating their biotransformation under aerobic conditions has been studied by several researchers (Yeh et al. 1999; Laha and Luthy 1992; Aronstein and Alexander 1992). The utilization of surfactant increases the solubility which overcomes the rate limiting step (Hassan and Sorial 2008). Researchers have found that addition of surfactants stimulated polycyclic aromatic hydrocarbon (PAH) biodegradation (Tiehm 1994; F. Volkerling et al. 1995; Boonchan et al. 1998). However, there are limited studies where surfactant was used for the dehalogenation in biofiltration system. The use of surfactants in BTF enhanced n-hexane (Hassan and Sorial 2009), toluene (Woertz et al. 2001), and styrene (Volkerling et al. 1997) degradation in fungi

biofilter. Yuan et al. (2010) also reported in their study that nonionic surfactant such as TX-100 greatly increased the dechlorination of chlorobenzenes (Yuan et al. 2010).

In the present work, an effort was made to evaluate the performance of anaerobic BTF for biodegradation of chloroform. The experimental plan was designed to operate the BTF in the presence and absence of co-metabolite and surfactant at different phases. The research also included the application of molecular tools for characterization of the microbial community diversity throughout the BTF. The BTF was used for the continuous removal of gaseous chloroform at three different phases and to gain an insight into the effects of halorespiration for dehalogenation of chloroform. The change in the biodiversity of the bacterial community during the three phases helps to identify the most abundant bacterial populations responsible for the dehalogenation of chloroform during the operation of the BTF.

2. Materials and Methods

2.1 Materials

Chloroform was used as a model THM compound; ethanol laden nitrogen gas stream was supplemented as co-metabolite and Tomadol 25 – 7 was used as a surfactant for the biodegradation. Chloroform with 99.8% purity obtained from Fisher Scientific (Pittsburgh, PA, USA), Ethanol with 99.5% purity obtained from Sigma Aldrich (St. Louis, MO, USA) and Tomadol 25 – 7 was obtained from Tomah products (Tomah products, LA). Chloroform is highly hydrophobic with a Henry's law constant, K_H of 3.5×10^{-3} atm.m³/mol, and the K_H value of a hydrophilic ethanol is known to be 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 extraction of bacterial strains was 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 Experimental Methods

2.2.1 Biotrickling Filter Bed experiment—The experimental set-up for BTF consisted of a cylindrical glass column with multiple sections providing a total length of 130 cm and an internal diameter of 7.6 cm. A schematic set up of the experimental system is shown in Fig. 1. The mid-portion of the column was packed with pelletized diatomaceous earth biological support media, to a depth of about 60 cm long (Celite® 6 mm R-635 Bio-Catalyst Carrier; Celite Corp., Lompoc, CA). The BTF was operated in a co-current mode where chloroform and co-metabolite laden gases, and liquid nutrient solution were introduced into the column from the top. The gas flow was controlled with two mass flow controllers (Sierra Instruments). The growth media of essential nutrients and vitamins was prepared with medium concentrations of 996 mg/L NH₄Cl, 414 mg/L KH₂PO₄, 390 mg/L MgCl₂.6H₂O, 280 mg/L CaCl₂.2H₂O, 2 mg/L FeCl₂.4H₂O, 4.79 mg/L CuSO₄.5H₂O, 6.53 mg/L MnSO₄.H₂O, 5.24 mg/L ZnCl₂, 4.58 mg/L CoCl₂.6H₂O, 0.32 mg/L B(OH)₃, 4.79 mg/L NiCl₂.6H₂O, 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 similar to the one used by Atikovic et al. (Atikovic et al. 2008) and Wu et al. (Wu et al. 2015).

The BTF was seeded with methanogenic microorganisms and maintained at 35°C. Initially, these bacteria were obtained from nutrient enriched solution kept under a blanket of nitrogen gas that was acclimated in our lab to chloroform in a 4 liter amber batch reactor for two months. The chloroform feed was step-wise increased from 5 to 50 ppmv within the two-month period. This inoculum was mixed in the ratio of 1:1 with other methanogenic bacteria acquired from another bioreactor that was treating food waste prior to seeding the BTF. A mixture of chloroform and ethanol (co-metabolite) was continuously fed into the BTF in a flowing nitrogen carrier gas at a flow rate of 0.5 L/min with a corresponding empty bed residence time of five minutes. The nutrient solution buffered at pH 7 was fed intermittently to the BTF bed by a solenoid valve at a rate of 2.0 L/day. The presence of co-metabolite enabled treatment strategies that stimulated biodegradation of chloroform, which alone was not enough to provide the carbon or energy benefit to the microorganism. To further improve, the removal efficiency of BTF's for chloroform degradation, a non-ionic surfactant (Tomadol 25 – 7) was used.

