In this study, the biodegradation of a mixture of two trihalomethane (THM) compounds, chloroform (CF) and dichlorobromomethane (DCBM), was evaluated using two laboratory-scale biotrickling filters (BTFs). The two BTFs, hereby designated as “BTF-A” and “BTF-B,” were run parallel and used ethanol as co-metabolite at different loading rates (LRs), and a lipopeptide-type biosurfactant that was generated by the gram-positive bacteria, Surfactin, respectively. The results using BTF-A showed that adding ethanol at a higher rate of 4.59 g/(m³ h) resulted in removal efficiencies of 85% and 87% for CF and DCBM, respectively. Conversely, for the same LR, the use of Surfactin without ethanol (BTF-B) showed comparable removal efficiencies of 85% and 80% for CF and DCBM, respectively. The maximum rate constant for CF and DCBM for the BTF-A was 0.00203 s⁻¹ and 0.0022 s⁻¹, respectively. For the same THMs LR, similar reaction rate constants resulted for the BTF-B. Further studies were conducted to investigate and understand the microbial diversity within both BTFs. The result indicated that for BTF with co-metabolite, Fusarium sp. was the most dominant fungi over 98% followed by F. Solani with less than 2%. F. oxysporum and Fusarium sp. were instead the dominant fungi for the BTF with Surfactin. Before introducing the Surfactin into the BTF, the batch experiment was conducted to evaluate the effectiveness of synthetic surfactant as compared to a biosurfactant (Surfactin). In this regard, vials with Surfactin showed better performance than vials with Tomadol 25-7 (synthetic surfactant).

KEYWORDS
biotrickling filter, co-metabolite, microbial diversity, Surfactin, trihalomethanes
exposure to THMs is inhalation and dermal contact during regular indoor and outdoor activities. The maximum allowable contaminant level in drinking water for total THMs is 0.08 ppm.2

Furthermore, air emissions, surface water discharges, underground injections, and releases to land are other possible sources of CF and other THMs other than disinfection. In the United States, direct release of THMs to the environment has been reported annually. For example, the US Environmental Protection Agency’s (EPA) Toxic Release Inventory (TRI) reported that in 2017 approximately 0.79 million lbs of CF and DCBM were released to the environment from industries.3 Presently, some water treatment plants use aeration in storage tanks in water distribution systems for reducing THMs.4 However, the emitted volatile compounds in the gas phase are not treated. To reduce air pollution further, the 1990 amendments to the Clean Air Act (1990) has established regulations, standards, guidelines, and codes of volatile organic compounds (VOCs) emissions that are more stringent. The Clean Air Act amendment helped the development of new processes and techniques to achieve stricter emission levels. The formation of THMs in drinking water also has highlighted the need for exploring other disinfectants and new treatment technologies.

In this work, CF and DCBM were taken as model DBPs for biodegradation study. Both are hydrophobic VOCs, which have higher Henry’s law constants.5 Trihalomethanes are recalcitrant to biodegradation. Hence, advanced oxidation, adsorption,6 air stripping,7 and catalytic and thermal oxidation8 are used to treat THMs. These treatment technologies either have limited effectiveness, are expensive, or may generate a secondary pollutant such as acid vapor.6 Due to their high Henry’s law constant,3 alternative approaches can be used to treat contaminated water, such as gas stripping9,10 or gas stripping combined with biological treatment. Biological treatment approaches for removal of VOCs have certain advantages over physical and chemical techniques including low-cost, effectiveness, higher safety, and eco-friendly.11 Biofiltration is a biological process that uses microorganisms to convert water-soluble VOCs into harmless by-products. Recently, there have been increasing interests in applying biofiltration for the removal of polluted waste-gas. The number of studies on the treatment of high flow rate and low VOC laden gas stream by using a biofilter system has risen.12-18 The technology is a combination of physiochemical and biological processes,19 where contaminants degraded by passing them through a bed filled with microorganisms that utilize the contaminant as an energy source. The degradation product of many VOCs are usually CO₂ and water vapor in aerobic operatic conditions. The process is considered a “greener” technology than the conventional thermal destruction techniques because it consumes a fraction of the energy and does not generate hazardous air pollutants. Biofiltration offers many potential advantages over existing control technologies, such as low installation and operation costs, low maintenance requirements, long life of the biofilter, and safe environmental operation. Thus, the biofilter functions efficiently and economically in removing low-level pollutants.20 It has been operated as a separate process21 or in combination with other technologies. For example, biofiltration has also been used in conjunction with other treatment technologies such as ozonation.22-25 In their recent work, Bacaro et al have expanded the treatment by ozonation with biofiltration studies to chloramines using a pilot scale system.26 Current reports show that the biofiltration process is easier to operate and is cost effective in removing VOC air pollutants.27 The main challenges in the biofiltration of hydrophobic compounds are their low bioavailability and high stability that result in low biodegradation.28 The mass transfer of pollutants, excess microbial populations, packing media, and moisture are the main factors.13,19 Although most studies show successful biodegradation of CF and DCBM in the liquid phase, there has been limited reported work on the use of biofiltration for the removal of CF and DCBM from gaseous streams.

