Occurrence and fate of endocrine disrupting antimicrobial triclocarban in municipal sludge during advanced anaerobic digestion using microwave pretreatment

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Abstract: Potential human and environment health impacts posed by antimicrobials and other emerging contaminants in wastewater sludge are creating challenges for the beneficial reuse or disposal of biosolids. Even though microwave (MW) irradiation has been studied extensively on sludge disintegration and performance of anaerobic digestion (AD) process, no previous studies have been conducted to investigate the effectiveness of MW pretreatment on the fate of the ubiquitous antimicrobial triclocarban (TCC) during AD. This study investigated the effect of MW pretreatment on the fate of the TCC that was already present in municipal sludge, during advanced AD under thermophilic and mesophilic conditions. The effects of final MW temperature and holding time configurations were studied employing by ten bench-scale ADs fed with mixed sludge at sludge retention times (SRTs) of 20, 12, and 6 days. TCC and six of its transformation products were analyzed in the total phase of each digesters' influent and effluent streams. Levels of TCC in the sludge significantly decreased (up to 64%) after MW pretreatment. Advanced AD by high-temperature MW pretreatment (160°C) accomplished an efficient reduction in the effluent TCC biosolids concentrations (up to 65% compared to the control digester). The non-chlorinated carbanilide (transformation product of TCC) was detected and quantified for the first time during conventional and MW-pretreated anaerobic sludge digestion. The formation of carbanilide in biosolids through reductive dechlorination could be an indicative of efficient and complete TCC transformation. This research demonstrated that AD using MW pretreatment could be used to reduce environmental concentrations of TCC in biosolids.

Keywords: wastewater sludge, anaerobic digestion, microwave, emerging contaminants, antimicrobials, transformation products.

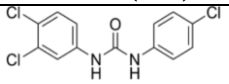
1. Introduction

Anaerobic digestion (AD) is commonly used as a sludge stabilization method due to the potential usage of methane-rich biogas. However, the slow and limited degradability of waste activated sludge (WAS) during the hydrolysis step (the rate-limiting) is the bottleneck of conventional AD with longer retention time requirements and lower organic degradation efficiencies. Pretreatment prior to AD can be used to solve existing limitations while resulting in smaller bioreactors. As an effective thermal pretreatment, microwave (MW) irradiation has become attractive with additional advantages (e.g. rapid heating, compactness, reduction in reaction times), in addition to higher improvements in pathogen destruction, digestate dewaterability, and methane production compared to mechanical pretreatments [1]. Agricultural use of biosolids is commonly accepted as an economical and environmentally responsible method of sludge disposal. However, there is a growing concern about the critical human and environment health threat of *emerging contaminants* (ECs) against sludge reuse for land application in addition to existing concerns about pathogens and heavy metals [2]. The elimination of a wide range of ECs is often insufficient in wastewater treatment plants (WWTPs) due to their design limitations. As an endocrine disrupting chemical, triclocarban (TCC) is a toxic, persistent, and bioaccumulative polychlorinated aromatic antimicrobial that is commonly used in many consumer and personal care products (e.g. bar soaps, toothpastes, cosmetics, detergents) in daily life [3]. Although TCC is first introduced to commerce in 1957, over the past two decades this compound has become popular worldwide as a powerful biocide [3]. This has been due to relaxed regulations, and widespread advertising and media reports emphasizing the potential for contact with harmful microorganisms in people's daily lives [3]. The aromatic nature and high chlorine content of TCC can cause not only resistance to biodegradation but also tendency for environmental persistence. Additionally, its limited water solubility along with significant lipophilicity can explain the potential for bioconcentration and bioaccumulation in the food web [4]. Selected physico-chemical properties of TCC are listed in Table 1. TCC has been frequently detected in different environmental matrixes as both parent and/or transformation products due to its partial elimination during water and wastewater treatment processes [5]. TCC has been selected as parent compound due to abundant existence in municipal treatment sludge, physico-chemical properties leading to *persistence, bioaccumulation and toxicity* to humans and ecosystems, and possible threats to the food chain through land application of biosolids [2, 3].

A comprehensive study by Venkatesan and Halden [6] reported that TCC was a high-production volume chemical, was ubiquitous in municipal biosolids (detected >1000 µg/kg dry weight (dw)) and determined it to be a priority chemical (one of the top 10 contaminants from the pool of 231 chemicals assayed in U.S. biosolids). TCC was the most abundant analyte with mean concentrations of 36,000 ± 8,000 ng/g dw (n = 5) in U.S. biosolids from the 2001 EPA national sewage sludge survey at 94 WWTPs [7]. Despite the excellent overall removal of TCC from the aqueous phase (97 ± 1%), a mass balance study conducted by Heidler et al. [8] for TCC at a WWTP

revealed that greater part of the TCC load remained during the treatment process and accumulated in dewatered sludge. AD operated at an sludge retention time (SRT) of 19 days did not promote TCC transformation, resulting in an accumulation of TCC in digested sludge with a mean concentration of 51,000 ± 15,000 ng/g dw [9]. In the study by Heidler and Halden [10], TCC was measured at a median concentration of 27,600 ng/g dw (between 4,700 - 63,000 ng/g dw) in anaerobically digested biosolids, and at comparatively lower levels in aerobically digested biosolids (between 16,400 - 19,600 ng/g dw) and undigested sludge (dewatering and/or lime treatment, between 21,600 - 43,200 ng/g dw), in 25 full-scale WWTPs (in 18 U.S. states).

Table 1: Selected physico-chemical properties of triclocarban

Property	**Triclocarban (TCC)**
Chemical structure	
CAS registration number	101-20-2
Molecular formula	$C_{13}H_9Cl_3N_2O$
Molecular weight (g/mol)	315.578
IUPAC name	3,4,4'-trichlorocarbanilide 1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)urea
Trade name	Solubacter
Use	Antiseptic and disinfectant
Chlorine content (weight %)	36.7[a]
Log K_{ow} (at 25°C, pH 7)	4.9[a]
Log K_{oc} (at 25°C, pH 7)	4.5[b]
pK_a	12.7[c]
Melting point (°C)	255-256[c]
Water solubility (mg/L at 25°C)	0.65 - 1.55[a]
Vapour pressure (mm Hg at 25°C)	3.61 x 10^{-9} [d]

Log K_{ow}: the logarithmic octanol-water partition coefficient, Log K_{oc}: organic carbon partition coefficient, pK_a: dissociation constant. [a]Halden and Paull [4], [b]Heidler and Halden [11], [c]Wu et al. [12], [d]Ying et al. [13].

The reductive dechlorination is known as a ubiquitous and important attenuation mechanism for the biotransformation of TCC into congeners of reduced chlorine content under anaerobic and reducing conditions, as previously mentioned in literature [8, 14-19]. In order for the core carbanilide structure to be available for biodegradation through detoxification reactions, higher chlorinated TCC and its manufacturing by-product 3,3',4,4'-tetrachlorocarbanilide (4-Cl-TCC) are transformed via dichlorocarbanilide (DCC) and monochlorocarbanilide (MCC) into carbanilide (NCC) by reductive dechlorination [15].

