OCCURRENCE AND FATE OF ENDOCRINE DISRUPTING ANTIMICROBIAL TRICLOCARBAN IN MUNICIPAL SLUDGE DURING ADVANCED ANAEROBIC DIGESTION USING MICROWAVE PRETREATMENT

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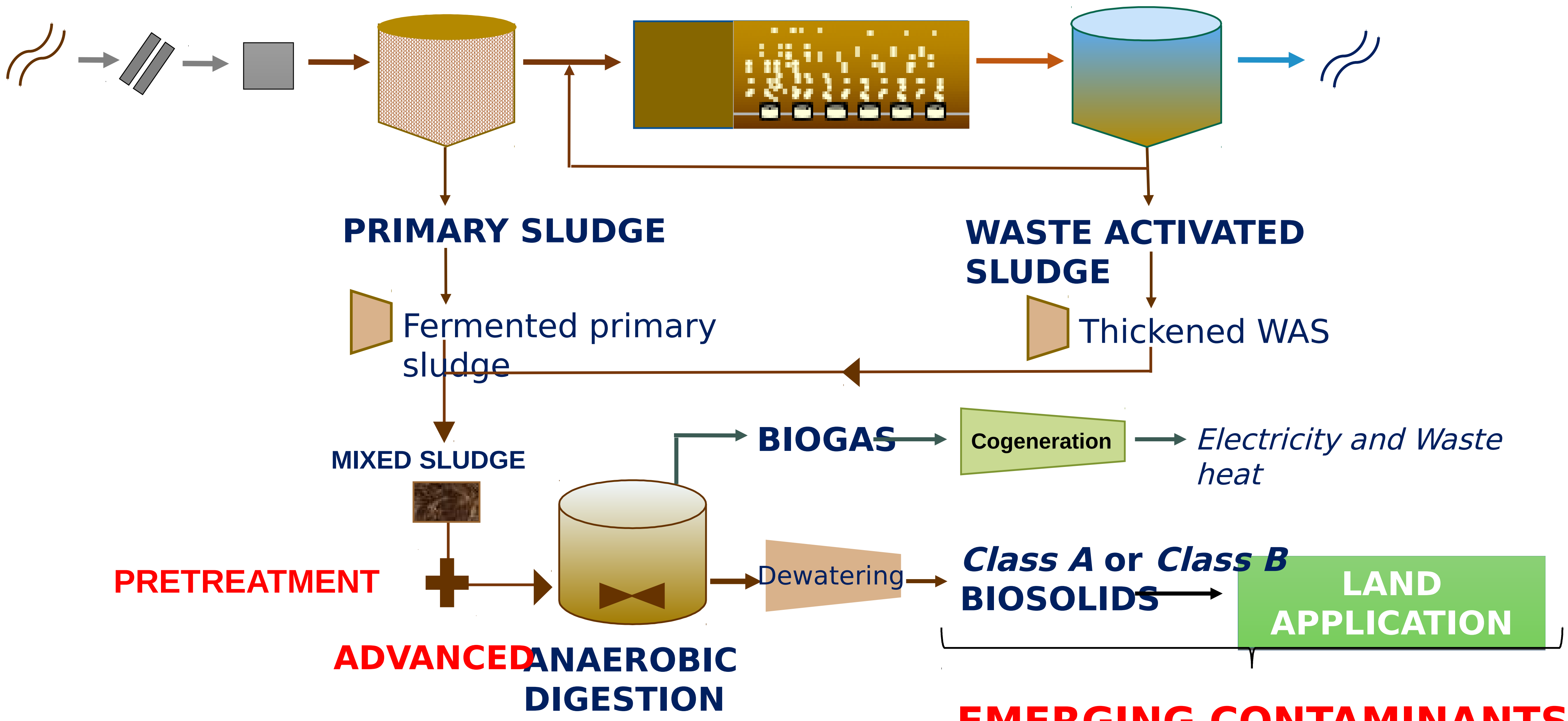
HERAKLION 2019, 26-29 June 2019, Heraklion, Crete Island, Greece

Emerging Concern in Biosolids



EMERGING CONTAMINANTS
(e.g. Pharmaceuticals and Personal Care Produc

Municipal Sludge Pretreatment Technologies

Chemical ⇨ *Ozonation*
Acidic & Alkaline
Peroxidation

Mechanical ⇨ *High pressure homogenization*
Ultrasonication
Pulsed electric field
Stirred ball mills

Biological ⇨ *Enzymatic*
Dual-stage anaerobic digestion

THERMAL

Conventional heating (~160°C, 30 min)

Microwave irradiation (2,450 MHz)

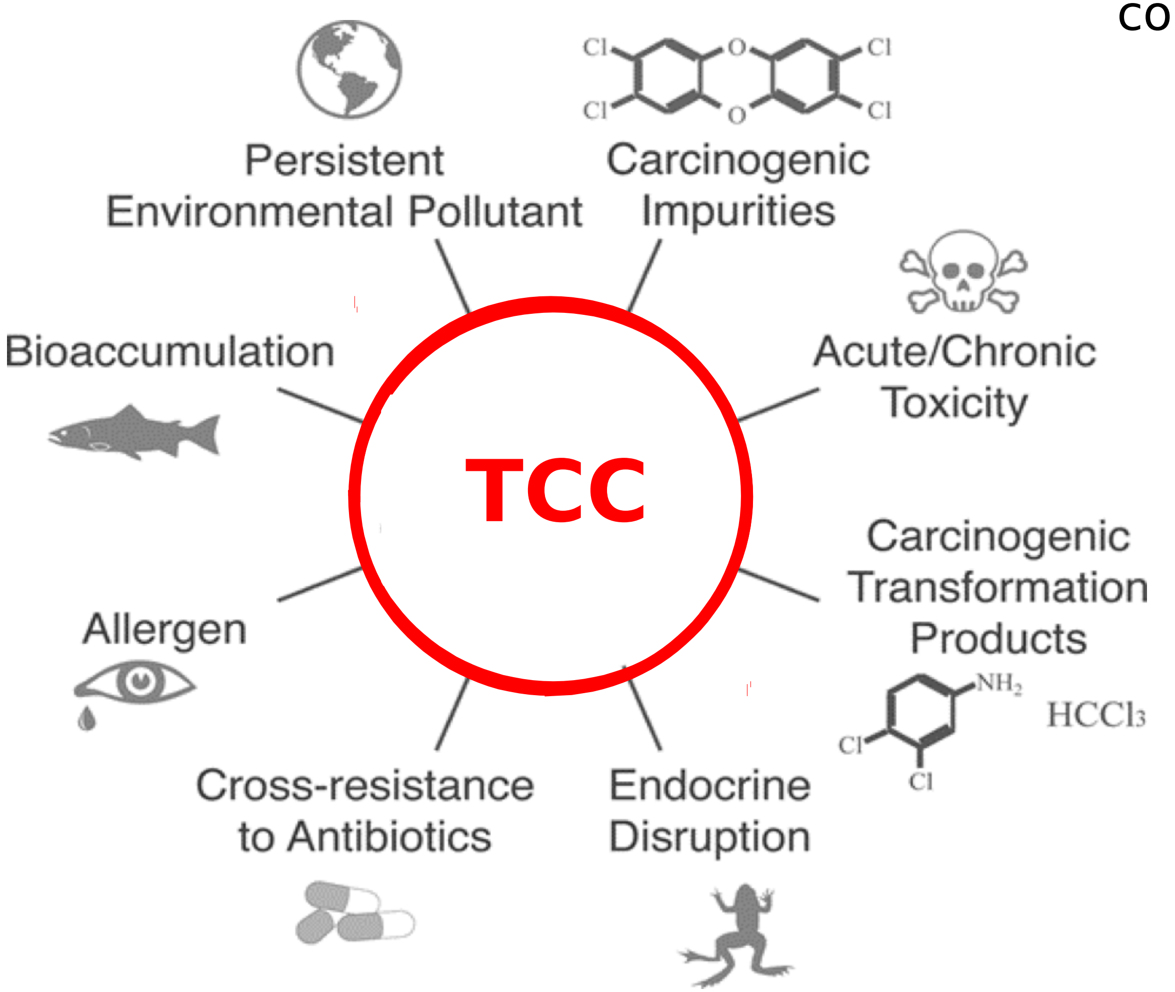
Radio frequency (13.56 MHz)