There are a few research studies in the literature that reported the effective use of the biofiltration for the treatment of a mixture of CF and other VOC gases.29,30 Balasubramanian et al29 studied how the elimination capacity (EC) and removal efficiency (RE) of biofiltration for treating a complex mixture of VOCs are affected by the inlet loading rate (LR) and empty bed residence time (EBRT). Their BTF was operated at both steady and transient conditions, for different inlet LRs, empty bed residence time, and inlet CF concentrations. As the rate of loading of CF mixed with nine other VOCs increased, there was a significant reduction in the RE of the biofilter.29 Other researchers, Yoon et al, have shown the degradation potential of CF along with nine VOCs compounds (benzene, toluene, m-xylene, o-xylene, styrene, trichloroethylene, isoprene, and dimethyl sulfoxide). In their work, they studied the effects of column temperature, gas residence time, and inlet concentrations on the removal of these compounds in a compost-packed biofilter. The study found out that the highest removal was for toluene, and the lowest removal was for CF.30 Furthermore, in a study of co-metabolism of THMs (including CF and DCBM) conducted with a nitrifying biofilm in biofiltration by Wahman et al31 obtained a CF removal ranging between 13% and 43%. Correspondingly, they reported that biofilters could degrade THMs with removals ranging from 7% to 24% that confirmed ammonia-oxidizing bacteria are capable of transforming the four regulated THMs.32

As discussed earlier, one of the challenges in using biofiltration technology is the bioavailability of the VOCs. Addition of synthetic or biological surfactants to the system could improve this limitation. Many researchers have studied the use of synthetic surfactants to enhance the RE of biofiltration. Tomadol 25-7 and saponins for n-hexane,33,34 Tween 20 for
toluene,\textsuperscript{35} and Triton X-100 for styrene\textsuperscript{36} were used for improving degradation in the fungi biofilter. However, the authors believe that the use of a biosurfactant such as Surfactin in a biofilter system has not been reported in the literature. Surfactin is a lipopeptide-type biosurfactant that is obtained from the gram-positive microorganism, \textit{Bacillus subtilis}.\textsuperscript{6} It is a supernatant of \textit{B. subtilis}. \textit{B. subtilis} is rod-shaped and gram-positive bacteria, which can tolerate extreme environmental conditions.\textsuperscript{6} Biological degradation of CF and DCBM is challenging because of their low water solubilities and low biodegradation rates that make them reluctant to treatment and persistent in drinking water. Because THM is a hydrophobic compound, they do not mix with water well, which affects their biodegradation rate within the biofilter and slow down their mass transfer into the liquid phase. Some researchers have replaced the microorganism consortium from bacteria to fungi that have a much higher surface area to increase their mass transfer to the water phase.\textsuperscript{37} Trickle bed biofilters that use fungi have shown to be useful for the treatment of hydrophobic volatile pollutants. Fungi are also resistant to drying out and are effective over a wide pH range including acidic condition. Therefore, fungi are not only more tolerant to pH fluctuations, but the aerial mycelia of fungi could also support the uptake of hydrophobic VOCs and accelerate their mass transfer from the air to the biofilm.\textsuperscript{15,37,38} However, there is limited published research that has used fungi in the traditional biofilter for insoluble compounds such as \textit{n}-hexane\textsuperscript{15} and alkyl benzenes.\textsuperscript{38} We have reported the application of biofiltration that uses fungi under acidic conditions to treat CF that resulted in the degradation of CF alone up to 81\% RE.\textsuperscript{39}

In this study, two BTFs were run in parallel, one using a co-metabolite, ethanol, and the other using biosurfactant, Surfactin. The first BTF (BTF-A) was studied by varying the LRs of the co-metabolites while feed concentration of THMs was kept constant. While the second BTF (BTF-B) run with surfactin at different THMs feed concentrations. To enhance the fungi growth, both BTFs were operating under an acidic environment. The microbial ecology within both BTFs was investigated to understand their performances better. Other parameters for measuring the performance of the BTFs such as reaction kinetics, carbon mass balance, and chemical oxygen demand (COD) removal versus nitrogen utilization were also examined.

## 2 MATERIALS AND METHODS

### 2.1 Volatile organic compounds and DNA extractions

The two THMs selected for this study were CF and DCBM, having 99.8\% purity, were obtained from Fisher Scientific (Pittsburgh, PA, USA). Ethanol with 99.5\% purity was acquired from Sigma Aldrich (St. Louis, MO, USA). Surfactin was obtained from Sigma Aldrich (St. Louis, MO, USA). All chemicals were used without any purifications. Chloroform and dichlorobromomethane, having Henry’s law constant at 25°C are $K_H$ of 3.67 $\times$ $10^{-3}$ and 2.12 $\times$ $10^{-3}$ atm·m$^3$/mol, respectively.\textsuperscript{40} Ethanol is highly miscible in water with a $K_H$ value of 5.1 $\times$ $10^{-6}$ atm·m$^3$/mol at 25°C.\textsuperscript{41} The first study was BTF-A done using ethanol as a co-metabolite. The second set of studies were conducted using BTF-B that was fed continuously with the same mixtures of THMs, and Surfactin was seeded without the presence of co-metabolite. 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