Although AD using MW pretreatment (i.e. advanced AD) has been examined extensively in terms of conventional performance parameters (i.e. organics removal, methane production) so far, to the best of our knowledge, *no study has investigated the effects of MW pretreatment on the fate of TCC in municipal/urban waste sludge streams.* To help to close this gap, the main objective of this study was to investigate how MW pretreatment along with AD can affect the environmental concentrations of TCC and its potential transformation products in municipal sludge. The concentrations of TCC and its six transformation products were analyzed in order to evaluate whether 1) TCC transformation occurs during MW sludge pretreatment itself, and 2) TCC removal (in terms of biotransformation and/or mineralization) takes place during advanced AD, and 3) the fate of TCC is dependent on MW pretreatment conditions, digester operating temperature and/or SRTs. Parallel to AD set-up/operation, an isotope dilution and ultra-high performance liquid chromatography/tandem mass spectrometry (UHPLC-MS/MS) based method was also developed for the simultaneous detection and quantification of TCC and six of its transformation products in the total phase of the digesters' influent (mixed sludge) and effluent (biosolids) streams.

2. Materials and Methods

2.1. Waste sludge collection

Waste sludge was collected bi-weekly from the Westside Regional Wastewater Treatment Plant (WRWTP) near West Kelowna, BC, Canada. This facility operates a three-phase modified Bardenpho process in sequential anaerobic, anoxic, and aerobic zones to remove carbon, nitrogen and phosphorus. WAS is settled in secondary clarifiers and thickened by dissolved air floatation to produce thickened WAS (TWAS). Primary sludge (PS) is thickened by gravity and is pumped into a fermenter where sludge becomes fermented primary sludge (FPS).

2.2. Experimental design and procedure

2.2.1. Microwave pretreatment and mixed sludge characteristics

A programmable bench-scale MW digestion system [ETHOS-EZ, Milestone Inc.] operating at 2,450 MHz with maximum power, temperature, and pressure of 1,200 W, 300°C, and 35 bar, respectively, was used. The unit has 12 Teflon pressure-sealed vessels (100 mL capacity each) rotating on a carousel and is equipped with ATC-400-CE temperature probe for monitoring the reference vessel temperature during its operation. Similar to common usage of patented pretreatment technologies in full-scale AD operations [20], MW pretreatment was applied to

TWAS only and then TWAS was fed to digesters after being mixed with un-pretreated (raw) FPS. In order to reduce pretreatment energy consumption per sludge dry weight, the solids content of TWAS samples was increased before MW irradiation from 4.0 ± 0.6 to 10.5 ± 0.5% TS (w/w) by centrifugation after the addition of a cationic polymer (Zetag® 7553, BASF Canada Inc.) solution (0.5% w/w). Dewatered WAS samples were irradiated to final MW temperatures of 80°C (low) and 160°C (high) at a constant heating ramp rate (2.25°C/min), then held for 1 min (fast) or 30 min (slow) to evaluate the effect of both MW temperature and holding time on target compounds in sludge/biosolids. Then, the dewatered WAS samples were diluted by centrate collected during centrifugation and mixed with un-pretreated (raw) FPS before being fed to the digesters (FPS:TWAS = 33:67% v/v). Five different mixed sludge samples were prepared for feeding the digesters: "Control" (a mixture of raw FPS and un-pretreated TWAS) and four MW-pretreated samples (a mixture of raw FPS and MW-pretreated TWAS) at different combinations. The basic characteristics of the mixed sludge samples are given in Table 2.

Table 2: Characterization of mixed sludge feed samples for semi-continuous flow anaerobic digesters[a]

Parameters	Feed				
	Un-pretreated	MW-pretreated			
	Control Mixed sludge	**MW 1 80°C-30min**	**MW 2 160°C-30min**	**MW 3 80°C-1min**	**MW 4 160°C-1min**
pH	5.63 ± 0.13[b]	5.64 ± 0.05	5.52 ± 0.20	5.65 ± 0.28	5.88 ± 0.06
TS (% by wt.)	3.94 ± 0.24	3.40 ± 0.35	3.37 ± 0.25	3.36 ± 0.31	3.54 ± 0.29
VS (% by wt.)	3.38 ± 0.20	2.90 ± 0.33	2.87 ± 0.24	2.89 ± 0.27	3.03 ± 0.26
[c]**TCOD** (mg/L/%VS by wt.)	15,505 ± 1,326	15,448 ± 980	14,732 ± 1,417	14,948 ± 2,030	14,962 ± 1,365
[c]**SCOD** (mg/L/%VS by wt.)	2,107 ± 444	2,917 ± 349	4,028 ± 688	2,653 ± 585	3,369 ± 347

[a]FPS: fermented primary sludge, TWAS: thickened waste activated sludge, MW: microwave, TS: total solids, VS: volatile solids, TCOD and SCOD: total and soluble chemical oxygen demand. Because centrifugation, pretreatment and resuspension of TWAS created slight differences in TS & VS concentrations of feed sludge for pretreated compared to control digesters, some of the parameters were reported after normalization based on the VS content (% w/w) in the samples. [b]Arithmetic mean ± standard deviation (from minimum 5 data points). [c]Samples were analyzed each time in duplicate.

2.2.2. Bench-scale anaerobic digester set-up

Side-armed Erlenmeyer flasks were used as semi-continuous flow ADs (fed once a day through the side-arm of the flask, 7 days/week) with total and liquid volumes of 2 and 1 L, respectively. The flasks were sealed with two-hole rubber stoppers for collection of digested sludge and biogas in Tedlar® bags. The collected volume of biogas was measured by a water-filled manometer daily. Mesophilic (MH) and thermophilic (TH) inocula were taken from existing bench-scale digesters which had been utilizing mixed sludge from a similar facility for more than one year. In order to expose the inocula to the MW-pretreated sludge, a total number of 10 bench-scale digesters were set-up with the acclimated inocula and run at TH (55 ± 1°C) and MH (35 ± 1°C) conditions in two temperature controlled reciprocal shakers (New Brunswick). Fig. 1 presents the schematic flow diagram of the experimental methodology for the ADs under different MW pretreatment scenarios.