This research

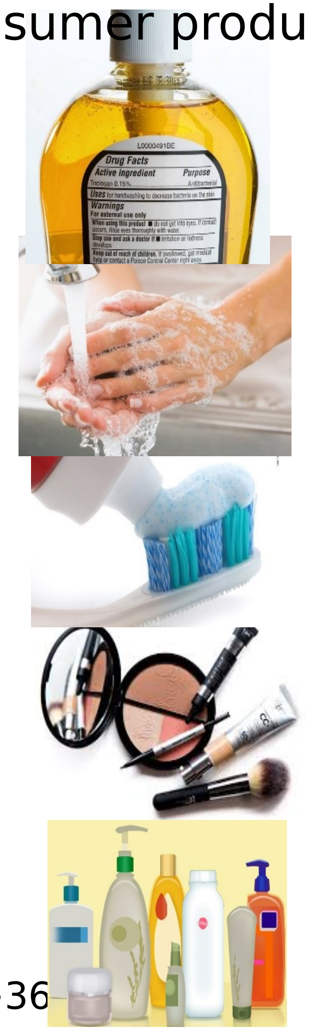
Tyagi, V. And Lo, S.L., 2011. *Rev Environ Sci Biotechnol.*, 10(3), 215-242.

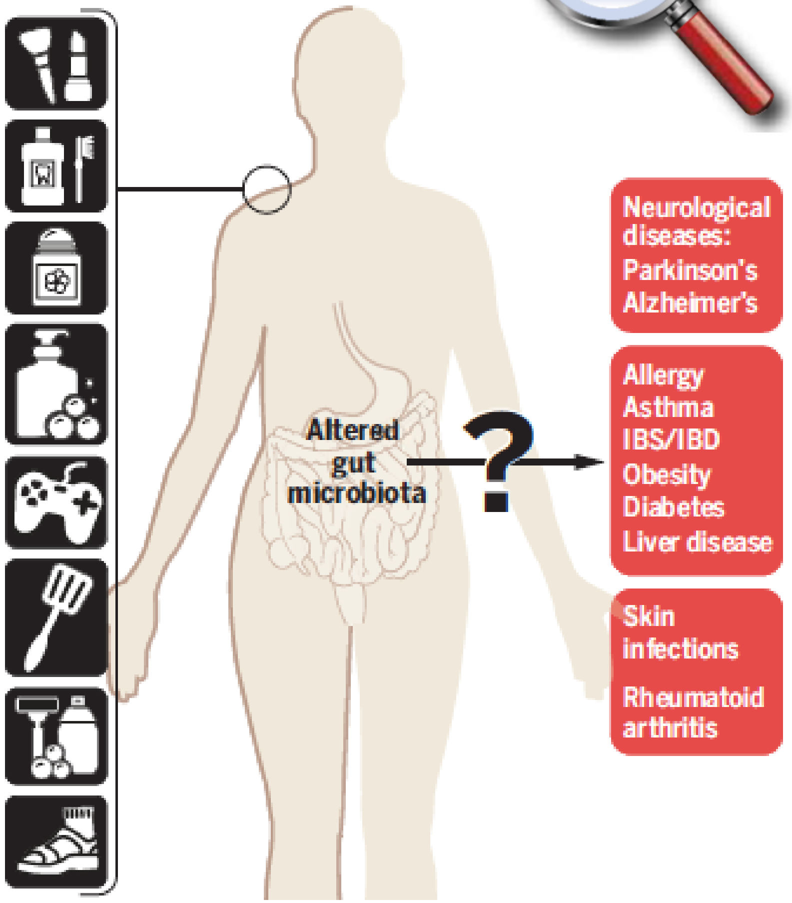
Hosseini Koupaie et al., 2017. *Water Res.*, 118: 70-81.

Why Triclocarban (TCC)?



used as an ingredient in commonly used in many consumer products



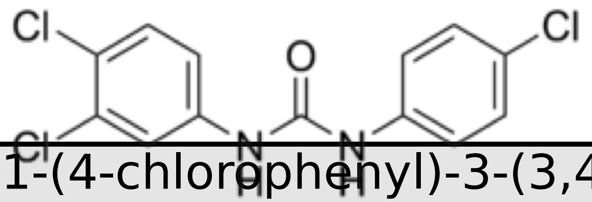


Halden, R.U., 2014. *Environmental Science & Technology*, 48, 3603−36

Yee, A.L. and Gilbert, J.A., 2016. *Science*, 353, 62

Triclocarban

Table. Selected physico-chemical properties of triclocarban.

Property	Triclocarban (TCC)
Chemical structure	
IUPAC name	1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)urea
Molecular formula	$C_{13}H_9Cl_3N_2O$
Molecular weight (g/mol)	315.578
Chlorine content (weight %)	36.7
Log K_{ow} (at 25°C, pH 7)	4.9
Log K_{oc} (at 25°C, pH 7)	4.5
pK_a (at 20°C)	12.7
Water solubility (mg/L at 25°C)	0.65 - 1.55
Vapour pressure (mm Hg at 25°C)	3.61×10^{-9}
Use	**Antiseptic and disinfectant**

1) Halden and Paull, 2005. *Environ Sci Technol*, 39(6), 1420-6. 2) Heidler and Halden, 2008. *Environ Sci Technol*, 42(17), 6324-6332. 3) Wu et al., 2009. *Journal of Agricultural and Food Chemistry*, 57(11), 4900-4905. 4) Ying et al., 2007. *Environmental Pollution*, 150(3), 300-305.

Target Compounds

Parent Compound

Triclocarban (TCC)

Transformation products

1) Dichlorocarbanilide (DCC)
2) Monochlorocarbanilide (MCC)
3) Carbanilide (NCC)
4) 3,3',4,4'-tetrachlorocarbanilide (4-Cl-TCC)
5) Monochloroanilines
6) 4-chlorocatechol

Motivation:

- *The effects of microwave (MW) pretreatment on the fate of TCC/by-products **are unknown** in municipal waste sludge streams.*

Research Objectives

MAIN OBJECTIVE:

To investigate how **MW pretreatment** along with **advanced anaerobic digestion** can affect the **environmental concentrations (NO SPIKING)** of **TCC and its transformation products** in digester influent/effluents streams

Variables

- **Pretreatment intensity: MW Temp.** [**80** and **160°C**] and
- **Digester temperature: Thermophilic** [**55 ± 1°C**] and
- **Digester sludge retention times (SRTs): 20, 12,** and **6 days**

METHODOLOGY

Wastewater Treatment Plant Selected

Westside Regional Wastewater Treatment Plant (West Kelowna

→ serves population of **44,500 people** and average daily flow capacity of **16,800m³/day**

Microwave Pretreatment Scenarios

Thickened WAS (TWAS) (~4% TS w/w)