2.2.2 Analytical methods—Gas and liquid samples were collected daily from the BTF system five days per week for the measurements of the ammonia -N the composition of feed and effluent gas/liquid streams. The total number of samples collected for phase I, II and III were 44, 97 and 95, respectively. Measurements of the effluent liquid pH, and organic matter, the gas flow pressure drop across the bed, and operating temperature were taken. Gas phase samples 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. The samples were analyzed for chloroform, ethanol, and by-products such as methane and carbon dioxide. They were injected into GC – HP, Column: HP, 608, 30 m X 530 µm film thickness, injection splitless through 1ml 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 °C/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. Retention time for chloroform was 3.8 min under the above conditions used. 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 N₂ make-up gas at a flow rate of 5 mL/min, a fuel gas flow (H₂) of 30 mL/min.

Liquid samples were collected from the effluent stream of BTF once a week and analyzed for volatile suspended solids (VSS) and total organic carbon (TOC). The samples were filtered through a 0.45 µm membrane filter (Whatman Co.) and analyzed for influent and effluent concentrations of ammonia, 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 by Standard Method 2540G (APHA 2005).

2.2.3 Microbial Community Molecular Analysis—Biofilm samples were collected from the 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. This method was previously done by other researchers (Zehraoui et al. 2014; Zhai et al. 2017). The samples consisted of about five media pellets covered with biomass and placed in O₂ free sampling tubes. All the samples collected were stored in a –20 °C freezer until being sent to molecular research laboratory (Molecular Research LP Shallowater, TX) for biological analysis. 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 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 RDP II (<http://rdp.cme.msu.edu>) and NCBI (www.ncbi.nlm.nih.gov) (DeSantis et al. 2006).

3. Results and discussion

3.1 Biotrickling Filter Performances

In this research, the effects of co-metabolite and surfactant on the performance of BTF were evaluated. Even though chloroform is a recalcitrant compound to biological transformation under conventional aerobic conditions, it can be transformed in the presence of a co-metabolite under aerobic and anaerobic environments (Zitomer and Speece 1995). The co-metabolite was mixed with chloroform stream to achieve higher removal efficiency by providing additional electron donor to the micro-organisms. Anaerobic dehalogenation of chloroform has been observed by different researchers by using methanogenic microbes with electron donating co-metabolites in reductive chloroform biotransformation (Mikesell and Boyd 1990; Bagley and Gossett 1995; Bouwer et al. 1981; Krone et al. 1989). Ethanol was used as a co-metabolite since it readily mixes with chloroform and water, and is non-toxic to microbial community. 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 $0.27 \text{ g}/(\text{m}^3 \cdot \text{hr})$. The operating conditions and different phases of operation are summarized in Table 1. It is worth noting that the removal efficiency of ethanol was always above 98% for the given loading rate conditions studied. Therefore, emphasis is placed on the performance of the BTF on chloroform removal. Daily dehalogenation rate of the BTF was used to track the performance and robustness of the BTF. Fig. 2 presents 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. The BTF was run for 44 days under the conditions of Phase I, and the average removal efficiency for this Phase was $49 \pm 8\%$, which provided an average EC of $0.13 \pm 0.02 \text{ g}/(\text{m}^3 \cdot \text{hr})$ (Table 1). Chloroform is used as an electron acceptor by the reducing bacteria and dehalogenation was enhanced by adding electron donor supply. In previously reported studies, co-metabolite assisted degradation of chloroform were all conducted in batch liquid phase reactions. Thus, there are limited studies on the use of co-metabolite for a continuous gas phase studies. Significant chloroform anaerobical degradation was obtained by Bouwer et al (1981) in seeded cultures with initial concentrations at less than 100 ppb and observed over 90% removal efficiency in the presence of methanol. It was also observed that lower fractional degradation of chloroform was obtained as the initial concentration increased. Gupta et al. (1996) investigated the use of acetic acid as a co-metabolite for chloroform biotransformation and achieved more than 99% of removal efficiency in a methanogenic environment in a chemostat (Gupta et al. 1996).