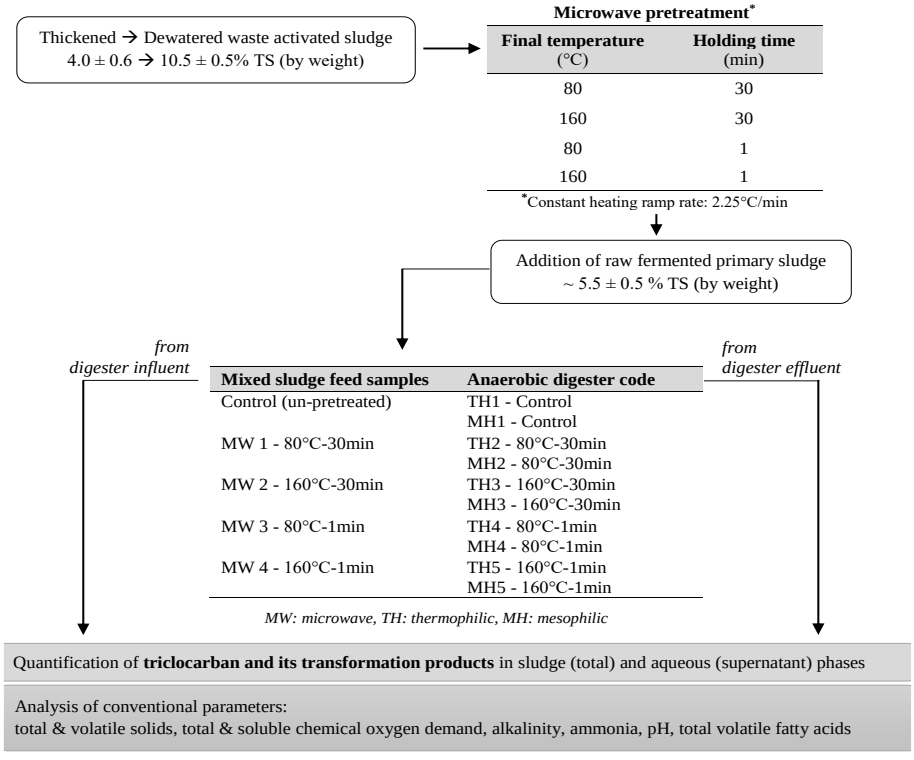


Fig. 1: Experimental methodology for anaerobic digesters under different microwave pretreatment scenarios

2.3. Analytical methods

Multiple parameters were measured from each digester's influent and effluent during each SRT at steady-state conditions for the quantification of the target compounds (analytes: parent compound (TCC) and its transformation products) and characterization of digester performance.

2.3.1. Quantification of TCC and its transformation products in sludge

TCC and its six transformation products [DCC, MCC, NCC, 4-Cl-TCC, monochloroanilines (sum of 3-chloroaniline and 4-chloroaniline), and 4-chlorocatechol] were quantified in total (sorbed + aqueous) phase of the digester influent and effluent streams. Three or four independent batches of samples were collected for the analysis of target analytes at each SRT during steady-state conditions and stored at -20°C until extraction and analysis.

Chemicals: TCC (101-20-2, ≥ 98.5% purity) was obtained from Sigma Aldrich (Oakville, Ontario) while isotopically labelled TCC (TCC-d_4) (1219799-29-7, > 99% atom deuterated) was obtained from C/D/N Isotopes Inc. (Pointe-Claire, Quebec). Its transformation products including MCC (2008-71-1), NCC (102-07-8, ≥ 97.5%), 4-Cl-TCC (4300-43-0), monochloroanilines (108-42-9, ≥ 99%), and 4-chlorocatechol (2138-22-9, ≥ 97%) were also purchased from Sigma-Aldrich while DCC (1219-99-4, ≥ 98%) was obtained from Oakwood Chemical (Estill, South Carolina). Stock solutions of native compounds were prepared in LC-MS grade methanol (MeOH) from Fisher Scientific (Ottawa, Ontario) and stored at -20°C in amber glass vials.

Sample preparation: Total phase samples were prepared and extracted according to the acid fraction procedure of the U.S. EPA Method 1694 with some modifications [21]. A flow chart that summarizes procedures for sample preparation, cleanup, and analysis of target analytes in sludge, is shown in Fig. 2. Peat moss was used as a reference method blank (0.25 ± 0.02 g dw), and was extracted and analyzed with each sample batch using the exact same procedure as samples to verify freedom from contamination.

Instruments: An UHPLC-MS/MS was used for the detection and quantification of all target analytes. A Waters™ ACQUITY UHPLC system was connected to a Waters™ Xevo TQD triple quadrupole MS equipped with an electrospray ionization (ESI) probe. Analytes were separated using a Waters™ Ethylene Bridged Hybrid C18 (2.1 x 50 mm, 1.7 µm) column and matching guard column. For UHPLC optimization, UHPLC aqueous mobile phases were either ultra-pure UHPLC grade water or ultrapure UHPLC grade water with either 5 mM of UHPLC grade ammonium acetate, or 10 mM ammonium bicarbonate. The organic phase for all methods were LC-MS grade MeOH. The MS/MS optimization was run for both ESI- and ESI+ modes for each analyte. Multiple reaction monitoring mode (MRM) was used for quantitative analysis that enables improved sensitivity and selectivity. Retention times of analytes were within ±15 seconds of a native compound as per U.S. EPA Method 1694. A summary of the optimized MS/MS parameters for each compound are listed in Table 3.

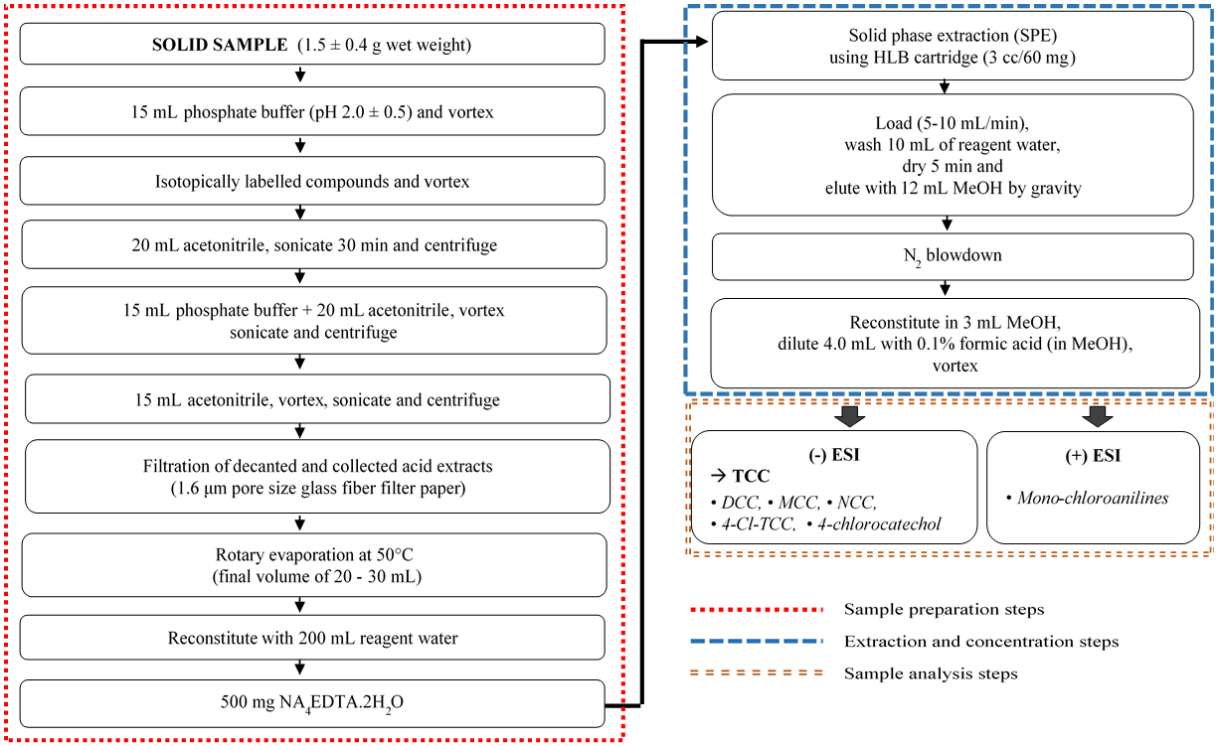


Fig. 2: Flow chart for determination of triclocarban and its transformation products in sludge (adapted from USEPA [21])