Dewatered TWAS (~ 11% TS w/w)
centrifugation after polymer solution addition

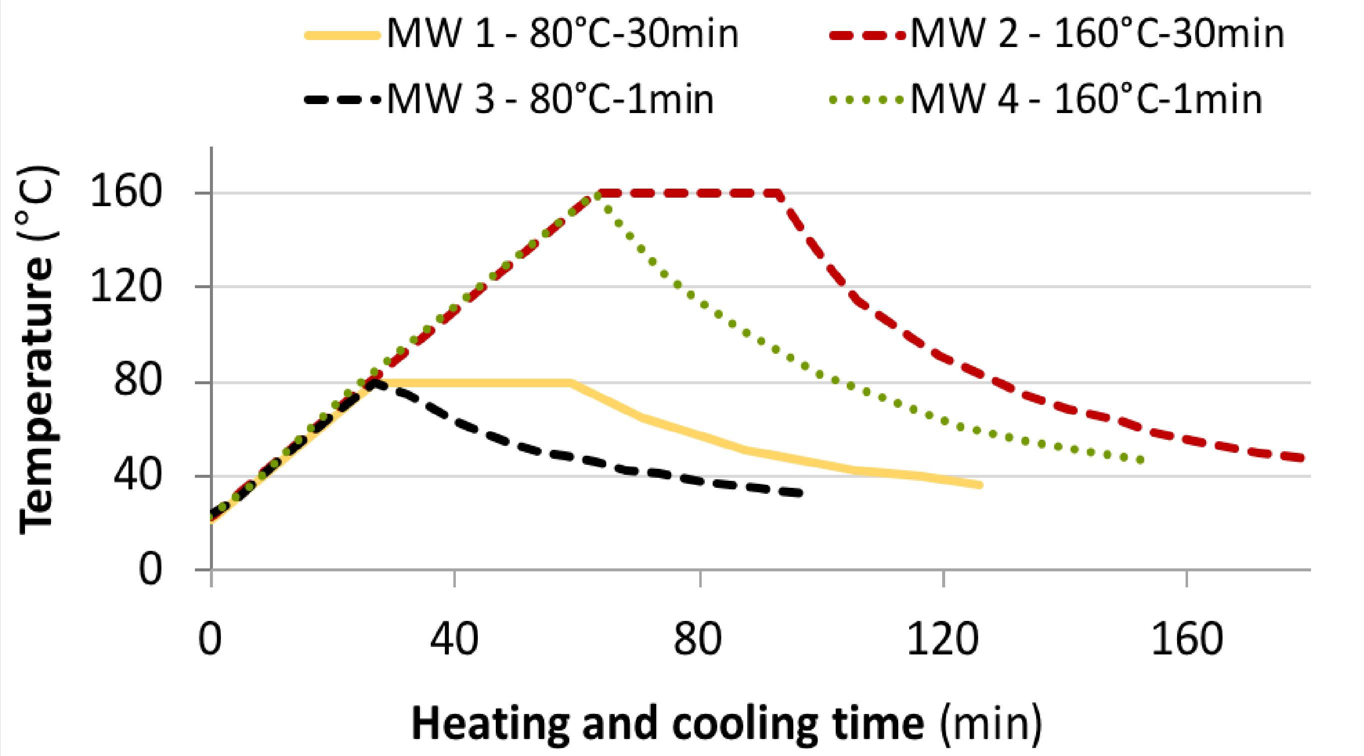
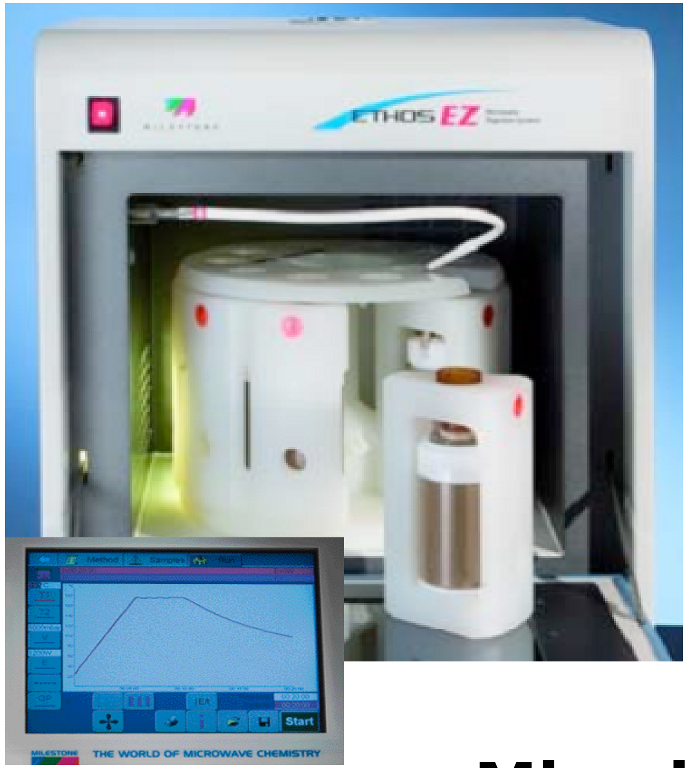


Fig. Heating & cooling profiles of **dewatered TWAS** at **2.25°C/min**.



+

Fermented Primary Sludge (3-5% TS w/w)

Mixed Sludge
(~3.5 % TS

Anaerobic Digestion Studies

1st acclimation stage
(acclimated to mixed sludge)

2nd acclimation stage
(acclimated to MW irradiated mixed sludge)

Operation/monitoring of 10 digesters at SRTs of 20, 12, 6 days

Total operation time: approx. 9 months

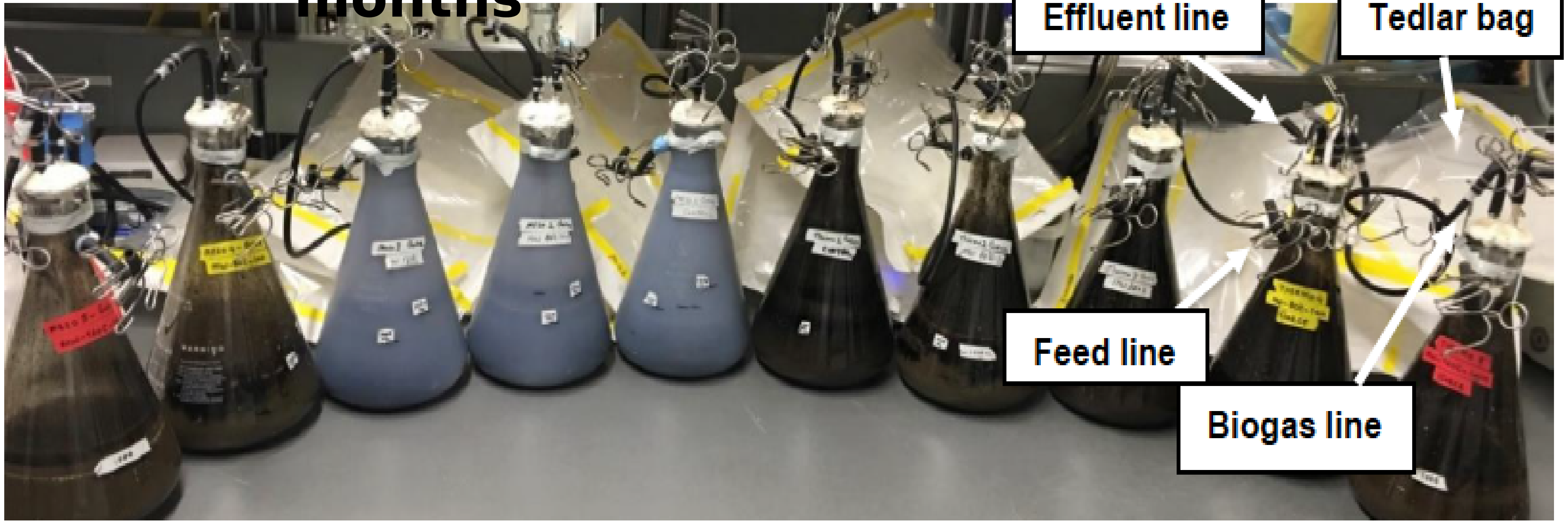


Fig. Ten bench-scale anaerobic digesters **semi-continuously fed** mode **(once a day, 7 days/week)**

Parameters Monitered

Table. Characterization of digester performance.