Figure 1 shows a schematic diagram of both biofiltration systems, BTF-A and BTF-B. The detail design parameters of the columns and packing materials were described by Zehraoui et al.\textsuperscript{42} Both BTFs were ran at an average temperature of 22 ± 1°C and operated in a co-current mode of both the feed gas and liquid flow downward. Biotrickling filter-A (BTF-A) was fed with THMs (CF + DCBM) to ethanol ranging from 1:5 (v/v) to 1:40 (v/v), whereas the LR of the THMs was maintained constant. For BTF-A, a microbial culture was obtained from previous biofiltration column and was seeded to the new column bed.\textsuperscript{39} However, BTF-B was fed at selected LRs ranging from 0.14 to 0.41 g/m$^3$·h for CF and 0.18 to 0.55 g/m$^3$·h for DCBM and no co-metabolite. A solution of microorganisms mixed with an enriched solution was prepared prior to the onset of the operating BTF-B. The solution contained THMs degrading microorganisms from the effluent liquid of BTF-A and 10 mg/L of Surfactin. Initially, the Surfactin was extracted from \textit{B. subtilis} strain #21332 (ATCC), which were grown at 30°C for 48 to 72 hours in lysogeny broth. One-liter cultures were rotated at 250 rpm in flat-bottom flasks until collection; cells were centrifuged at 12,000 g’s for 20 minutes to separate the Surfactin containing the supernatant from the bacteria. The spent growth media containing Surfactin was decanted and applied to the enriched solution without further purification after \textit{B. subtilis} cells were removed. The solution was applied to BTF-B by pouring it on the media and leaving the solution to saturate media for 2 hours before draining it out. Once the seeding process was
completed, the enriched Surfactin solution in the BTF was acclimated at a pH = 4 by using sodium formate buffered with HCl. A stream of air laden with CF and DCBM was fed into the column continuously for 45 days until the growth of new biomass was visible.

The operations of both BTFs included stagnation type of biomass control strategy, where the system was stopped for two days per week. During the stagnation period, all inflow streams including VOCs, nutrients, and feed air were stopped. The technical effectiveness of stagnation on the performance of the biofiltration and biomass growth control was shown in previous studies. The BTFs were run under aerobic conditions using air as carrier gas flowing at the 0.5 L/min and corresponding EBRT of 5 minutes. The hydraulic residence time and trickling velocity for both BTFs were 979 minutes and 0.031 cm/min, respectively. A buffered nutrient solution containing nitrate as a nitrogen source was supplied at an average rate of 2.0 L/day. The composition of the nutrient solution was used according to Hassan et al. For BTF-A, liquid mixtures of THMs and ethanol were injected via two separate syringe pumps in a series and vaporized into the air stream. However, for BTF-B, only liquid CF and DCBM with 1:1 (v/v) ratio were injected via syringe pump and Surfactin was seeded to the BTF bed.

2.3 Sampling and analysis

Liquid and gas samples from each BTF were collected 5 days per week. Gas samples were collected from inlet and outlet streams at different ports along the column. Liquid samples from the inlet and outlet streams were collected to measure concentrations of the total organic carbon, nitrate, and pH. Measurements of gas temperatures along the bed and the pressure drop between the inlet and exit port were taken daily. Gas samples were collected using gas-tight syringes from all ports along both BTF columns and injected into GC equipped with 1-ml sample loop, DB-5 column, and a flame ionization detector (FID). The gas sample compositions were analyzed for the levels of CF, DCBM, ethanol (only for BTF-A) and levels of by-product CO2. Detail gas phase analysis was described in our previous paper. Liquid samples collected once a week from the outlet of the BTF for volatile suspended solids (VSSs) and total organic carbon analysis. Membrane filters (0.45 μm in size, Whatman Co.) were used to separate influent and effluent concentrations of dissolved total and inorganic carbon, nitrate, and VSS. Nitrate – N concentration from the liquid phase measured using the AccuMate single-ion electrode sensor. A Shimadzu total organic carbon analyzer model TOC - L (Shimadzu Corp., Tokyo, Japan) was used to determine dissolved total and inorganic carbon content of the liquid samples. The VSS was conducted according to Standard Method 2540G.
2.4 | Microbial community molecular analysis

To determine the profile of the microbial community of each BTF under the different operating conditions, biofilm samples were collected from the first port of each column, Port 2, as shown in Figure 1. Biofilm samples were collected at the end of each test phase for the molecular biology analysis. The method facilitates the simultaneous assessment of microbial 16S rRNA diversity by using PCR and reverse transcription-PCR. Gel electrophoresis of the microbial community analysis differentiated the active component (rRNA derived) from the total bacterial diversity (ribosomal DNA derived). Additional information on the microbial analysis can be found in our previous report. The analysis was done at the Molecular Research LP (www.mrdnalab.com, Shallowater, TX, USA) that includes DNA sequencing using an Ion Torrent Personal Genome Machine (PGM) according to the manufacturer’s guidelines.