Table 3: Instrumental analysis parameters

Compound	Ion Source	Parent ion (m/z)	Cone potential (V)	Fragment ion (m/z)	Collision Energy (V)	Capillary voltage (kV)	
						[a]*ESI-*	[b]*ESI+*
Triclocarban (TCC)	ESI-	312.9	22, 31	125.9	22	0.6	0.6
Transformation products:							
Dichlorocarbanilide (DCC)	ESI-	278.9	26	126.0	12	0.6	0.6
Monochlorocarbanilide (MCC)	ESI-	245.0	24	125.9	10	0.6	0.6
Carbanilide (NCC)	ESI-	211.0	28	92.0	10	0.6	0.6
3,3',4,4'- tetrachlorocarbanilide (4-Cl-TCC)	ESI-	348.8	40	159.5	36	2.5	2
Monochloroanilines	ESI+	127.9	32	93.0	16	1.5	4
4-chlorocatechol	ESI-	143.0	33	107.0	17	1.5	4
Triclocarban-d_4 (TCC-d_4)	ESI-	317.0	44	129.9834 159.9541	24, 14	Varies	Varies

[a]negative ionization mode, [b]positive ionization mode.

Method validation: All compounds were quantified using a multi-point calibration curve prepared from native compounds (minimum of 8 and maximum of 12 calibration points), while isotope dilution was employed to account for analyte losses during sample preparation, extraction and instrumental error during analysis. Good linearity was achieved with a coefficient of determination (R^2) value equal or higher than 0.98 for all the compounds. The instrument limit of detection (LOD) and limit of quantification (LOQ) were determined using peak signal to noise ratio of 3 and 10 or greater in native standards or 9 in samples, respectively. Recoveries for most compounds were within the EPA recommended range of 70 - 130% with some exceptions [21]. Compound-specific LODs, LOQs, reporting limits (RLs), recoveries and are given in Table 4. Repeated and intra-day injections of identical samples had relative standard deviations ranging from 3.1 to 9.6%.

Table 4: Validation of developed method

Compound	**LOD**[a] (ppb)	**LOQ**[b] (ppb)	**RL**[c] (ppb)	**Recovery** (%)
Triclocarban (TCC)	0.08	0.24	0.54	112.4
Transformation products:				
Dichlorocarbanilide (DCC)	0.08	0.25	0.52	118.9
Monochlorocarbanilide (MCC)	0.10	0.25	0.55	258.3
Carbanilide (NCC)	0.16	0.55	0.72	136.8
3,3',4,4'-tetrachlorocarbanilide (4-Cl-TCC)	0.70	0.95	1.43	n/a
Monochloroanilines	0.12	0.45	0.61	91.55
4-chlorocatechol	0.87	0.99	1.16	163.49

[a]LOD: limit of detection, [b]LOQ: limit of quantification, [c]RL: reporting limit.

2.3.2. Characterization of conventional parameters

Measurements of total and volatile solids (TS and VS), alkalinity and ammonia were performed as defined in Standard Methods [22] procedures 2540 B, 2540 E, 2320B and 4500-NH_3 D, respectively. Colorimetric total and soluble (< 0.45 μm) chemical oxygen demand (COD) measurements were conducted according to Standard Methods [22] procedure 5250 D and measured at a 600 nm wavelength. Total volatile fatty acids (VFAs: summation of acetic, propionic and butyric acids, < 0.22 μm) and biogas composition were analyzed using an Agilent 7890A Gas Chromatograph (GC) with flame ionization detector [23] and an Agilent 7820A GC with a thermal conductivity detector [24], respectively.

2.3.3. Statistical analysis

The experimental data were analyzed using *Minitab™ 17 statistical software*. Tests for statistically significant effects of the independent variables were performed by an analysis of variance (ANOVA) using either a One-Way ANOVA or a General Linear Model as linear multiple regression considering a 95% confidence level ($\alpha = 0.05$).

3. Results and Discussion

The operation of 10 digesters was first started at a conventional AD SRT of 20 days under the corresponding organic loading rate (OLR) of 1.45 ± 0.13 g VS/L/d and continued for a period of 67 days after the steady-state conditions (less than ± 10% variation in daily biogas production, biogas composition, TS and VS concentrations for each digester) were established during approximately first 30 days. Afterwards, the SRT of each digester was reduced to 12 days and the operation was sustained for a duration of 49 days under OLR of 2.50 ± 0.21 g VS/L/d at steady-state. Finally, the lowest SRT of 6 days was applied and maintained during 32 days after reaching steady-state under OLR of 5.18 ± 0.31 g VS/L/d. Summary of the steady-state performance parameter results of anaerobic digesters at the SRTs of 20, 12, and 6 days are listed in Table 5.

Table 5: The steady-state performance of anaerobic digesters at the SRTs of 20, 12, and 6 days[a]