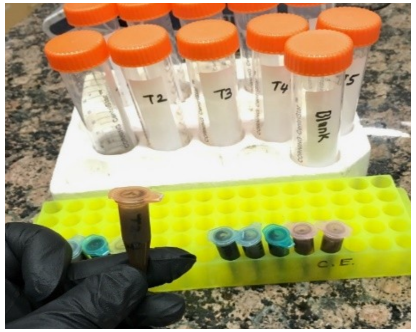
Sample	Parameter
Digester influent/effluent streams	• Total/volatile solids • Total/soluble chemical oxygen demand, biopolymers • pH, alkalinity, ammonia • Total VFAs (acetic, propionic and butyric acids) • **Target TCC parent/transformation compounds** • Dewaterability, fecal coliforms, heavy metals
Digester headspace	• Biogas volume • Biogas composition (CH_4, CO_2, N_2, O_2)

- **Statistical Analysis** using **Analysis of Variance (ANOVA)** using "*Minitab™ 17 statistical software*"

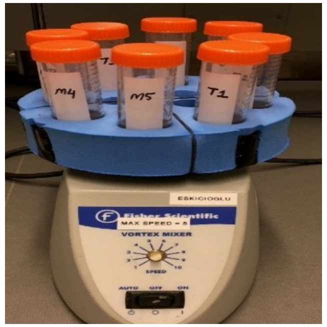
Conventional performance data: G. Kor-Bicakci, E. Ubay-Cokgor, C. Eskicioglu, 2019

thod development for target compound

Quantification of TCC and its Transformation Products in Sludge

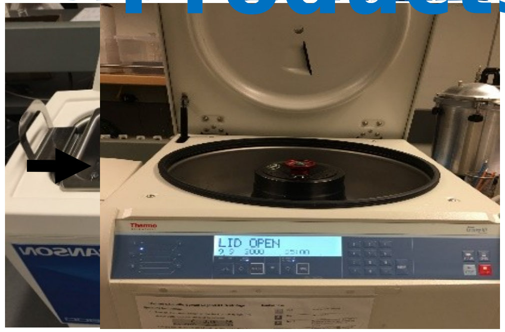


Total phase samples



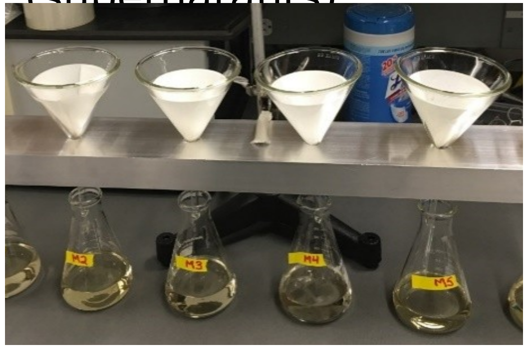
Vortex

Ultrasound sonication



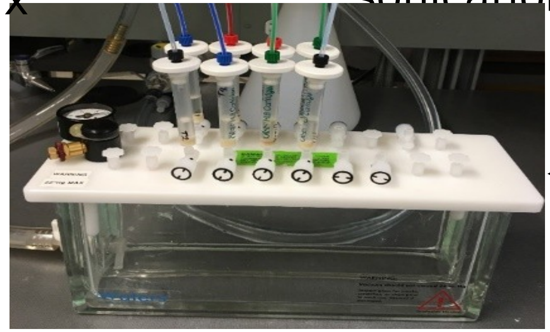
Centrifugation

Acid extracts (supernatants)

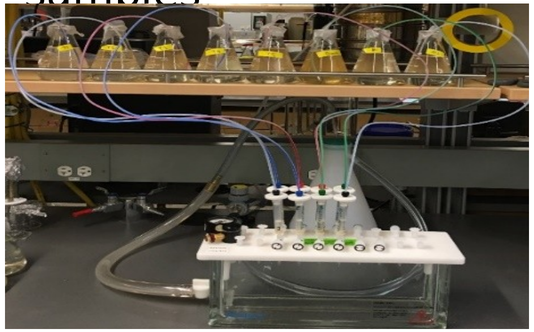


Filtration

Rotary evaporator

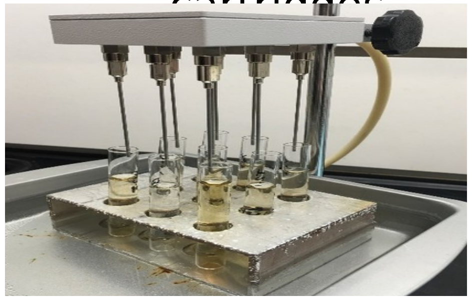


HLB cartridges



Loading of samples

Elution with MeOH by gravity



N_2 blowdown

Extracts in vials

UHPLC-ESI-MS/MS

RESEARCH FINDINGS

Environmental Occurrence of TCC in Mixed Sludge

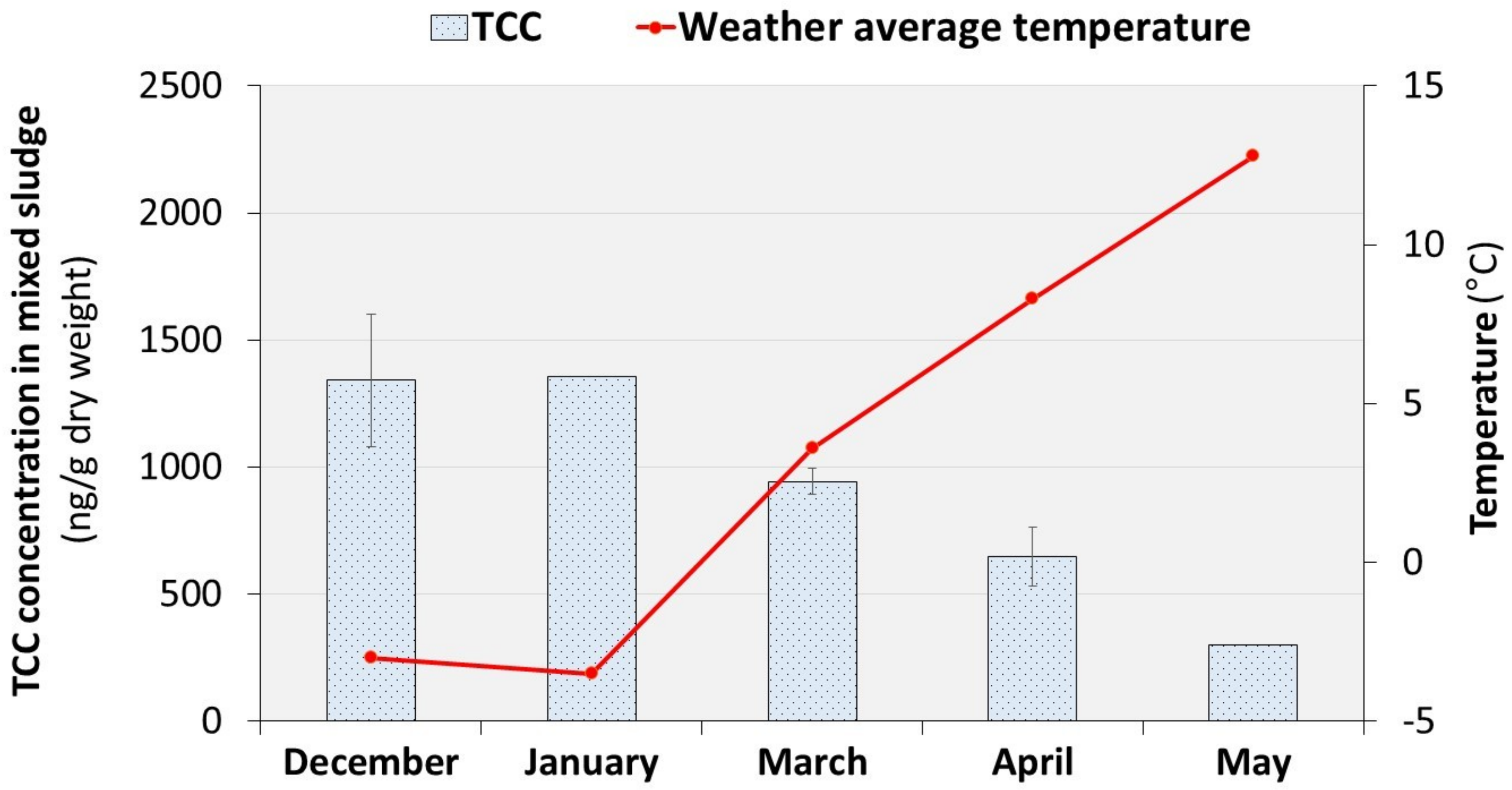


Fig. Seasonal fluctuation in occurrence of triclocarban in different sample collection periods together with weather averages.