3 | RESULTS

3.1 | Comparison of synthetic and bio surfactant for the solubility of CF

The goal of this batch study was to compare the widely used synthetic surfactant against biosurfactant on CF’s degradation. The procedure utilized for this study was similar to the one conducted by Hassan et al. The experiment was performed by placing 200 mL of acidic nutrient solution enriched with fungi in 250 mL amber vials. Two kinds of surfactants, namely, Tomadol 25-7 (synthetic surfactant) and Surfactin (biosurfactant), were used for this study. Each surfactant concentration was at 10 mg/L prepared in a replica of three vials for estimating the precision. Additionally, the nutrient solution with no surfactant added mixed with CF was used as a control. The CF concentration in all vials was 25 μg/L. The vials were capped with a 24-mm replacement mini-inert valve (Supelco, PA). The vials were then continuously mixed in a tumbler maintained isothermally at 22 ± 1°C. A 1-mL gas sample was drawn from the headspace of each vial using gas-tight syringes and analyzed for CF and degradation by-products concentrations on days 1, 3, 7, and 10. Figure 2 displays CF concentration in the headspace obtained from the control, and the two surfactants were containing vials (Tomadol 25-7 and Surfactin). It is shown in Figure 2 that the vials with Surfactin showed better performance than that with Tomadol 25-7. One of the reasons could be that Surfactin is less toxic than the other synthetic surfactants. The controls showed no change in concentration with time. Additionally, the vials with Surfactin produced 30% more carbon dioxide than the ones with Tomadol 25-7. Hence, the addition of Surfactin demonstrated the extent of the bioavailability of CF for biodegradation by the microbes.

3.2 | BTF performances

3.2.1 | Performance of biotrickling filter-A (BTF-A)

For tests on BTF-A, the inlet air stream carried a mixture of CF, DCBM mixed with a co-metabolite to supplement the biodegradation. Ethanol is added as a co-metabolite that represent a low-cost and readily degradable carbon source. The RE of ethanol was more than 98% for all tests used as a co-substrate, and emphasis is placed on the performance of the BTF for removal of CF and DCBM. During Phase I, total mixtures of THMs was 5 ppmv (2.5 ppmv each component) and
ethanol concentration of 25 ppmv providing a ratio of 1:5 (v/v) (THMs: ethanol). The corresponding CF and DCBM LRs were 0.14 and 0.18 g/(m³-h), respectively.

The operational details for the different phases of the BTF-A including the rate of influent loading and concentrations, days of operation, the corresponding RE, and average ECs are given in Table 1. Removal efficiency and elimination capacity are calculated using the following equations.

\[ \text{RE} = \left( \frac{C_0 - C}{C_0} \right) \times 100 \]  

\[ \text{EC} = \frac{Q}{V} (C_0 - C) \]  

where \( C_0 \) and \( C \) are the initial and final THMs concentration, \( Q \) is the air flow rate, and \( V \) is the volume of the bed.

Figure 3A displays a box plot showing the removal efficiencies for the different LRs of the BTF-A operations. The plot shows the 10th and 90th percentiles of the data as endpoints of the error bars. The limits of the boxes denote the lower and upper quartiles of the data, and the lines within the boxes show the median.

In Phase I, the BTF was run for 36 days. During this Phase, CF removal ranged from 48% to 87% and DCBM ranged from 61% to 88%, providing average removal efficiencies of 73 ± 11% and 77 ± 10%, respectively. The average EC for CF and DCM was 0.11 ± 0.02 g/(m³-h) and 0.14 ± 0.03 g/(m³-h), respectively (Table 1). In Phase II, the mixtures of THMs - ethanol ratio was raised to 1:10 (v/v) by increasing only the LR of ethanol. CF removal ranged from 63% to 85% and DCBM ranged from 65% to 88% providing average removal efficiencies for CF and DCBM of 77 ± 6% and 78 ± 6%, respectively. The corresponding ECs were 0.12 ± 0.02 and 0.15 ± 0.02 g/(m³-h), respectively. Phase II was run for 41 days. Mixtures of THMs to ethanol ratio in Phase III was increased to 1:20 (v/v), which provided ethanol LR of 2.30 g/(m³-h), while maintaining the same LR for both CF and DCBM. During Phase III, the CF removal ranged from 60% to 88%, and DCBM ranged from 63% to 88% providing average removal efficiencies for CF and DCBM of 79 ± 8% and 80 ± 7%, respectively. In this case, it showed a slight increase with respective ECs of 0.12 ± 0.01 g/(m³-h) for CF and 0.16 ± 0.02 g/(m³-h) for DCBM. Because the feed stream and nutrient trickling into BTF had downward co-current flow, there was a larger biomass accumulation on the upper segment of the BTF. The biomass grew with the increase of VOCs LRs because of the enhanced cell synthesis and other VOCs. The higher amounts of biofilm and cell accumulation in the top sector of the BTF were observed by other researchers studying the biofiltration of toluene and other VOCs. The weekly stagnation abetted in controlling this excessive biomass formation and pressure drop. A similar biomass controlling technique was used for the biofiltration of n-hexane and CF.