	Thermophilic					Mesophilic				
Parameters	TH1 Control	TH2 80°C-30min	TH3 160°C-30min	TH4 80°C-1min	TH5 160°C-1min	MH1 Control	MH2 80°C-30min	MH3 160°C-30min	MH4 80°C-1min	MH5 160°C-1min
					SRT = 20 days					
VS removal efficiency (%)	52 (2.0;16)	54 (3.8;16)	56 (5.0;16)	53 (4.0;16)	54 (5.8;16)	47 (1.8;16)	51 (4.4;16)	56 (2.9;16)	49 (3.4;16)	52 (7.2;16)
[c]Daily specific methane yield (mL CH_4/g VS_{fed}/d)	372 (22;67)	391 (19;67)	390 (22;67)	396 (29;67)	393 (26;67)	366 (26;67)	387 (17;67)	386 (17;67)	382 (22;67)	409 (22;67)
[d]Alkalinity (mg/L/% VS by wt. as $CaCO_3$)	3,412 (145;5)	3,455 (258;5)	3,323 (215;5)	3,111 (74;5)	3,332 (486;5)	2,373 (92;5)	2,583 (262;5)	3,125 (220;5)	2,371 (231;5)	2,647 (565;5)
[d]Ammonia (mg N/L/% VS by wt.)	1,050 (120;5)	1,084 (99;5)	1,119 (64;5)	1,001 (89;5)	1,127 (109;5)	791 (106;5)	877 (93;5)	991 (99;5)	824 (96;5)	1,044 (163;5)
[d,e]SCOD (mg/L/% VS by wt.)	1,578 (158;9)	1,627 (234;9)	2,377 (220;9)	1,706 (303;9)	1,783 (149;9)	300 (24;9)	408 (230;9)	959 (62;9)	300 (29;9)	592 (85;9)
					SRT = 12 days					
VS removal efficiency (%)	53 (1.8;14)	53 (4.0;14)	53 (2.7;14)	51 (1.0;14)	53 (3.8;14)	49 (1.5;14)	48 (4.8;14)	51 (2.6;14)	45 (2.8;14)	52 (3.7;14)
[c]Daily specific methane yield (mL CH_4/g VS_{fed}/d)	343 (21;49)	373 (22;49)	367 (17;49)	365 (13;49)	379 (27;49)	331 (17;49)	361 (21;49)	376 (30;49)	340 (19;49)	371 (20;49)
[d]Alkalinity (mg/L/% VS by wt. as $CaCO_3$)	3,265 (41;3)	3,159 (78;3)	3,184 (264;3)	3,081 (46;3)	3,205 (246;3)	2,329 (124;3)	2,409 (79;3)	2,814 (176;3)	2,320 (79;3)	2,730 (327;3)
[d]Ammonia (mg N/L/% VS by wt.)	897 (44;3)	888 (67;3)	996 (114;3)	917 (86;3)	1,014 (107;3)	686 (68;3)	742 (73;3)	936 (67;3)	780 (12;3)	907 (77;3)
[d,e]SCOD (mg/L/% VS by wt.)	1,469 (195;7)	1,530 (295;7)	2,633 (453;7)	1,438 (240;7)	2,047 (333;7)	312 (68;7)	533 (129;7)	1,224 (320;7)	484 (156;7)	672 (173;7)
					SRT = 6 days					
VS removal efficiency (%)	52 (3.0;9)	52 (2.4;9)	53 (3.8;9)	52 (2.7;9)	55 (2.5;9)	45 (3.6;9)	47 (1.8;9)	53 (2.4;9)	46 (3.7;9)	51 (3.5;9)
[c]Daily specific methane yield (mL CH_4/g VS_{fed}/d)	319 (17;32)	317 (16;32)	318 (17;32)	378 (13;32)	384 (20;32)	346 (18;32)	369 (16;32)	384 (25;32)	354 (24;32)	368 (16;32)
[d]Alkalinity (mg/L/% VS by wt. as $CaCO_3$)	2,888 (109;3)	2,440 (192;3)	2,589 (392;3)	2,439 (243;3)	2,665 (353;3)	1,990 (164;3)	1,850 (84;3)	2,220 (275;3)	1,862 (111;3)	2,117 (130;3)
[d]Ammonia (mg N/L/% VS by wt.)	781 (32;3)	709 (107;3)	798 (158;3)	739 (114;3)	845 (145.;3)	538 (2.3;3)	504 (44;3)	636 (85;3)	532 (56;3)	632 (72;3)
[d,e]SCOD (mg/L/% VS by wt.)	2,474 (263;5)	2,906 (175;5)	4,535 (687;5)	2,421 (585;5)	3,373 (547;5)	515 (74;5)	488 (50;5)	1,452 (179;5)	508 (10.1;5)	1,108 (122;5)

[a]SRT: sludge retention time, TH: thermophilic, MH: mesophilic, VS: volatile solids, SCOD: soluble chemical oxygen demand. [b]Data represent arithmetic mean of measurements (standard deviation; number of data points). [c]at standard temperature and pressure (0°C, 1 atm). [d]Because centrifugation, pretreatment and resuspension of TWAS created slight differences in TS & VS concentrations of feed sludge for pretreated compared to control digesters, some of the parameters were reported after normalization based on the VS content (% w/w) in the samples. [e]Samples were analyzed each time in duplicate.

3.1. Environmental occurrence of TCC and its transformation products in mixed sludge

The concentrations of TCC in the mixed sludge were in the range of 300 - 1,800 ng/g dw with an average of 1,030 ± 470 ng/g dw, as shown in Fig. 3. These concentration values are similar with previous results conducted in Canada [25, 26]. For instance, in the Saskatoon WWTP (Saskatchewan), the concentrations of TCC in the mixed sludge samples (detection frequency: 3 out of 3) ranged from 1,550 to 1,760 ng/g dw (the median: 1,680 ng/g dw) [25]. Guerra et al. [26] also reported that the concentration ranges for TCC in six samples (detection frequency: 6 out of 6) collected from two Canadian WWTPs were between 1,800 - 5,200 ng/g dw in the PS and 1,100 - 2,900 ng/g dw in the TWAS. According to study by Narumiya et al. [27], among the 47 pharmaceutical and personal care products (PPCPs), TCC was one of the highest concentrations (in the range of 1,200 - 1,940 ng/g dw) in the solid phase of thickened sludge collected from target treatment plants in Japan.

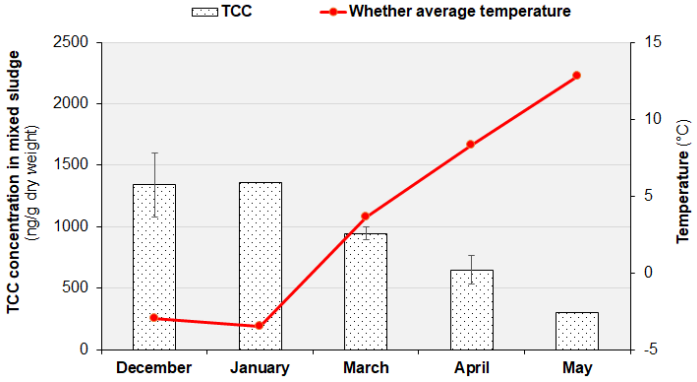


Fig. 3: Seasonal fluctuation in occurrence of triclocarban in different sample collection periods together with weather averages

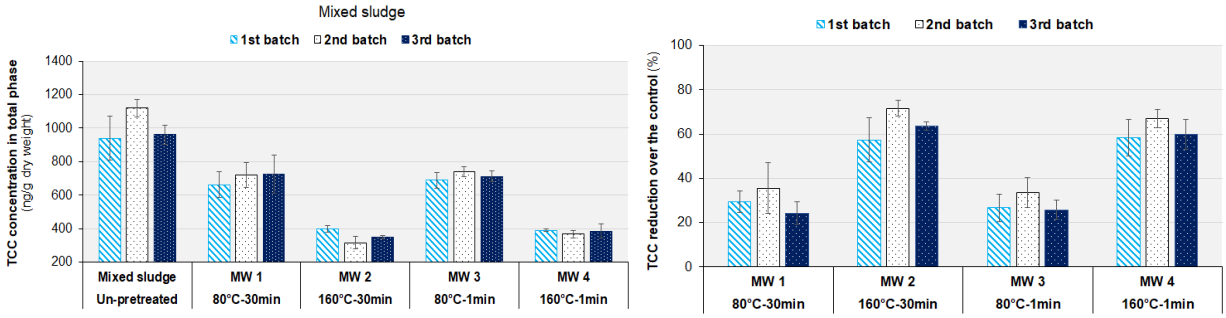
As seen in Fig. 3, higher TCC concentrations were observed in the samples collected from WRWTP in December and January (as colder temperatures) in comparison to those collected in warmer temperatures (p-value = 0.172 > 0.05). The fluctuation in TCC concentrations of sludge samples collected could be attributed to seasonal differences. TCC removals in biological nutrient removal process (production of older sludge caused by longer SRT) at WRWTPs could be higher during warm temperatures compared with cold temperatures, most probably influencing the levels of TCC in sludge. The higher removal (as biotransformation/mineralization) of TCC at warmer temperatures could be explained by enhanced microbial activity at higher temperatures [28-30]. In the study conducted by Guerra et al. [26], the concentration ranges of TCC analyzed in nine WAS samples (with 100% of detection) collected from three Canadian WWTPs (using extended aeration treatment) was found as 1,100 - 3,700 ng/g dw in cold temperatures and 870 - 4,200 ng/g dw in warm temperatures (p-value > 0.05). The mass balance study by Lozano et al. [31] indicated that the concentrations of TCC did not change through conventional activated sludge process, but around 18% TCC decrease was observed through the nitrification & denitrification processes. According to authors, this might have been caused by higher degradation rates during nitrification-denitrification (with longer hydraulic retention times and SRTs) than the secondary treatment.