between 300 and 1,800 ng/g dw

average concentration of TCC 1,030 ± 470 ng/g dw

Transformation products

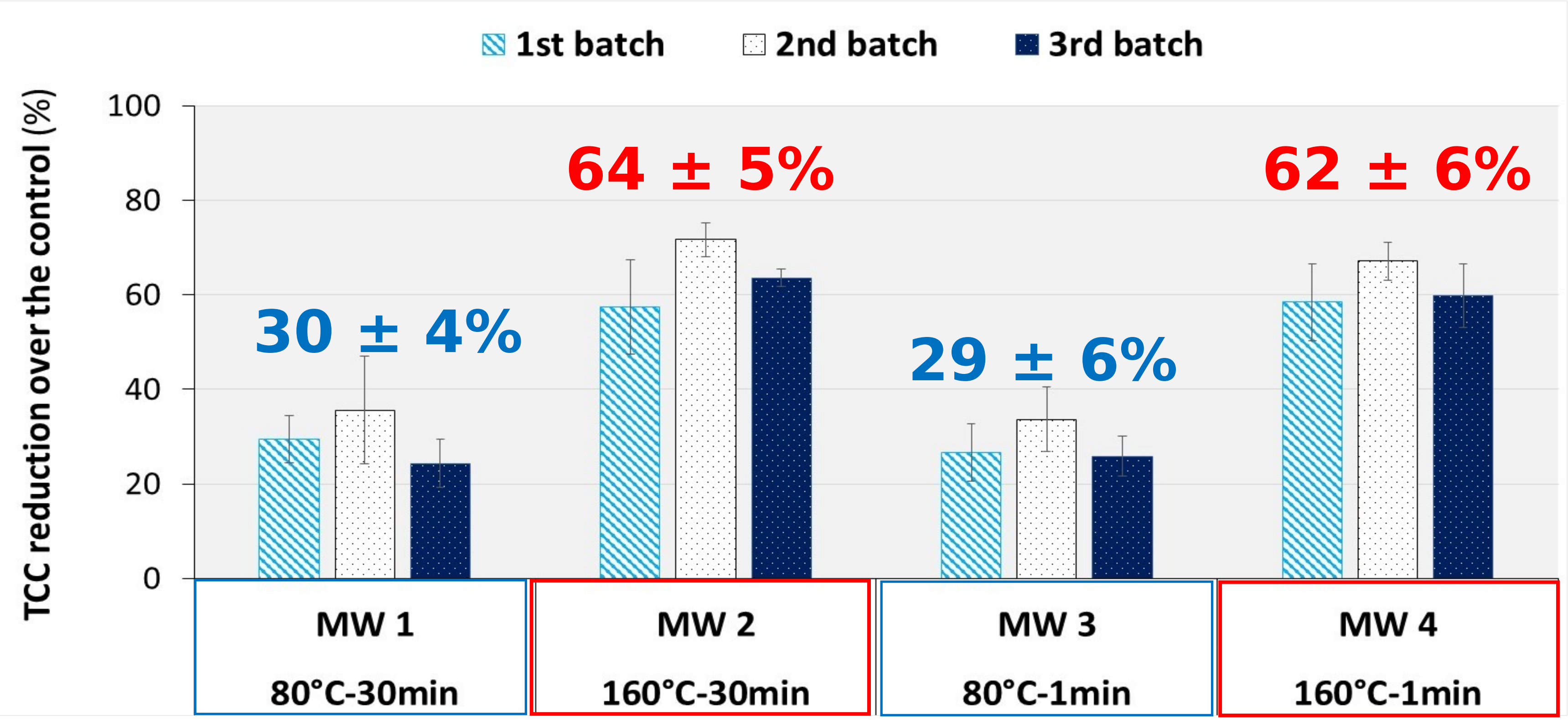
4-Cl-TCC
MCC
monochloroanilines
4-chlorocatechol

below the limit of detection (LOD)

DCC and NCC

below the limit of quantification (LOQ)

Effect of MW Pretreatment on Fate of TCC in Mixed Sludge



MW final temperature

MW holding time

Fig. Effect of MW pretreatment on reduction of triclocarban concentrations in pretreated over the **un-pretreated mixed sludge (control).**