On day 110 (Phase IV), the LR of ethanol was increased to 4.59 g/(m³-h). This phase was run for 38 days, and the removal for CF ranged from 67% to 94%, and for DCBM, the range was 72% to 95%. During this phase, the average removal efficiencies for CF and DCBM increased to 85 ± 6% and 87 ± 6%. Similarly, in this phase, stagnation was used to control the observed high-pressure drops across the system. The pressure drops during this phase could be due to the high biomass accumulation within the bed, which created the BTF short circuit. The mixtures of THMs-ethanol ratio of 1:40 (v/v) provided a higher EC of 0.13 ± 0.01 and 0.17 ± 0.01 g/(m³-h) for CF and DCBM compared to the other previous phases (Table 1). The result also showed that the increase in the LRs of the co-metabolite enhanced the performances of the BTF. Similar observation was made in our previous studies, which were conducted for the removal of CF alone in the presence of ethanol. Although our previous research focused on a single solute (only CF), the result confirmed that as the concentration of ethanol increased, the RE of CF also increased accordingly. Similarly, Wahman et al reported that...
FIGURE 3 Performance of BTF-A and BTF-B. A, The four phases of BTF-A: (Phase I) 1:5 (v/v) ratio of THMs mixture (chloroform and dichlorobromomethane) to ethanol (co-metabolite), (Phase II) 1:10 (v/v) ratio of THMs to ethanol, (Phase III) 1:20 (v/v) ratio of THMs to ethanol, and (Phase IV) 1:40 (v/v) ratio of THMs to ethanol; B, The three phases of BTF-B: (Phase I) 5 ppmv of THMs, (Phase II) 10 ppmv of THMs, and (Phase III) 15 ppmv of THMs. BTF, biotrickling filter; THM, trihalomethane; CF, chloroform; DCBM, dichlorobromomethane

the use of co-metabolic biofilters seeded with three different mixed cultures.\textsuperscript{50} The biofilter media that was used in their study expanded to include granular activated carbon obtained from an operating drinking water treatment. The batch degradation kinetic tests were conducted to evaluate the biofilm’s ability to remove THMs. As a result, the system was able to remove CF and DBCM up to 18\% and 75\%, respectively. However, this current study demonstrated that higher removal efficiencies could be obtained for both CF and DCBM by using fungi BTF under an acidic condition.

3.2.2 Performance of BTF-B

Table 2 describes the details of the operation of BTF-B. Biotrickling filter-B (BTF-B) was run at various influent concentrations of mixtures of CF and DCBM without co-metabolite. The two components were at equal ratios 1:1 (v/v) with total concentration ranging from 5 to 15 ppmv with corresponding LRs of 0.14 to 0.55 g/(m\textsuperscript{3}·h). Figure 3B represents a statistical summary of the RE as a box plot at different LRs of the BTF. In Phase I, after the acclimation period, the BTF was run for 60 days, and the average removal efficiencies obtained for CF and DCBM were 85\% and 80\%, respectively, which afforded average ECs of 0.13 ± 0.01 g/(m\textsuperscript{3}·h) and 0.16 ± 0.02 g/(m\textsuperscript{3}·h), respectively (Table 2).

On day 61 (Phase II), the concentration of the mixture was raised to 10 ppmv (LRs of 0.27 and 0.37 g/(m\textsuperscript{3}·h)) where both removal efficiencies for CF and DCBM decreased to 75\% and 74\%. Their corresponding ECs were 0.20 ± 0.02 and 0.27 ± 0.02 g/(m\textsuperscript{3}·h). In this phase, the BTF ran for 55 days. During Phase III, after increasing the concentrations to 15 ppmv (LRs of 0.41 and 0.55 g/(m\textsuperscript{3}·h), the system was left to run for 46 days. The obtained removal efficiencies were 69\% and 68\% for CF and DCBM, respectively. As shown in (Table 2), the RE (69\%) was less than the rest of the previous phases (74\%-85\%). This could be due to the toxicity effect of THMs on the microbes at 15 ppmv. The result showed that as the concentration THMs increased, the RE for CF and DCBM decreased.
TABLE 2 Operating conditions for biotrickling filter-B (BTF-B) for degrading equal mixtures of trihalomethanes (THMs), chloroform (CF) and dichlorobromomethane (DCBM), at different loading rates

| Experimental conditions | I | Phases | II | III |
|-------------------------|---|--------|---|-----|
| THMs concentration (ppmv) | 5 | 10     | 15 |     |
| Operation time (days)   |   | 60     | 55 | 46  |
| THMs                    | CF| DCBM   | CF| DCBM |
| Removal efficiency (%)  | 85 ± 9 | 80 ± 7 | 75 ± 6 | 74 ± 7 |
| Loading rate (g/m³·h)   | 0.14 | 0.18   | 0.20 ± 0.02 | 0.27 ± 0.02 |
| Elimination capacity (g/m³·h) | 0.13 ± 0.01 | 0.16 ± 0.02 | 0.30 ± 0.02 | 0.37 ± 0.02 |