To further improve the dehalogenation of chloroform, a nonionic surfactant was mixed with the nutrient feed solution. Hence, in Phase II, a surfactant was added along with the co-metabolite. A biodegradable nonionic surfactant, Tomadol 25 - 7 was mixed with the nutrient solution to obtain a concentration of 75 mg/L that corresponds to 0.5 of the critical micelle concentration. It is worthwhile to note that the behavior of this BTF was very similar to the reported performance of n-hexane trickle bed air biofilter using Tomadol 25 - 7 (Hassan and Sorial 2008). The microorganisms utilize surfactants as substrates for energy and nutrients during biodegradation process (Ying 2006). Phase II test was run for 97 days to give the microbes sufficient time to acclimate and attain a stable condition. The increased run time allowed the BTF more cell production and increase of biomass throughout the bed. As shown in Fig. 2, this condition resulted in an increase of removal efficiency to $64 \pm 8\%$ with EC of $0.17 \pm 0.02 \text{ g}/(\text{m}^3 \cdot \text{hr})$ (Table 1). In a previous study, the use of Tomadol 25 - 7 improved the performance of BTF by doubling the elimination capacity for n-hexane, allowing a robust operation, and decreasing the fluctuation of the effluent stream n-hexane concentrations (Hassan and Sorial 2008). The amount of methane produced in our research, during Phases I and II, were 0.15 mg/day and 0.25 mg/day, respectively, which illustrated the more enhanced performance of the BTF in the presence of surfactant.

During phase III, the BTF was augmented with surfactant only without a co-metabolite. This phase has the same operating conditions as Phase II and was run for 95 days. The removal efficiency was higher than that of Phase I and the overall dehalogenation of chloroform was high in the absence of ethanol. The removal efficiency was $60 \pm 7\%$ with EC of $0.16 \pm 0.02 \text{ g}/(\text{m}^3 \cdot \text{hr})$ (see Table 1). The methane production for phase III was 0.17 mg/day. The drop in

the performance compared to the previous Phase (II) could be due to the limitation of biomass growth as was depicted by measurements of VSS.

3.2. Dehalogenation Pathways and Kinetics for the Different Phases

The reductive dehalogenation of chloroform along the BTF was measured weekly by collecting gas samples 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 (i.e., ports 2–5 Fig. 1). During the reductive dehalogenation process (see Supplementary data, Appendix A), the hydrodehalogenation and hydrogenation of chloroform can be hypothesized as in Fig S1 (Supplementary data, Appendix B). 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:

$$\ln \left(\frac{C}{C_o} \right) = kt \quad (1)$$

Where t is time, C and C_o are the effluent and the influent concentration, respectively, and k reaction rate constant (seconds^{-1}). Fig. 3 shows the reaction rate constant for the BTF for the three phases of reactions. The error bars represent the standard deviations of three replicas. The results in the figure show higher reaction rate of 0.002 s^{-1} was obtained for Phase II as compared to Phase I, whereas the reaction rate did not change significantly between Phase II and Phase III. The addition of surfactant prompted higher reduction rate constant. This could be due to the co-metabolism and dissolution effects of the surfactants that resulted in excess biomass retention within the BTF bed. As discussed in the performance section, these values correlated with efficiencies of the BTF for the different phases. The higher reaction rate was obtained due to the enhancement and improvement gained from the co-metabolite and surfactant added to the system, which increased microbial activity within the biofilter bed. The results correspond well with the increase in chloroform removal efficiency during Phase II and III.

3.3 Carbon Mass Balance

The cumulative CO_2 equivalent of for this research is presented in Fig. 4. To study the carbon cycle within the bed for both the liquid and gaseous phases, all the carbon sources and products were measured. The influent consisted of gaseous concentrations of chloroform and ethanol, plus aqueous inorganic and organic carbon. The effluent included aqueous inorganic and organic carbon, a carbon equivalence of volatile suspended solids, gaseous carbon dioxide, gaseous methane and a carbon equivalence of chloroform and ethanol concentrations. 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 carbon recovery obtained in this research was 63% with a

standard deviation of 10% and similar carbon recovery was reported for n-hexane as single VOC with surfactant (Hassan and Sorial 2010). To avoid toxicity to the microbes, chloroform was supplied to the BTF at a relatively lower flow rate, which resulted in the lower recovery. The loss of influent and effluent carbon was produced as biomass within the BTF (Hassan and Sorial 2010). 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 $C_{4.9}H_{9.4}NO_{2.9}$ (Bruce and Perry 2001). These compositions were used as the basis for relating the ammonia consumed in building up new biomass to estimate the amount of biomass retained within the BTF. A two-tailed t-test was performed to compare the results of the carbon consumed and the biomass produced. The test generated a 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 BTF are the gas phase concentrations of the influent and effluent chloroform and ethanol concentration, and effluent gaseous carbon dioxide and methane. 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.