Considering reductive dechlorination mechanism, TCC's higher chlorinated congener 4-Cl-TCC and its lesser monochlorinated congener MCC were not detected (below the LOD) in the mixed sludge samples. Additionally, its toxic transformation products monochloroanilines and the microbial by-product 4-chlorocatechol were not detected in this study. On the other hand, its lesser di- and non-chlorinated congeners DCC and NCC were detected in the mixed sludge samples, but their concentrations were below the LOQ.

3.2. Effect of MW pretreatment on fate of TCC in mixed sludge

The impact of MW pretreatment on concentrations of TCC and its transformation products in the total phase of un-pretreated (mixed sludge) and MW-pretreated mixed sludge were studied with three independent batches of samples. An average concentration of TCC in the un-pretreated mixed sludge was found as 1,010 ± 100 ng/g dw, as seen in Fig. 4a. TCC concentrations reduced significantly (p-value < 0.05) when different aforementioned combinations of MW pretreatment were applied to TWAS samples (and then were mixed with raw FPS). Similar TCC concentrations (705 ± 35 and 715 ± 30 ng/g dw, p-value = 0.145 > 0.05) were obtained from "MW1-80°C-30min" and "MW3-80°C-1min" scenarios, respectively (Fig. 4a). In addition to reduction of TCC concentrations during pretreatment at 80°C compared to mixed sludge, average TCC levels also further decreased to 370 ± 30 ng/g dw when the samples were irradiated at 160°C for 30 and 1 min (p-value = 0.297 > 0.05). Pretreatment scenario couples of "MW1 vs. MW3" and "MW2 vs. MW4" accomplished average TCC reductions (over the mixed sludge) of "30 ± 4 vs. 29 ± 6%" and "64 ± 5 vs. 62 ± 6%", respectively (Fig. 4b). According to the results, the final MW temperature appeared to have a decisive factor in TCC concentrations' reduction (over mixed sludge), compared to holding time (p-value = 0.000 < 0.05). After MW pretreatment process, 4-Cl-TCC, MCC, monochloroanilines, and 4-chlorocatechol were not detected in mixed sludge while DCC and NCC were detected but not quantified, as in the un-pretreated mixed sludge.

When compared to other studies, the study of Armstrong et al. [32] also found significant reduction of TCC concentrations from dewatered mixed sludge during *Cambi Thermal Hydrolysis Process*™ at 150 - 180°C for 30 min (0.37 - 0.95 MPa). Average concentrations of TCC before thermal hydrolysis ranged from 6,816 to 7,368 ng/g dw whereas they ranged from 67.5 to 89.9 ng/g dw after treatment. Ross et al. [33] also investigated the impact of temperature and time on the removal of TCC from wastewater biosolids in batch thermal processing/pyrolysis experiments. In the study, concentration of TCC decreased from 4,000 to 2,000 ng/g dw at 100°C, while TCC was removed at 200°C (to below the LOQ: 100 ng/g dw). At 500°C, TCC was removed (<100 ng/g dw) from biochar after pyrolysis reaction time of 2.5 min. Based on the data obtained from this study and other limited information in the literature, further research is required to determine which mechanism is responsible for the significant reduction of TCC during pretreatment at high temperatures and to accomplish a more comprehensive evaluation of all potential transformation products of TCC which can be produced during MW pretreatment.



(a) **(b)**

Fig. 4: Effect of microwave pretreatment on (a) triclocarban concentrations in the total phase of mixed sludge, and (b) reduction of triclocarban concentrations in pretreated over the un-pretreated mixed sludge

3.3. Fate of TCC and its transformation products during conventional anaerobic digestion

During conventional AD (control: without MW pretreatment), TCC was detected in the total phase of digested sludge (biosolids) at an average concentration of 1,765 ± 105 and 1,770 ± 175 ng/g dw under TH and MH conditions, respectively at an SRT of 20 days (Table 6). At the following SRT of 12 days, similar TCC concentration values were also obtained from both "TH1-Control" and "MH1-Control" digesters (p-value = 0.901 > 0.05). When the SRT was reduced to 6 days, the concentrations of TCC was slightly increased in both TH and MH digestates (p-value = 0.981 > 0.05). Based on these results, the digester SRT did not have a statistically significant effect on TCC concentrations in the conventional AD operated at either digester operating temperature (p-value = 0.430 > 0.05). In spite of the fluctuations in TCC concentrations observed in the feed sludge samples during the different sample collection periods, TCC concentrations measured in AD biosolids did not change depending on the seasonal temperature change observed in the different SRTs.

Table 6: Environmental triclocarban (TCC) and carbanilide (NCC) concentrations in the anaerobic digesters' effluents at steady-state conditions[a]

		Concentration (ng/g dry weight)	
Compound	**SRT**	TH1 - Control	MH1 - Control
TCC	20 days	1765 ± 105 (4)[b]	1770 ± 175 (4)
	12 days	1795 ± 200 (4)	1780 ± 100 (4)
	6 days	1900 ± 100 (3)	1900 ± 175 (3)
NCC	20 days	505 ± 55 (4)	-[c]
	12 days	395 ± 25 (4)	-[c]
	6 days	255 ± 15 (3)	-[c]

[a]SRT: sludge retention time, TH: thermophilic, MH: mesophilic. [b]arithmetic mean ± standard deviation (number of data points). [c]below the quantification limit.

The TCC concentrations in biosolids analysed were similar to results of previous studies [25, 30]. For example, TCC was found in all 31 treated sludge samples and biosolids (100% occurrence) collected from the different region of Canada with the median concentrations of 1,930 ng/g dw [25]. Specifically, median concentrations of TCC in the anaerobically digested (MH temperature) biosolids ranged from 1,850 to 3,130 ng/g dw (the median: 1,930 ng/g dw) in the Saskatoon WWTP (Saskatchewan). In the study by Guerra et al. [30], TCC concentrations in biosolids which were obtained from four WWTPs in Canada, were between 1,200 and 8,900 ng/g dw (n = 24) with a median of 2,900 ng/g dw.