4 DISCUSSION OF THE RESULTS

4.1 Performance comparison for BTF-A and BTF-B

For similar inlet concentrations of CF and DCBM, BTF-B offered higher EC and performance as compared with BTF-A. The highest RE obtained for BTF-A was at Phase IV when the ethanol loading reached 4.59 g/(m³·h). At this point, the removal efficiencies for CF and DCBM were 85% and 87%, respectively. However, BTF-B provided RE of 85% for CF and 80% DCBM at a similar THMs concentration (5 ppmv) without co-metabolite. This behavior indicates that the significance of the presence of Surfactin in degrading the THMs. As can be seen from Table 2, the presence of Surfactin enhanced the bioavailability of the THMs by facilitating their biotransformation. Furthermore, it can be concluded that BTF-B could tolerate high LRs (0.14 g/m³·h for CF and 0.18 g/m³·h for DCBM) and reasonable EC (0.13 and 0.17 g/(m³·hr) for CF and DCBM, respectively) without adding a co-metabolite. For chemical industries that emit VOCs including ethanol and chlorinated compounds, ethanol serves as co-substrate to enhance biodegradation. Balasubramanian studied the biodegradation using BTF of CF along with a mixture of VOCs, including ethanol that was found in the emission stream of the pharmaceutical plants. Therefore, this study demonstrated that BTF could be a useful technology for degrading THMs when emissions of VOCs include ethanol or can be used with Surfactin without a co-metabolite if emissions do not contain ethanol. Due to their acceptance and less toxicity, bio-surfactants such as Surfactin have the advantage over synthetic surfactants.

4.2 Biodegradation kinetics of CF and DCBM for the different phases

For both BTF-A and BTF-B, gas phase samples were drawn from each port one day after the stagnation period every week to evaluate the reaction rate kinetics of CF and DCBM for the various VOCs LRs. Kinetics analysis was conducted to ensure uniformity of biomass throughout the BTF bed. The samples were taken along the BTFs bed. The analysis was conducted based on other similar studies. By assuming a plug flow reactor model for the BTF, the degradation kinetics of CF and DCBM was developed. For a pseudo-first-order reaction rate,

\[ \ln C = -kt + \ln C_0, \]

where \( C \) represents the concentration of exit stream THMs at time \( t \), \( C_0 \) represents the influent concentration of each species, and \( k \) is the reaction rate constant. The concentration data of CF and DCBM in each reactor was fit with residence time using an unweighted linear regression, and the slope of the regression was reported as the pseudo-first-order rate constant. The results of the reaction rate constant for the BTF-A and BTF-B are presented in Figure 4A,B. The reaction rate constant values for the four phases of BTF-A ranged from 0.0016 s⁻¹ to 0.00203 s⁻¹ for CF, and 0.0017 s⁻¹ to 0.0022 s⁻¹ for DCBM (Figure 4A). On the other hand, the reaction rate constants for the BTF-B ranged from 0.0012 s⁻¹ to 0.00203 s⁻¹ for CF and from 0.0014 s⁻¹ to 0.002 s⁻¹ for DCBM (see Figure 4B). The highest reaction rate constant was observed in Phase IV of BTF-A at 0.00203 s⁻¹ for CF and 0.0022 s⁻¹ for DCBM, and these results correlate with the increase of ethanol LR. Bacterial growth was enhanced by the addition of ethanol as the co-substrate, which fostered the rates of biodegradation of the THMs. However, for BTF-B, higher reaction rates of CF and DCBM were obtained during Phase I at 0.00203 s⁻¹ for CF and 0.0020 s⁻¹ for DCBM (Figure 4B). The lowest reaction rate constant for CF (0.0012 s⁻¹) and DCBM (0.0014 s⁻¹) were recorded at a higher LR of BTF-B. The removal kinetics observed in this study showed a similar trend for CF reported by Palanisamy et al., trichloroethylene removal by Chheda and Sorial, and n-hexane, benzene, and methanol by Zehraoui and Sorial. The highest reaction rate constant achieved in this study is less than the values for
trichloroethylene (0.027 s\(^{-1}\)) and the mixture of n-hexane, benzene, and methanol (0.025 s\(^{-1}\)) studied in similar fungi BTF operated under acidic condition. The degradation rate constants for CF and DCBM decreased as the inlet concentrations increased, which is contradictory to the true first-order kinetics.

In comparison, both BTF-A and BTF-B have similar reaction rate constants for CF and DCBM at lower concentrations of 5 ppmv. The results shown in Figures 4A and 4B indicated that comparable higher reaction rates for CF and DCBM were obtained for Phase IV of BTF-A and Phase I of BTF-B as compared to the other phases of both BTFs. The degradation rate constant for CF attained in this study was 13% higher than the 0.0018 s\(^{-1}\) reported in our previous work. These results showed that the presence of DCBM did not inhibit the degradation of CF.