3.4 Microbial ecological analyses and correlation

As a microbial degradation process, biofiltration is based on the ability of microorganisms to degrade organic and inorganic compounds. Webster et al. (1997) studied the microbial ecosystems in compost and granular activated carbon biofilters and found out both biofilters dominated by gram-negative bacteria (Webster et al. 1997). Although, gram negative bacteria has been reported as the dominant bacterial community of some biofilters, few studies have been done on the microbial diversity of BTF (Bruce and Perry 2001; Webster et al. 1997). Similarly, Amann et al. (1995) suggested that up to 99 % of the microorganisms that are active in biofilters are not culturable by traditional cultivation methods (Amann et al. 1995). Hence, in order to understand and analyze the microbial community structure and the diversity of the BTF during the dechlorination processes, molecular methods have been proven to be powerful tools for analyzing the diversity and structure of the microbiome (Fakruddin and Mannan 2013). In this research, Ion Torrent system was used to examine the impact of the presence and absence of cometabolite and surfactant on the bacterial community structure in the BTF. To get a high diversity of microbes, inocula usually come from digested activated sludge or previously cultivated microflora (Wagner et al. 2002). Initially, in this research microbes were acclimated from chloroform based culture and methanogenic bacteria from food waste. Overall, the BTF bacterial communities consisted of mainly betaproteobacteria (45%) following deltaproteobacteria (19%), synergistia (8%), alphaproteobacteria (7%) and gammabacteria, Fig. 2S (Supplementary data, Appendix C) is based on 97% identity of 16S rRNA gene sequences in class level. Table 2 shows dominant groups in species level (97% identity), their taxonomical class, potential metabolic role and related receptors. The community dominant compositions were *A. oryzae* and *A. restricta*, *Geobacter* spp. & *G. sulfurreducens*, *Smithella* spp, *Azonexus* spp, *Aminobacterium* spp.

and *Anaerobaculum mobile*. Especially, *A. oryzae* and *A. restrica*, *Geobacter* spp. and, *Smithella* spp. 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) was reported for perchlorate remediation and *Smithella* spp. utilize to degrade propionate first to acetate and then to butyrate (Dolfing 2013). Both have been studied in the biological removal of perchlorate and propionate anaerobically (Hutchison et al. 2013; Dolfing 2013). Similarly, Anderson et al. (2003) reported that *Geobacter* species can be important agents for in situ bioremediation of uranium (Anderson et al. 2003). Bae et al. (2007) studied about the species of *A. restrica* and found out that it's a nitrogen-fixing bacterium. Within these dominant species, the bacteria with the highest relative abundances across all the samples were *A. oryzae*, *Geobacter* spp., *G. sulfurreducens* and *Smithella*.

Fig. 5 shows that the relative abundance and the diversity of the anaerobic microbial community observed in the BTF for samples collected from port 2 (see Fig. 1). 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, no significant sequences of *A. restrica* were retrieved, the relative abundance of *Geobacter* spp. reduced to 13%. On the other hand, the amount of *A. pyruvatiphilus* decreased to less than 1%, while *A. oryzae* showed a significant increase to 76% and *Azonexus* spp. and *Smithella* spp. were significantly detected in 0.6% and 3%, respectively. During Phase III, *A. oryzae*, *Smithella* spp., *Clostridium saccharoperbutylacetonicum* and *Geobacter* spp. were the dominant species with the relative abundance of 50%, 14%, 5% and 5%, respectively. With the addition of surfactant in the BTF system, the growth of *A. oryzae* was greatly enhanced. Furthermore, the addition of the surfactant has inhibited the growth of chloroform degrading species like *A. restrica* which were the dominant species during Phase I. This effect was clearly noticed when the feed stream chloroform was supplemented with a co-metabolite with a surfactant in the BTFs during Phase II, where the concentration of *A. oryzae* increased significantly from 21% to 76%. Furthermore, the presence of co metabolite during Phase II increased the growth of *A. restrica* 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. Hence, in Phase II, where ethanol was combined with Tomadol, the average removal efficiency was 64% and at this higher efficiency, the abundance of *A. oryzae* also increased to 76%. The percentage of *A. oryzae* for Phase I and III are 21% and 50%, respectively. It is, therefore, speculated that *A. oryzae* could be the primary bacteria for the dehalogenation of chloroform under conditions of this research.