4-Cl-TCC, MCC, monochloroanilines, 4-chlorocatechol → below the LOD

DCC and NCC → below the LOQ

Fate of TCC and its Metabolites during Conventional AD

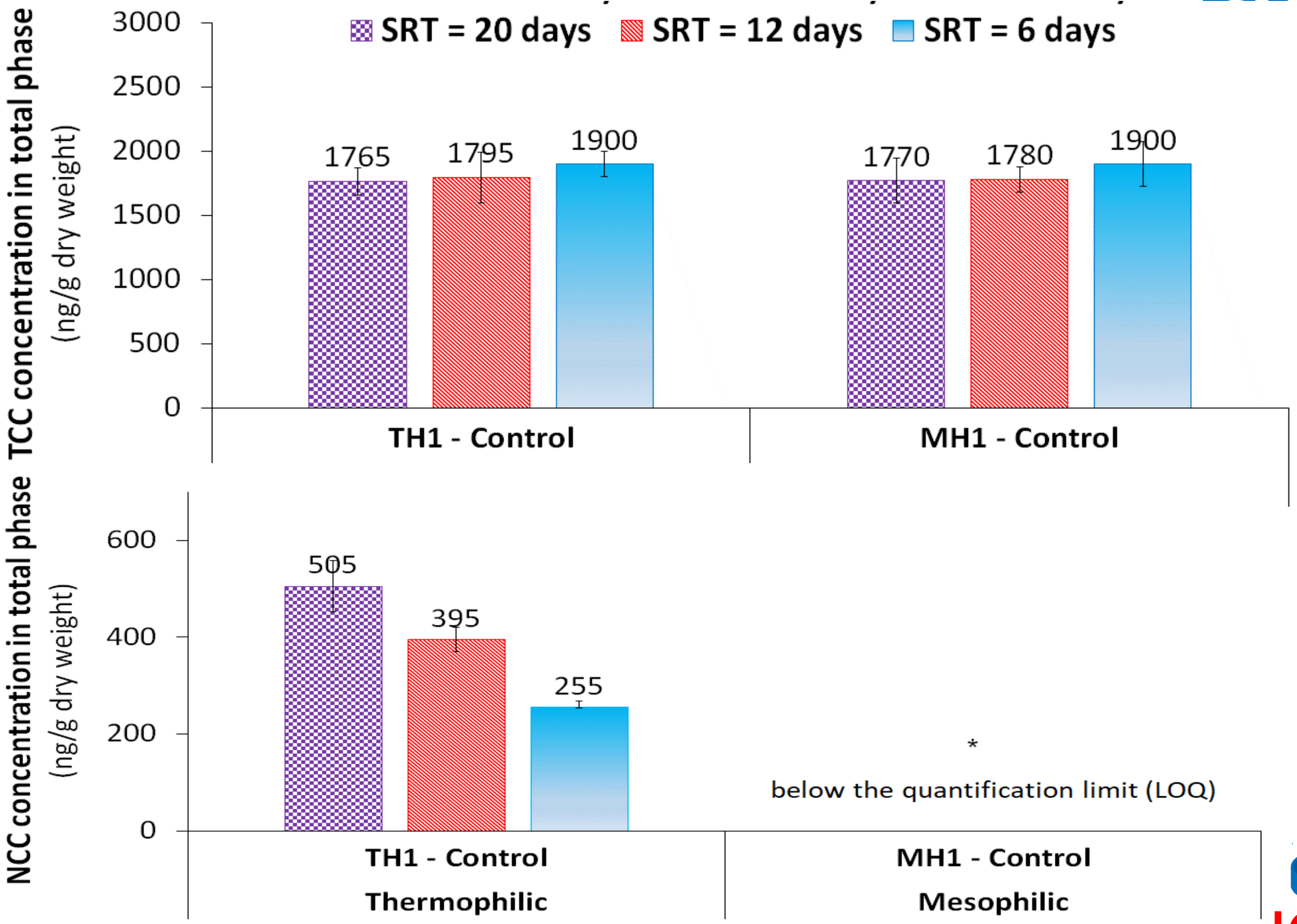


Fig. Average concentrations of TCC and NCC of the TH and MH digesters' effluents at SRTs of 20, 12, and 6 days (n = 4)

TCC: 1,765 – 1,900 ng/g dw

SRT

NCC: 505 – 255 ng/g dw

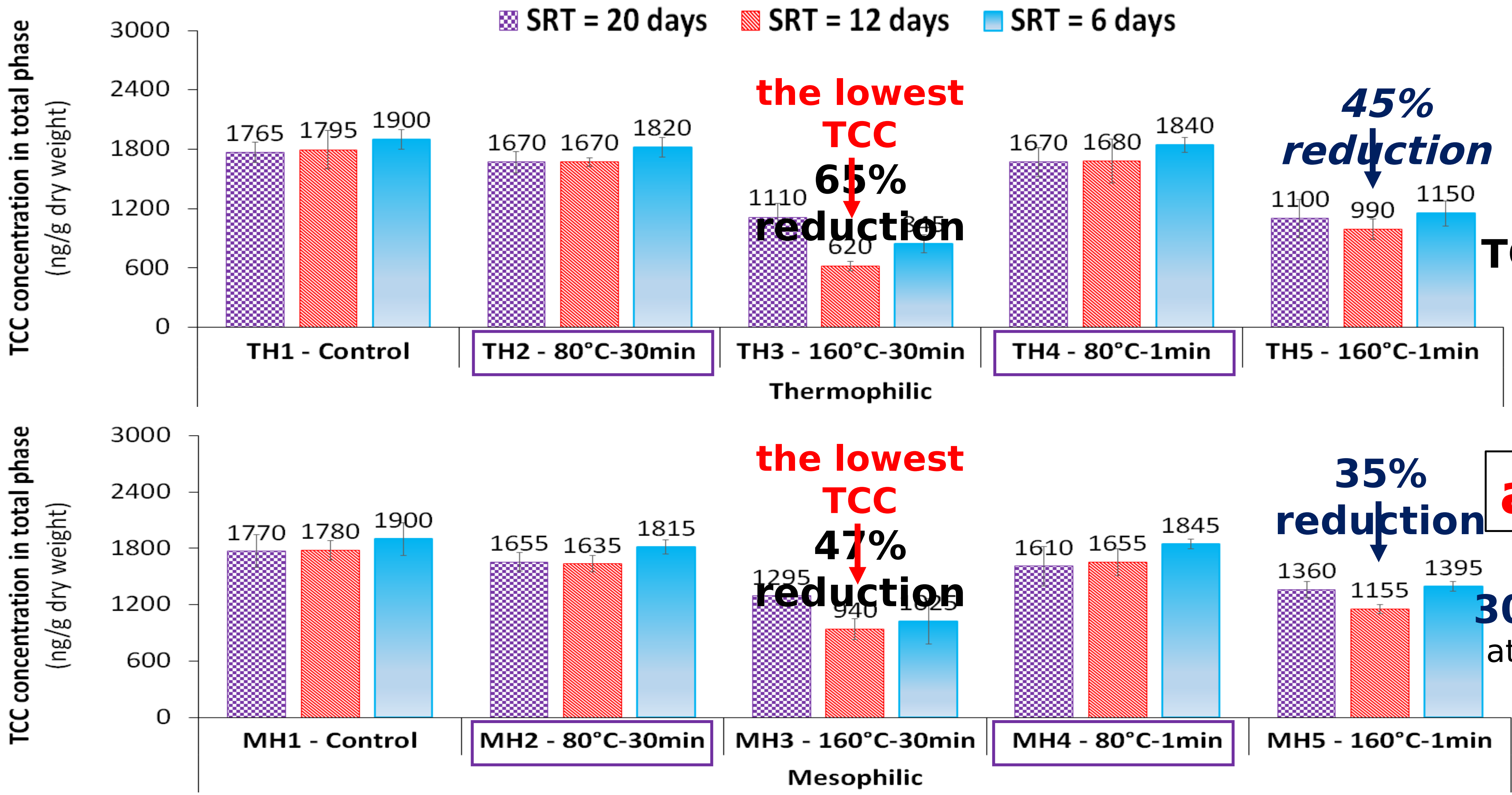
SRT ↓ NCC concentration ↓

the first study

TCC DCC MCC **NCC**

via ***complete reductive dechlorination***

Fate of TCC during Advanced AD



at 80°C

TCC reduction < 10%

~~holding time~~

at 160°C

holding time

30 min > 1 min

at SRTs 12 & 6 days

TH >> MH

Fig. Average concentrations of TCC in total phase of the TH and MH digesters' effluents (n = 4).

Conclusions

- Compared to the control AD, **advanced AD** using **MW pretreatment** was found to be **effective in decreasing TCC levels in** digester influent/effluent streams (biosolids),
- **Most intensive MW pretreatment (160°C/30 min)** showed **the highest impact** on TCC removal from mixed sludge at SRT of 12 days,
- **Seasonal changes** had an **impact on removal of TCC** during **wastewater treatment process at the WWTP,**
- **Higher TCC transformation to NCC occurred** at thermophilic temperature via **potential dechlorination mechanism** compared to mesophilic temperature.

Acknowledgements

TÜBİTAK

International Research Fellowship Program, Turkey (221

NSERC CRSNG

Collaborative Research and Development Grant, Canada
(CRDP J462765-13)

REGIONAL DISTRICT of CENTRAL OKANAGAN

Westside Regional Wastewater Treatment Plant Staff

thank you!

Why Triclocarban Selected?

- High-production volume chemical (the worldwide annual production: ~1,500 ton)
- Being **priority chemical** candidates
- Abundant existence in **municipal treatment sludge and biosolids** (detected >1000 μg/ kg dry solids)

  - physico-chemical properties

  - possible threats to the food chain through land application of biosolids

 among the top 10 compounds detected in **biosolids** which were taken from WWTPs in U.S. (in the 231 chemicals assayed)

 among the top 10 contaminants of American **rivers** in U.S. Geological Survey (in the 95 contaminants from a 139 streams across 30 states)

Venkatesan, A.K., and Halden, R.U., 2014. *Nature, Scientific Reports,* 4, 3731. Halden et al., 2017. *Environmental Health Perspectives*, 125(6), 064501.
Kolpin et al., 2002. *Environ. Sci. Technol.* 2002, 36, 1202-1211.