### 4.3 Carbon mass balance for BTF operation

Figure 5A presents the cumulative CO\(_2\) equivalents of ethanol, CF, DCBM, and the nutrients entering and leaving the BTF-A. All the carbon sources, and products in the inlet and exit gas and liquid streams were measured. The gaseous inlet stream contains THM, the co-substrate, and the exit gaseous stream may contain unreacted reactants and the by-product, CO\(_2\). The feed and effluent liquid streams consisted of organic and inorganic carbon and the carbon equivalent of VSSs. The molar CO\(_2\) equivalence of all the carbon components was computed as the cumulative input and output on successive time are presented in Figure 5A, where the carbon recovery for BTF-A was 55%. Likewise, Figure 5B shows the cumulative CO\(_2\) equivalent of CF, DCBM, and the nutrients entering and leaving the BTF-B. A similar carbon recovery of 55% was obtained for this BTF. The recovery reported from our previous work for single solute CF for similar four phases was 63%. It is postulated that the difference in the carbon content between the influent and effluent streams accumulated over the test period produced the biomass within both BTFs. This hypothesis justifies the loss of carbon to the amount of biomass accumulated within each bed. The acidic condition in the BTF afforded the growth of cellular filamentous fungi that can be presented by C\(_9\)H\(_{15}\)O\(_5\)N. The nitrate consumption in the growth of the fungal biomass was used to estimate the cellular compositions of the biomass retained within the BTFs. A statistical \(t\) test was conducted to verify the consumption of carbon within the BTFs correlates with the growth of biomass for BTF-A and BTF-B. The statistical test confirms (\(p\) value <0.05) that the link between the biomass production in the BTF bed and the carbon retained have high statistical significance.

### 4.4 Chemical oxygen demand (COD) and nitrogen utilization

Assimilation of NO\(_3^-\) and NH\(_4^+\) by microorganism is essential for their growth and development. In this work, nitrate was provided as a macronutrient for the microorganism. Daily measurements of NO\(_3^-\)–N were taken for samples obtained from the influent and effluent steams. The overall BTF performance and utilization of nitrogen were calculated from the influent and effluent liquid NO\(_3^-\)N streams. The complete oxidation of ethanol, CF, and DCBM under strong oxidizing
conditions produces carbon dioxide (CO₂), water (H₂O), and HCl (from CF). The COD for any organic compound can be theoretically calculated from balanced equations presented in Equations (4) to (6), where C₂H₅OH, CHCl₃, and CHCl₂Br stands for ethanol, CF, and DCBM, respectively.

\[
C₂H₅OH + 3O₂ → 2CO₂ + 3H₂O \quad (4)
\]

\[
2CHCl₃ + 2H₂O + O₂ → 2CO₂ + 6HCl \quad (5)
\]

\[
2CHCl₂Br + 2H₂O + O₂ → 2CO₂ + 4HCl + 2HBr \quad (6)
\]

The mass ratio of COD and VOC for ethanol was 2.09 (Equation (4)). Likewise, from Equations (5) and (6), the mass ratios for CF and DCBM were 0.13 and 0.1, respectively. The difference between COD of the influent and effluent (for both gas and liquid streams) used to calculate the net COD. Figure 6A,B shows CODremoval/Nutilization (CODR/NU) at different phases for BTF-A and BTF-B. The lowest CODR/NU ratios for BTF-A was 5.12 (Phase I) for a total LR of 0.89 g/(m³·h). The highest ratio was obtained in Phase IV, which was 15.46 for the LRs of 4.91 g/(m³·h). The LRs to the BTF-B have a significant influence on all phases (see Figure 6A).

In the case of BTF-B, Phase I produced a CODR/NU ratio of 14.75 for a total VOC LR of 0.32 g/(m³·h). It then reduced to 11.31 for a total LR of 0.64 g/(m³·h) in Phase II with lower performance than Phase I (see Figure 6B). During Phase III, for the total LR of 0.96 g/(m³·h), the corresponding CODR/NU ratio reduced further to 11.04, which again corresponds to the lowest performance of the BTF (see Figure 6B). In this BTF, as we increased the THMs concentration, the respected CODR/NU ratio also reduced following its performance. The maximum reported CODR/NU value for single CF was 14.5.39

In conclusion, the use of Surfactin in the BTF system can result in providing the same mass ratio of CODR/NU by giving a high-yield VOC that contributed significantly to high nitrogen consumption.
Microbial ecological analyses and correlation

Ion Torrent Personal Genomics Machine™ (PGM) system was used to study the microbial diversity structure of both BTFs. Samples for the microbial analysis were collected from each BTF after re-acclimation to each phase and when stability was achieved. Microorganisms are the core component of biofiltration. Figure 7A,B provides the fungi community diversity observed in BTF-A and BTF-B for samples collected from each BTF from the top port (port 2) port respectively.

**BTF-A**: On Phase I, the BTF was running under acidic conditions and fed with 5 ppmv of equal mixtures of THMs (2.5 ppmv CF and 2.5 ppmv DCBM) and 25 ppmv of ethanol; the most dominant species at port 2 was *Fusarium sp.*, with relative abundance of 98.4% (Figure 7A). During Phase II, when the BTF was fed with more ethanol (50 ppmv), the amount of *Fusarium sp.* showed a slight increase to 98.5%. In Phase III, when the co-metabolite increased, *Fusarium sp.* dominates by 98.7%. In Phase IV, with higher ethanol (200 ppmv) concentration, the BTF was again dominated by *Fusarium sp.* by 98.6%. In this phase, a slight percentages of *F. oxysporum* and *F. solani* have been observed with a small abundance of 0.5% and 0.4%, respectively (Figure 7A).