Conclusion

This research evaluated the effectiveness of BTF for the degradation of chloroform under anaerobic condition. The BTFs performance for the chloroform was evaluated for three phases, namely, in the presence of co-metabolite (Phase I), in the presence of co-metabolite and surfactant (Phase II) and the presence of surfactant only (Phase III). Enhanced removal efficiency was obtained when surfactant was added to the system together with ethanol (Phase II), which provided a removal efficiency of 64% with EC of 0.17 g/(m³.hr) for a

loading rate of 0.27g/(m³.hr). Consequently, higher reaction rate constants were attained in this phase as compared to the other two phases. The results of the microbial analysis showed a diverse group of bacterial community in the biofilter. From the biological community structure investigation, the presence of co-metabolite supports the growth of *A. restricta* species than others. A mixture of co-metabolite and surfactant benefits the growth of *A. oryzae* and *Geobacter spp.* However, the absence of co metabolite greatly encourages *Smithella spp* more than *A. oryzae* and *Geobacter spp.* The increase in the percentage of *A. oryzae* also correlates with the removal efficiency of chloroform. The overall results obtained for the BTF showed clearly that using both co-metabolite and surfactant had effectively enhanced the biodegradation of chloroform due to providing more favorable conditions for the growth of bacteria colonies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The work conducted was partly supported by the contract number EP11C000147 obtained from the EPA Path Forward Innovation Project from the EPA-University of Cincinnati Grants Program.

References

- Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol reviews. 1995; 59(1):143–169.
- Anderson RT, Vrionis HA, Ortiz-Bernad I, Resch CT, Long PE, Dayvault R, et al. Stimulating the in situ activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer. App Environ Microbiol. 2003; 69(10):5884–5891.
- APHA. Standard methods for the examination of water and wastewater. American Public Health Assoc. and Water Environment Federation; Washington, DC, USA: 2005.
- Aronstein BN, Alexander M. Surfactants at low concentrations stimulate biodegradation of sorbed hydrocarbons in samples of aquifer sands and soil slurries. Environ Toxicol Chem. 1992; 11(9): 1227–1233.
- Atikovic E, Suidan MT, Maloney SW. Anaerobic treatment of army ammunition production wastewater containing perchlorate and RDX. Chemosphere. 2008; 72(11):1643–1648. [PubMed: 18586300]
- ATSDA, A. f. T. S. a. D. R. [Accessed 09/10/2016 Accessed September, 05 2016] Toxic Substances Portal - Chloroform. 2015. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=53&tid=16>
- Bagley DM, Gossett JM. Chloroform degradation in methanogenic methanol enrichment cultures and by *Methanosarcina barkeri* 227. Appl Environ Microbiol. 1995; 61(9):3195–3201. [PubMed: 7574627]
- Balasubramanian P, Philip L, Bhallamudi SM. Biotrickling filtration of complex pharmaceutical VOC emissions along with chloroform. Biores Technol. 2012; 114:149–159. DOI: 10.1016/j.biortech. 2012.03.035
- Becker JG, Freedman DL. Use of cyanocobalamin to enhance anaerobic biodegradation of chloroform. Environ Sci Technol. 1994; 28(11):1942–1949. [PubMed: 22175937]
- Boonchan S, Britz ML, Stanley GA. Surfactant-enhanced biodegradation of high molecular weight polycyclic aromatic hydrocarbons by *Stenotrophomonas maltophilia*. Biotechnol Bioeng. 1998; 59(4):482–494. [PubMed: 10099362]
- Bouwer EJ, Rittmann BE, McCarty PL. Anaerobic degradation of halogenated 1-and 2-carbon organic compounds. Environ Sci Technol. 1981; 15(5):596–599. [PubMed: 22283955]