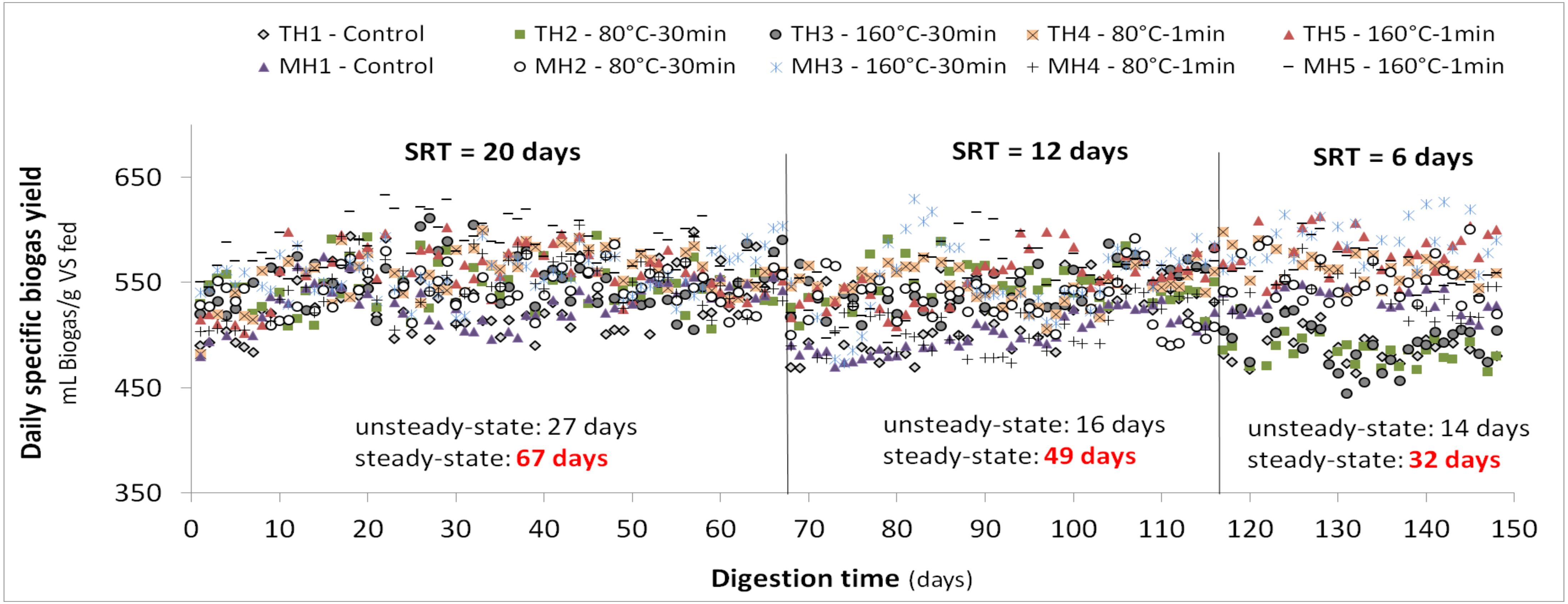
Biogas Production

All digesters ~ **STABLE**

OLRs 1.45 - 5.20 g VS/L of digester/d

Specific biogas production 500 - 550 mL biogas/ g VS$_{fed}$

CH_4 content of biogas: **65 - 72%**



Improvements: 2-19% over controls

G. Kor-Bicakci, E. Ubay-Cokgor, C. Eskicioglu, 2019. *Energy*, 168, 782–

Fate of NCC during Advanced AD

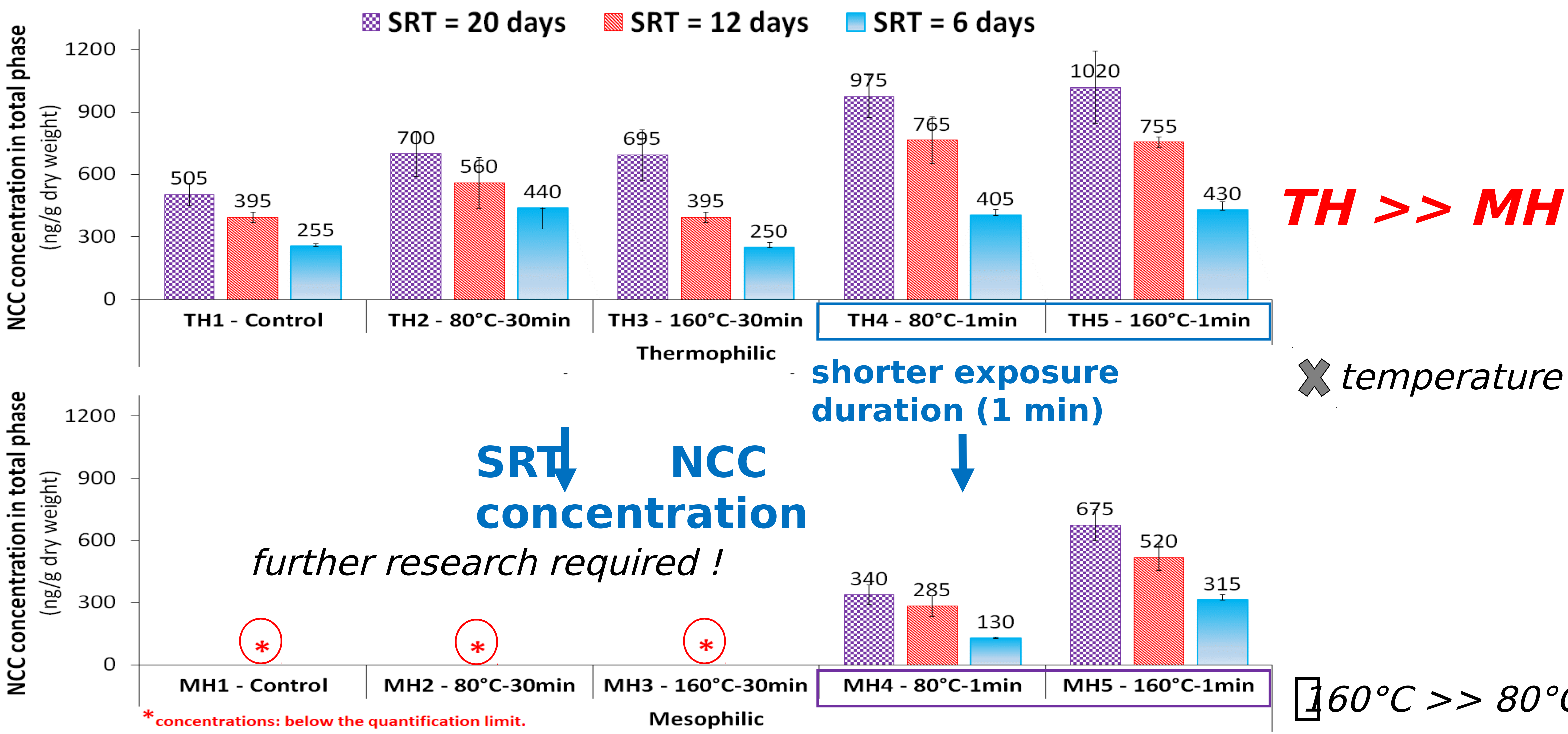


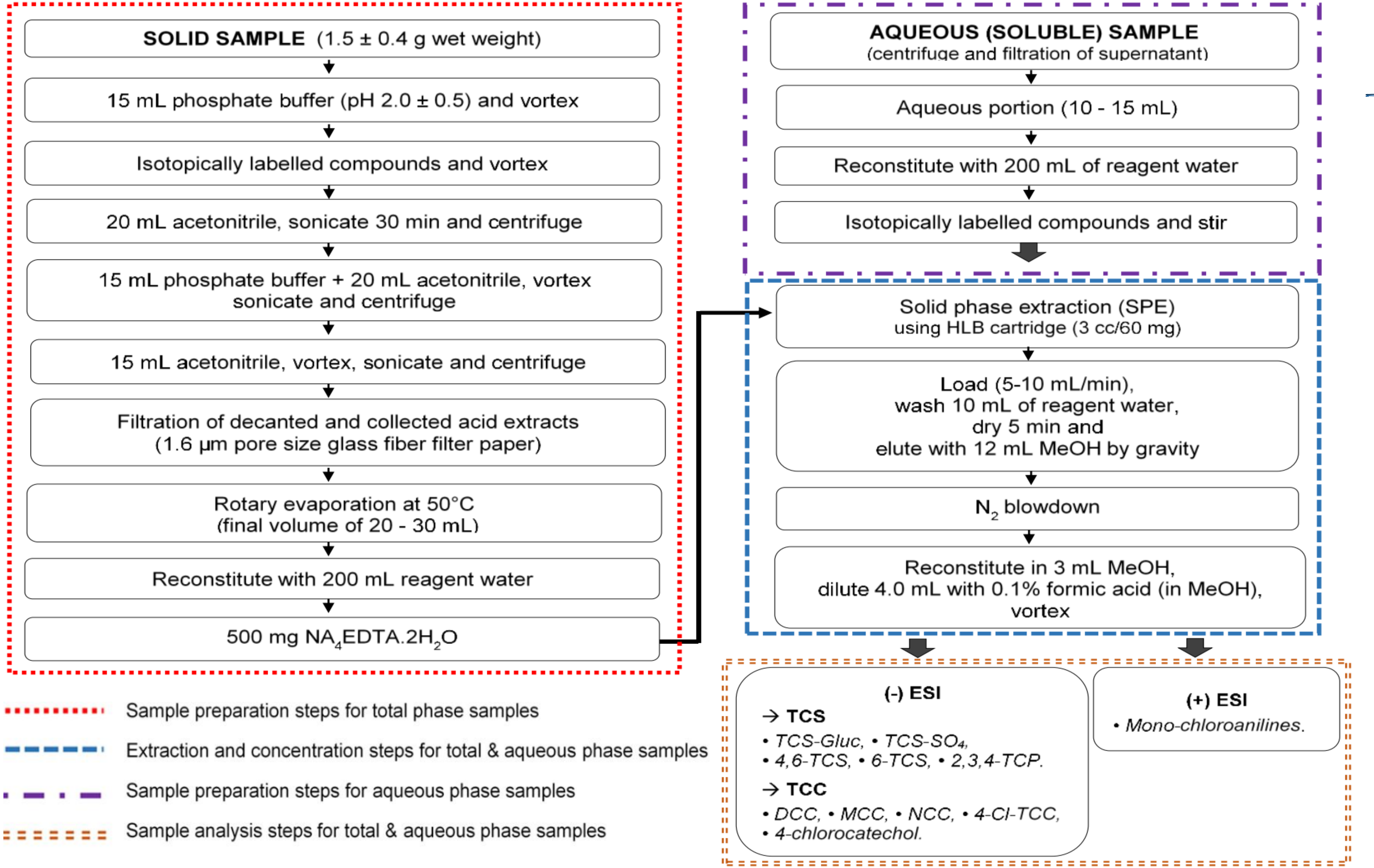
Fig. Average concentrations of NCC in total phase of the TH and MH digesters' effluents (n = 4).

Compound	Total phase LOD[a] (ppb)	Total phase LOQ[b] (ppb)	Total phase RL[c] (ppb)	Total phase Recovery (%)	Soluble phase LOD[a] (ppb)	Soluble phase LOQ[b] (ppb)	Soluble phase RL[c] (ppb)	Soluble phase Recovery (%)
Triclocarban (TCC)	0.08	0.24	0.54	112.4	0.11	0.28	0.68	72.1
Dichlorocarbanilide (DCC)	0.08	0.25	0.52	118.9	0.09	0.25	0.50	74.8
Monochlorocarbanilide (MCC)	0.10	0.25	0.55	258.3	0.10	0.24	0.53	64.9
Carbanilide (NCC)	0.16	0.55	0.72	136.8	0.06	0.25	0.64	61.80
3,3',4,4'-tetrachlorocarbanilide (4-Cl-TCC)	0.70	0.95	1.43	n/a	0.53	0.99	1.47	n/a
Monochloroanilines (sum of 3-CA & 4-CA)	0.12	0.45	0.61	91.55	0.09	0.56	0.90	76.40
4-chlorocatechol	0.87	0.99	1.16	163.49	0.57	0.94	1.11	n/a
Triclosan (TCS)	0.33	0.50	1.10	112.50	0.16	0.42	0.98	109.2
Triclosan O-β-D-glucuronide	1.44	5.22	5.46	107.24	1.27	1.64	6.08	n/a
Triclosan-*O*-Sulfate	0.12	0.31	0.50	317.64	0.63	0.86	1.04	n/a
Tetra-III	0.58	0.95	4.92	110.3	1.37	4.77	5.05	n/a
Penta	0.09	0.25	0.46	105.18	0.64	1.09	5.16	n/a
2,3,4-trichlorophenol (2,3,4-TCP)	0.26	0.57	0.69	114.2	0.10	0.30	0.60	n/a

[a]LOD: limit of detection, [b]LOQ: limit of quantification, [c]RL: reporting limit, n/a: not available.

Table 3.10 : Sample analysis methods and their aqueous and organic phases for target analytes.

Method	Compounds	Aqueous Phase	Organic Phase