**BTF-B**: On Phase I, the BTF was running under acidic conditions and fed with 5 ppmv of equal mixtures of THMs (2.5 ppmv CF and 2.5 ppmv DCBM) and 25 ppmv of ethanol; the most dominant species at port 2 was *Fusarium sp.*, with relative abundance of 98.4% (Figure 7A). During Phase II, when the BTF was fed with more ethanol (50 ppmv), the amount of *Fusarium sp.* showed a slight increase to 98.5%. In Phase III, when the co-metabolite increased, *Fusarium sp.* dominates by 98.7%. In Phase IV, with higher ethanol (200 ppmv) concentration, the BTF was again dominated by *Fusarium sp.* by 98.6%. In this phase, a slight percentages of *F. oxysporum* and *F. solani* have been observed with a small abundance of 0.5% and 0.4%, respectively (Figure 7A).
**BTF-B**: In Phase I, the BTF was run with 5 ppmv of equal mixtures of CF and DCBM (2.5 ppmv each) without co-metabolite. The most dominant species were *F. oxysporum* and *Fusarium sp.*, with a relative abundance of 78.1% and 21.1%, respectively (Figure 7B). During Phase II, when the BTF was fed with increased mixtures at 10 ppmv, the amount of *F. oxysporum* increased to 84.2% and the amount for *Fusarium sp.* reduced to 14.4%. In this phase, *Fusarium sp.* reduced by 6.6% from the previous phase. In Phase III, when the influent concentration of the THMs mixture was increased to 15 ppmv, *F. oxysporum* dominated by 66.9% followed by *Fusarium sp.* of 30.5%. In this phase, the amount of *Fusarium sp.* increased by almost 16% as compared with Phase II. The amount of *F. oxysporum* was also at lowest abundancy than *Fusarium sp.* In addition, it was observed in Figure 7B that this phase has more *Fusarium sp.* than the other two previous phases. It is speculated that when the overall RE reduced, the dominant fungi (*F. oxysporum*) of BTF-B showed a significant reduction, which benefited the growth of *Fusarium sp.* Another reason may be that the higher concentration of THM's (15 ppmv) caused an adverse effect increasing the toxicity for *F. oxysporum* strain or it could be that *Fusarium sp.* thrived to survive more efficiently in higher THM environments relative to *F. oxysporum*. In comparison with BTF-A, it is interesting to observe that the most dominant fungi in BTF-B bed were *F. oxysporum*, whereas in BTF-A, it was *Fusarium sp.* Hence, it can be concluded that *F. oxysporum* was the dominant fungi for the degradation of CF and DCBM without co-metabolite, and *Fusarium sp.* was the abundant fungi with co-metabolite.

### 5 | CONCLUSION

Batch studies conducted to compare the effectiveness of the biosurfactant, Surfactin, to synthetic surfactant (Tomadol 25-7) indicated the superiority of Surfactin over Tomadol 25-7. The vials with Surfactin could eliminate more than 18% as compared with the vials with Tomadol. Besides, these vials produced 30% more carbon dioxide than the ones with Tomadol. The biofiltration study was then conducted to investigate the effect of co-metabolite, and Surfactin for the removal of CF and DCBM. The results from BTF-A revealed that the co-metabolite-to-THM ratio was a determining factor for the BTF performance; as the co-metabolite levels increased, the RE also increased.

In the absence of co-metabolite and presence of Surfactin (BTF-B), the performance of the BTF with respect to CF was similar to the performance of BTF with co-metabolite (BTF-A). However, the performance of the DCBM was slightly lower. The kinetics study also revealed that reaction rate constants for both CF and DCBM were similar in both BTFs for the LRs of 0.14 and 0.18 g/(m³·h), respectively. One should note that the BTF in presence of Surfactin (BTF-B) could sustain higher LRs of THMs as compared with the BTF with co-metabolite (BTF-A). Furthermore, the carbon recoveries for both BTFs were similar and the carbon loss could be attributed to a buildup of more biomass within each BTF. The use of Surfactin in the BTF system does not require the addition of co-metabolite and thus reduces the cost of treatment. Microbial analysis indicated that *Fusarium sp.* and *F. oxysporum* were the most dominant and abundant fungi responsible for the degradation of CF and DCBM.

In conclusion, industries, which emit VOCs with ethanol, can use biofiltration technology where ethanol will act as co-metabolite (BTF-A). However, industries that emit VOCs without the presence of ethanol can utilize biofiltration technology where the system was seeded by a biosurfactant such as Surfactin (BTF-B). Although this research does not include the combined effect of ethanol and Surfactin in the BTF, an experiment is underway to determine the combined outcome. Overall, biofiltration could provide more trust in the removal of THMs because treatment facilities prefer not installing more expensive options to ensure compliance with regulatory rules.

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