- Bruce, ER., Perry, LM. Environmental biotechnology: principles and applications. Vol. 400. New York: McGrawHill; 2001.
- Capone KA, Dowd SE, Stamatas GN, Nikolovski J. Diversity of the human skin microbiome early in life. *J Investigative Dermatol.* 2011; 131(10):2026–2032.
- Cappelletti M, Frascari D, Zannoni D, Fedi S. Microbial degradation of chloroform. *Appl Microbiol Biotechnol.* 2012; 96(6):1395–1409. [PubMed: 23093177]
- Clark RM, Adams JQ, Lykins BW Jr. DBP control in drinking water: cost and performance. *J Environ Eng.* 1994; 120(4):759–782.
- DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl and Environ Microbiol.* 2006; 72(7):5069–5072. [PubMed: 16820507]
- Dolfing J. Syntrophic propionate oxidation via butyrate: a novel window of opportunity under methanogenic conditions. *Appl and Environ Microbiol.* 2013; 79(14):4515–4516. [PubMed: 23787899]
- Dowd SE, Sun Y, Wolcott RD, Domingo A, Carroll JA. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned Salmonella-infected pigs. *Foodborne Pathog and Dis.* 2008; 5(4):459–472.
- Durmishi BH, Reka AA, Gjulađin–Hellonlb T, Ismaili M, Srbinovski M, Shabani A. Disinfection of Drinking Water and Trihalomethanes: A Review. *International J Advan Res Chem Sci (IJARCS).* 2015; 2(11):45–56.
- Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinform.* 2010; 26(19): 2460–2461.
- Edwards DA, Luthy RG, Liu Z. Solubilization of polycyclic aromatic hydrocarbons in micellar nonionic surfactant solutions. *Environ Sci Technol.* 1991; 25(1):127–133.
- Egli C, Scholtz R, Cook AM, Leisinger T. Anaerobic dechlorination of tetrachloromethane and 1, 2-dichloroethane to degradable products by pure cultures of *Desulfobacterium* sp and *Methanobacterium* sp. *FEMS Microbiol Letters.* 1987; 43(3):257–261.
- Egli C, Stromeyer S, Cook AM, Leisinger T. Transformation of tetra- and trichloromethane to CO₂ by anaerobic bacteria is a non-enzymic process. *FEMS Microbi Letters.* 1990; 68(1–2):207–212.
- Egli C, Tschan T, Scholtz R, Cook AM, Leisinger T. Transformation of tetrachloromethane to dichloromethane and carbon dioxide by *Acetobacterium woodii*. *Appl Environ Microbiol.* 1988; 54(11):2819–2824. [PubMed: 3145712]
- Eren AM, Zozaya M, Taylor CM, Dowd SE, Martin DH, Ferris MJ. Exploring the diversity of *Gardnerella vaginalis* in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. *PloS one.* 2011; 6(10):e26732. [PubMed: 22046340]
- Fakruddin M, Mannan K. Methods for analyzing diversity of microbial communities in natural environments. *Ceylon J Sci (Biol Sci).* 2013; 42(1)
- Gupta M, Sharma D, Suidan MT, Sayles GD. Biotransformation rates of chloroform under anaerobic conditions—I. Methanogenesis. *Water Res.* 1996; 30(6):1377–1385.
- Hassan AA, Sorial G. Biological treatment of benzene in a controlled trickle bed air biofilter. *Chemosphere.* 2009; 75(10):1315–1321. [PubMed: 19345397]
- Hassan AA, Sorial GA. n-Hexane biodegradation in trickle bed air biofilters. *Water, Air, & Soil Pollut.* 2008; 8(3–4):287–296.
- Hassan AA, Sorial GA. A comparative study for destruction of n-hexane in trickle bed air biofilters. *Chem Eng J.* 2010; 162(1):227–233.
- Houel N, Selleck RE, Pearson FH. Air stripping of chloroform from water. *J Environ Eng Divis.* 1979; 105(4):777–781.
- Hutchison JM, Poust SK, Kumar M, Cropek DM, MacAllister IE, Arnett CM, et al. Perchlorate reduction using free and encapsulated *Azospira oryzae* enzymes. *Environ Sci Technol.* 2013; 47(17):9934–9941. [PubMed: 23924304]
- Kim Y, Semprini L, Arp DJ. Aerobic cometabolism of chloroform and 1, 1, 1-trichloroethane by butane-grown microorganisms. *Bioremediation J.* 1997; 1(2):135–148.