Regarding the lower chlorinated TCC analogs, the concentration of DCC was below the LOQ while the concentration of MCC was below the LOD, in the control digesters' effluents. The non-chlorinated NCC detected and quantified in the total phase of digested control sludge samples collected from TH digesters at different SRTs whereas NCC was detected but not quantified under MH temperature. At an SRT of 20 days, NCC was measured at an average concentrations of 505 ± 55 ng/g dw in the digester of "TH1-Control", as shown in Table 6. The biosolids concentration values of NCC decreased when the SRT was reduced to 12 and 6 days. The statistically significant effect of digester SRT on concentrations of NCC was confirmed by One-Way ANOVA (*p*-value = 0.001< 0.05). It was underlined by earlier studies that a long SRT in the biological systems increase the adaptation of different kinds of microorganisms and also the enrichment of some specific populations which can excrete enzymes able to degrade some types of organic compounds [28, 34]. This may be a result of the biotransformation of parent compound TCC into NCC at longer SRTs. However, previous studies literature could not detect NCC in biosolids collected from conventional digesters even though the higher chlorinated derivatives of TCC have been detected and quantified in biosolids [10, 15]. Thus, this research may be the first study which assesses the environmental concentrations of NCC in biosolids during conventional AD operated at TH temperature at different SRTs. The study by Pycke et al. [15] was the first documentation that TCC dechlorination was occurred significantly in the sewage system and/or WWTPs. According to their study, TCC-dechlorination products DCC and MCC were detected in raw and digested sludge collected from 14 different WWTPs across the U.S. As a strong linear correlation was found between DCC and MCC levels (Pearson's r = 0.99), it was concluded that if the transformation of TCC to DCC (as the first step of TCC dechlorination) took place, the conversion of DCC into MCC (as second step) occurred equally. However, because the non-chlorinated NCC was not detected in any biosolids sample, it remains unclear whether TCC dechlorination was indeed slow and incomplete (with no NCC formation), or whether NCC was readily degraded and thus complete dechlorination of TCC may have occurred during wastewater treatment. Considering previous studies in literature [14-19], it can be concluded that the higher chlorinated TCC was able to be converted into non-chlorinated NCC through complete reductive dechlorination mechanism (TCC → DCC → MCC → NCC) during conventional AD in this study. On the other hand, 4-Cl-TCC, monochloroanilines, and 4-chlorocatechol were not detected in control digestate samples.

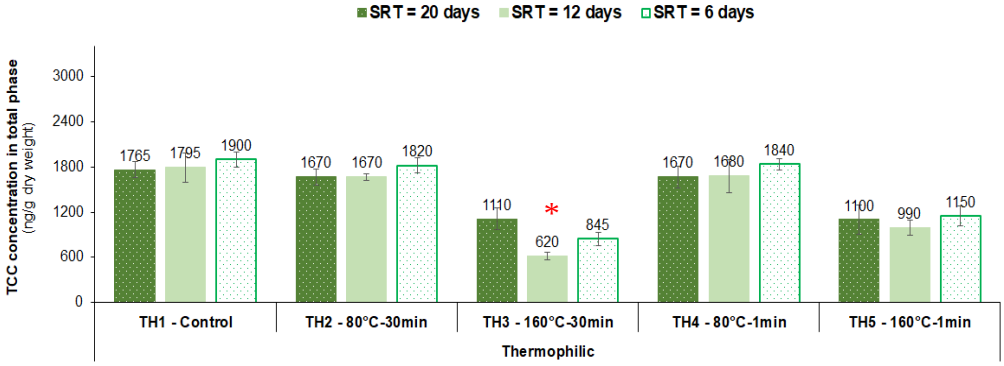
In the study by Heidler and Halden [10], DCC and 4-Cl-TCC was detected in digested sludge for the first time. The median concentration of DCC was found as 1,500 ng/g dw in anaerobically digested biosolids, whereas 470 and 420 ng/g dw in aerobically digested and undigested sludge, respectively. Conversely, 4-Cl-TCC was found in anaerobically digested sludge at a median value of 1,600 ng/g dw, whereas 2,500 and 3,000 ng/g dw in aerobically digested and undigested sludge, respectively [10]. Another study by Pycke et al. [15] investigated the concentration fluctuations of TCC and its transformation products, human metabolites, manufacturing by-products in anaerobically digested sludge samples for 12 months from a single WWTP (n = 16) in the U.S. According to the results obtained, the concentration changes were minimal and rarely differed significantly (α = 0.01) for TCC (7 ± 5%), human metabolites 2'-hydroxytriclocarban and 3'-hydroxytriclocarban (12 ± 11 and 31 ± 31%, respectively), and the manufacturing by-product 4-Cl-TCC (13 ± 12%). However, the differences in concentrations during a 12-month period became more prominent for the microbial products DCC and MCC, with changes ranging from 4 to 53% and from 12 to 180%, respectively.

3.4. Effect of MW pretreatment on fate of TCC and its transformation products during advanced anaerobic digestion

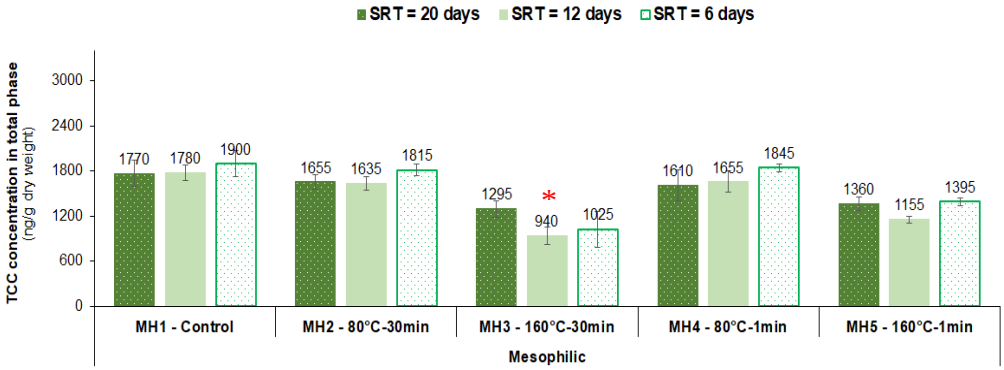
The combined effect of anaerobic sludge digestion with MW pretreatment (i.e. advanced AD) on TCC concentrations in the digested sludge were investigated in the effluents of MW-pretreated digesters. The advanced digesters, which were fed with MW-pretreated sludge at 80°C for different holding times (30 and 1 min), under both TH and MH temperatures had similar TCC concentrations in their effluents at SRTs of 20 and 12 days (*p*-value = 0.959 > 0.05). For instance, the average concentrations of TCC in total phase of the advanced digester couples of "TH2-80°C-30min & TH4-80°C-1min" and "MH2-80°C-30min & MH4-80°C-1min" were "1,670 ± 105 & 1,670 ± 145 ng/g dw" in TH temperature and "1,655 ± 100 & 1,610 ± 200 ng/g dw" in MH temperature, respectively, at an SRT of 20 days (Fig. 5). When the SRT was reduced to 6 days, TCC concentrations uniformly increased in both TH and MH conditions (*p*-value = 0. 985 > 0.05). These advanced ADs displayed a limited reduction in concentrations of TCC (<10%) compared to the respective control digesters during the operations of three SRTs (Fig. 5). These findings demonstrated that MW holding time did not have a statistically significant effect (*p*-value = 0.872 > 0.05) on the total phase of TCC concentrations at the same MW temperature of 80°C.