A	Triclocarban (TCC) • *Dichlorocarbanilide (DCC)* • *Monochlorocarbanilide (MCC)* • *Carbanilide (NCC)* • *4-chlorocatechol* Triclosan (TCS) • *Triclosan O-β-D-glucuronide* • *Triclosan-O-Sulfate* • *Tetra-III* • *Penta* • *2,3,4-trichlorophenol (2,3,4-TCP)*	5 mM ammonium acetate solution	Methanol
B	• *3,3',4,4'-tetrachlorocarbanilide (4-Cl-TCC)*	10 mM ammonium bicarbonate solution	Methanol
C	• *Monochloroanilines (3-CA and 4-CA)*	Ultra-pure water	Methanol


SOLID SAMPLE (1.5 ± 0.4 g wet weight)
15 mL phosphate buffer (pH 2.0 ± 0.5) and vortex
Isotopically labelled compounds and vortex
20 mL acetonitrile, sonicate 30 min and centrifuge
15 mL phosphate buffer + 20 mL acetonitrile, vortex sonicate and centrifuge
15 mL acetonitrile, vortex, sonicate and centrifuge
Filtration of decanted and collected acid extracts (1.6 μm pore size glass fiber filter paper)
Rotary evaporation at 50°C (final volume of 20 - 30 mL)
Reconstitute with 200 mL reagent water
500 mg $NA_4EDTA.2H_2O$
AQUEOUS (SOLUBLE) SAMPLE (centrifuge and filtration of supernatant)
Aqueous portion (10 - 15 mL)
Reconstitute with 200 mL of reagent water
Isotopically labelled compounds and stir
Solid phase extraction (SPE) using HLB cartridge (3 cc/60 mg)
Load (5-10 mL/min), wash 10 mL of reagent water, dry 5 min and elute with 12 mL MeOH by gravity
N_2 blowdown
Reconstitute in 3 mL MeOH, dilute 4.0 mL with 0.1% formic acid (in MeOH), vortex
(-) ESI
→ TCS
• TCS-Gluc, • $TCS\text{-}SO_4$,
• 4,6-TCS, • 6-TCS, • 2,3,4-TCP.
→ TCC
• DCC, • MCC, • NCC, • 4-Cl-TCC,
• 4-chlorocatechol.
(+) ESI
• Mono-chloroanilines.
Sample preparation steps for total phase samples
Extraction and concentration steps for total & aqueous phase samples
Sample preparation steps for aqueous phase samples
Sample analysis steps for total & aqueous phase samples