- Krone UE, Laufer K, Thauer RK, Hogenkamp HP. Coenzyme F430 as a possible catalyst for the reductive dehalogenation of chlorinated C1 hydrocarbons in methanogenic bacteria. *Biochem.* 1989; 28(26):10061–10065. [PubMed: 2559772]
- Laha S, Luthy RG. Effects of nonionic surfactants on the solubilization and mineralization of phenanthrene in soil–water systems. *Biotechnol Bioeng.* 1992; 40(11):1367–1380. [PubMed: 18601093]
- Leson G, Winer AM. Biofiltration: an innovative air pollution control technology for VOC emissions. *J Air & Waste Manage Assoc.* 1991; 41(8):1045–1054. [PubMed: 1958341]
- Li J, Blatchley ER. Volatile disinfection byproduct formation resulting from chlorination of organic–nitrogen precursors in swimming pools. *Environ Sci Technol.* 2007; 41(19):6732–6739. [PubMed: 17969688]
- Mahon S. Biofiltration for the Treatment of Recalcitrant Volatile Organic Compounds in Polluted Air Streams. *Air & Water Pollut.* 2013
- McCulloch A. Chloroform in the environment: occurrence, sources, sinks and effects. *Chemosphere.* 2003; 50(10):1291–1308. [PubMed: 12586161]
- Mikesell MD, Boyd SA. Dechlorination of chloroform by *Methanosarcina* strains. *Appl and Environ Microbiol.* 1990; 56(4):1198–1201. [PubMed: 16348167]
- Novak P, Daniels L, Parkin G. Enhanced dechlorination of carbon tetrachloride and chloroform in the presence of elemental iron and *Methanosarcina barkeri*, *Methanosarcina thermophila*, or *Methanosaeta concillii*. *Environ Sci Technol.* 1998; 32(10):1438–1443.
- Picardal FW, Arnold RG, Couch H, Little A, Smith M. Involvement of cytochromes in the anaerobic biotransformation of tetrachloromethane by *Shewanella putrefaciens* 200. *Appl Environ Microbiol.* 1993; 59(11):3763–3770. [PubMed: 8285682]
- Richardson SD, DeMarini DM, Kogevinas M, Fernandez P, Marco E, Lourencetti C, et al. What's in the pool? A comprehensive identification of disinfection by-products and assessment of mutagenicity of chlorinated and brominated swimming pool water. *Environ Health Perspec.* 2010; 118(11):1523.
- Shemer H, Narkis N. Trihalomethanes aqueous solutions sono-oxidation. *Water Res.* 2005; 39(12):2704–2710. [PubMed: 15985276]
- Swanson KS, Dowd SE, Suchodolski JS, Middelbos IS, Vester BM, Barry KA, et al. Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. *The ISME J.* 2011; 5(4):639–649. [PubMed: 20962874]
- Tiehm A. Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants. *Appl and Environ Microbiol.* 1994; 60(1):258–263. [PubMed: 8117081]
- USEPA. [Accessed 09/10/2016] Accessed 09/10/2016 [2016] Ground Water and Drinking Water: Table of Regulated Drinking Water Contaminants. 2016. <https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants>. <https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants>
- Volkerling Breure A, Rulkens W. Microbiological aspects of surfactant use for biological soil remediation. *Biodegradation.* 1997; 8(6):401–417. [PubMed: 15765586]
- Volkerling F, Breure AM, van Andel JG, Rulkens WH. Influence of nonionic surfactants on bioavailability and biodegradation of polycyclic aromatic hydrocarbons. *Appl Environ Microbiol.* 1995; 61(5):1699–1705. [PubMed: 16535016]
- Wagner M, Loy A, Nogueira R, Purkhold U, Lee N, Daims H. Microbial community composition and function in wastewater treatment plants. *Antonie Van Leeuwenhoek.* 2002; 81(1–4):665–680. [PubMed: 12448762]
- Webster TS, Devinny JS, Torres EM, Basrai SS. Microbial ecosystems in compost and granular activated carbon biofilters. *Biotechnol Bioeng.* 1997; 53(3):296–303. [PubMed: 18633984]
- Woertz J, Kinney K, Szaniszló P. A fungal vapor-phase bioreactor for the removal of nitric oxide from waste gas streams. *J Air & Waste Manage Assoc.* 2001; 51(6):895–902.
- Wu S, Yassine MH, Suidan MT, Venosa AD. Anaerobic biodegradation of soybean biodiesel and diesel blends under methanogenic conditions. *Water Research.* 2015; 87:395–402. [PubMed: 26454635]
- Yeh DH, Pennell KD, Pavlostathis SG. Effect of Tween surfactants on methanogenesis and microbial reductive dechlorination of hexachlorobenzene. *Environ Toxicol Chem.* 1999; 18(7):1408–1416.