The level of reduction in TCC concentrations at advanced digesters, which were fed with high-temperature MW-pretreated mixed sludge (160°C), were considerably higher compared to that of the digesters pretreated at 80°C, under both TH and MH temperature. As seen in Fig. 5a, the digester of "TH3-160°C-30min" had an average TCC concentration of 1,100 ± 145 ng/g dw in its effluent whereas the digester of "TH2-80°C-30min" had 1,670 ± 105 ng/g dw at an SRT of 20 days (*p*-value = 0.003 < 0.05). At the same SRT, the AD couples of "TH3-160°C-30min & TH5-160°C-1min" and "MH3-160°C-30min & MH5-160°C-1min" had similar TCC concentrations in their biosolids (*p*-value = 0.960 > 0.05 for TH temperature and *p*-value = 0.867 > 0.05 for MH temperature). At the following SRT of 12 days, the lowest TCC concentration (620 ± 50 ng/g dw) was obtained from the digester

of "TH3-160°C-30min" operated at TH temperature, resulted in the highest TCC reduction (65%) compared to the respective control (1,795 ± 200 ng/g dw) (Fig. 5a). Similarly, under MH conditions, an average TCC concentration reduced to 940 ± 115 ng/g dw in the digester's effluent of "MH3-160°C-30min", with the TCC reduction of 47% compared to control digester (1,780 ± 200 ng/g dw), as shown in Fig. 5b. When the digester operating temperatures were compared, the statistical analysis confirmed that the TCC concentrations in the digester of "TH3-160°C-30min" was significantly lower than that of in the digester of "MH3-160°C-30min" (p-value = 0.006 < 0.05). It can be clearly seen that the effect of MW holding time on TCC concentrations in biosolids had a more pronounced at digesters pretreated at 160°C while operated at SRTs of 12 days (p-value = 0.009 < 0.05) and 6 days (p-value = 0.016 < 0.05).



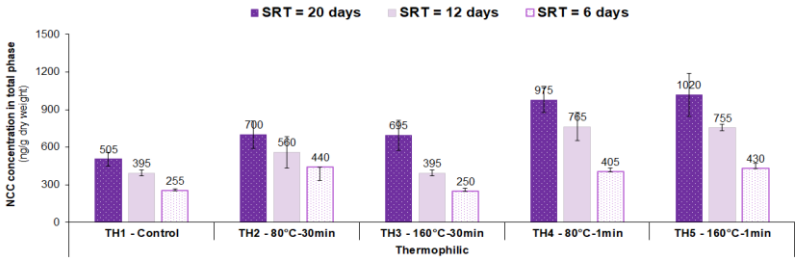
(a)



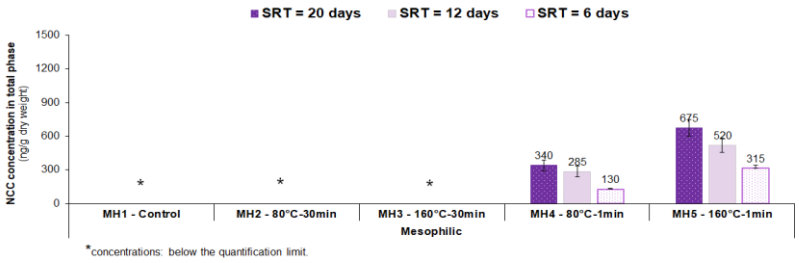
(b)

Fig. 5: Average concentrations of triclocarban in the total phase of (a) thermophilic and (b) mesophilic digesters' effluents during steady-state at solid retention times of 20, 12, and 6 days
(*the best scenario representing the combined effect of anaerobic digestion with MW pretreatment)

Although the DCC and MCC were not quantified in MW-pretreated digested sludge samples, non-chlorinated NCC was detected and quantified in the total phases of TH and MH advanced digesters' effluents at different SRTs. This study is the first to demonstrate that TCC dechlorination can take place significantly in the thermally pretreated biosolids at different digester operating temperature and SRTs. During the AD operations at steady-state conditions, NCC concentrations in the total phase of biosolids collected from advanced digesters under TH temperature were increased as the SRT was increased, as seen in Fig. 6a. The statistically significant effect of digester SRT on the formation of non-chlorinated NCC in MW-pretreated biosolids was also proven by a One-Way ANOVA (p-value = 0.000 < 0.05). At prolonged SRTs, the increased contact time between microorganisms and compounds might have resulted in higher TCC biodegradation and higher NCC transformation in the digesters. It was also emphasized by an earlier study that the increasing SRT during activated sludge treatment process enhanced TCC degradation rates [35]. When the MW holding time compared (30 vs. 1 min), the average NCC concentrations in the digesters which were pretreated at different final MW temperatures for 1 min, were more discernible (p-value = 0.004 < 0.05) (Fig. 6a). Moreover, no statistically significant differences in terms of NCC concentrations were observed between the digesters of "TH4-80°C-1min" and "TH5-160°C-1min" at the each SRT (p-value = 0.695 > 0.05). Regarding MH temperature, the concentrations of NCC were below the LOQ in the advanced digesters pretreated for 30 min, at the each SRT. These results emphasize the need for further investigation because it remains unclear whether TCC transformation was slow without NCC formation or whether NCC was readily biodegradable with complete TCC transformation during advanced AD. According to a One-Way ANOVA results, the differences in NCC concentrations monitored in the digesters which were pretreated at different MW temperatures for short exposure duration (1 min), were not statistically significant (p-value = 0.131 > 0.05).



(a)



(b)

Fig 6: Average concentrations of carbanilide (NCC) in the total phase of (a) thermophilic and (b) mesophilic digesters' effluents during steady-state at solid retention times of 20, 12, and 6 days

4. Conclusion

The following conclusions can be drawn based on the experimental data and analysis:

- MW pretreatment enhanced the removal of persistent TCC from mixed sludge by 29 - 64%,
- Final MW temperature had substantial impact on TCC reduction from mixed sludge when compared to MW holding time,
- Seasonal changes may have had an impact on the removal of TCC during biological process at the WWTP, most likely influencing the levels of TCC in undigested sludge,
- Compared to the control AD, advanced AD using MW pretreatment was found to be effective in decreasing TCC levels in biosolids,
- Final MW temperature had a statistically significant effect on TCC removal in the advanced ADs operated at SRTs of 20, 12, and 6 days under both TH & MH temperatures (p-value < 0.05),
- Under TH and MH conditions, low-temperature (MW-80°C) advanced digesters had limited reductions in the total phase concentrations of TCC (less than 10%) over the respective controls during the operations of three SRTs. However, especially at SRT of 12 days, the biosolids concentrations of TCC in MW-pretreated digesters at 160°C were significantly lower than that of in MW-pretreated digesters at 80°C (65% reduction at TH temperature and 47% reduction at MH temperature compared to their respective controls),
- Among the six transformation products of TCC monitored, the environmental concentrations of NCC was quantified for the first time in biosolids. Considering unquantified concentrations of DCC and MCC in biosolids samples, the formation of non-chlorinated NCC in biosolids via reductive dechlorination could be an indicative of efficient and complete TCC transformation,
- Higher TCC transformation occurred at TH temperature via potential dechlorination mechanism compared to MH temperature.

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