- Ying GG. Fate, behavior and effects of surfactants and their degradation products in the environment. *Environ International*. 2006; 32(3):417–431.
- Yoon IK, Kim CN, Park CH. Optimum operating conditions for the removal of volatile organic compounds in a compost-packed biofilter. *Korean J Chem Eng*. 2002; 19(6):954–959.
- Yoon IK, Park CH. Effects of gas flow rate, inlet concentration and temperature on biofiltration of volatile organic compounds in a peat-packed biofilter. *J Biosci Bioeng*. 2002; 93(2):165–169. [PubMed: 16233182]
- Yu Z, Smith GB. Chloroform dechlorination by a wastewater methanogenic consortium and cell extracts of *Methanosarcina barkeri*. *Water Res*. 1997; 31(8):1879–1886.
- Yuan S, Wen H, Wu X, Chen J, Wang L. Effect of nonionic and cationic surfactants on the dechlorination kinetics and products distribution of various polychlorinated benzenes by Cu/Fe particles. *Sep Purif Technol*. 2010; 74(1):130–137.
- Zehraoui A, Kapoor V, Wendell D, Sorial GA. Impact of alternate use of methanol on n-hexane biofiltration and microbial community structure diversity. *Biochem Eng J*. 2014; 85:110–118.
- Zhai J, Wang Z, Shi P, Long C. Microbial Community in a Biofilter for Removal of Low Load Nitrobenzene Waste Gas. *PloS one*. 2017; 12(1):e0170417. [PubMed: 28114416]
- Zitomer DH, Speece RE. Methanethiol in nonacclimated sewage sludge after addition of chloroform and other toxicants. *Environ Sci Technol*. 1995; 29(3):762–768. [PubMed: 22200286]

Figure 1.
Schematic diagram of the Biotrickling Filter (BTF)

Figure 2.

Performance of the BTF in the three phases. Phase I (44 days) presence of cometabolite, phase II (97 days) presence of cometabolite and surfactant, phase III (95 days) presence of surfactant with no cometabolite.

Figure 3.

Reaction rate constants for chloroform in three phases. Three data sets have been collected per phase and the error bars present the standard deviations for these replicas. Phase I: Chloroform with Co metabolite (Ethanol), Phase II: Chloroform with Co metabolite (Ethanol) and nonionic surfactant and Phase III: Chloroform with nonionic surfactant.

Figure 4.
Carbon mass balance: Cumulative carbon input and output as CO₂ equivalent in mole for the BTF

Figure 5.

Bacterial community diversity for the three conditions for samples collected at the top port of the biofilter. Phase I: Chloroform with Co metabolite (Ethanol), Phase II: Chloroform with Co metabolite (Ethanol) and nonionic surfactant and Phase III: Chloroform with nonionic surfactant.

Table 1

Operating condition for the BTF degrading chlorform under anaerobic condition

Operating Conditions	Phases		
	I <i>with Co metabolite no Surfactant</i>	II <i>with Co metabolite and Surfactant</i>	III <i>No Co metabolite with Surfactant</i>
Influent Chloroform concentration, ppmv	5		
Empty Bed Residence Time, min	5		
Operation time, days	44	97	95
Average Chloroform Removal Efficiency (%)	49 ± 9	64±8	59±8
Loading Rate (g/m ³ . h)	0.27	0.27	0.27
Elimination Capacity (g/m ³ . h)	0.13 ± 0.02	0.17 ±0.02	0.16 ±0.02

Table 2

Summary of the community diversity along with their metabolic characteristics

Bacteria	Taxonomic classification (Class)	Metabolic role	Electronic receptor
<i>Azospira suillum</i> , <i>A. oryzae</i> , <i>Azonexus fungiphilus</i> , <i>Azonexus spp.</i>	Betaproteobacteria	dissimilatory reduction of perchlorate and chlorate	humic substances and nitrate and metals
<i>A. restricta</i>	Betaproteobacteria	- metabolizes b-hydroxybutyrate, c-hydroxybutyrate, D,L-lactate and putrescine as growth substrates -capacity of nitrogen fixation and reduction acetylene to ethylene	D,L. Lactic acid, Putrescine and B. bhydroxybutyric acid
<i>Geobacter spp.</i>	Deltaproteobacteria	Oxidize organic compounds and metals, including iron, radioactive metals and petroleum compounds	iron oxide or other available metals
<i>Smithella spp.</i>	Deltaproteobacteria	anaerobic, syntrophic, propionate-oxidizing bacteria	fumarate
<i>Anaerobaculum mobile</i>	Synergistia	anaerobic, peptide-fermenting	Crotonate, Amino acid
<i>Aminobacterium</i>	Synergistia	an amino acid-degrading anaerobe isolated from anaerobic sludge	Amino acid