Supporting information

Triclocarban influences antibiotic resistance and alters anaerobic digester microbial community structure

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Section S1. Quantifying Triclocarban in Biomass

TCC concentrations were measured by liquid chromatography-mass spectrometry (LC/MS). Briefly, 4 µg of ^{13}C-labed TCC (Cambridge Isotope Laboratories, Inc, Andover, MA, USA) were added to a 5 mL sample of wet biosolids. The sample was allowed to dry in a crucible for 72 hours at 35°C. The mass of the dried biomass was quantified and then extracted into approximately 20 mL of methanol by using an Accelerated Solvent Extraction System (Dionex ASE 350, Thermo Scientific, Sunnyvale, CA, USA). The extraction protocol was modified from Anger *et al.*, to thoroughly remove TCC and TCS with methanol and acetone [1]. For extraction, dried biosolids was placed into a clean ASE cell. The cell was heated to 60°C and held at a pressure of 1500 psi; it was heat cycled twice to this temperature and then flushed with 60% of the extraction cell volume.

Micropollutant concentrations from the ASE extracts were determined by injecting 20 µL into a Shimadzu LCMS-2020 (Shimadzu, Addison, IL, USA). Chromatography was performed with a Phenomenex Luna C18 column (3 µm particle size, 150 x 3 mm). The flow rate was 400 µL/min using mobile phase A of 100% HPLC grade water and mobile phase B of 100% methanol. The method began at 80% methanol and increased linearly over 13 minutes to 100% methanol. The mass to charge ratios used for detection of TCC and ^{13}C-TCC were 313 and 319, respectively. Concentrations were determined by using a seven-point standard curve.

Table S1: TCC results and recoveries from extraction

Day	Sample	13-C TCC Recovery	Corrected TCC (mg/kg)	Difference from nominal concentration
0	Seed	58%	27	NA
33	Background	76%	25	18%
47	Control	57%	0.8	NA (Target conc = 0)
47	Low-Immediate	46%	126	3.1%
47	Medium-Immediate	44%	420	6.6%
47	High Immediate	50%	692	19%
110	Control	56%	0	NA (Target conc = 0)
110	Background	43%	31	3.3%
110	Low-Gradual	56%	131	0.8%
110	Medium- Gradual	45%	448	0.4%

Section S2. Nutrient Media Fed to Anaerobic Digesters

Table S2- Nutrient Feed Recipe

Constituent	(mg/L)
NH_4Cl	400
$MgSO_4.7H_2O$	195
KCl	400
$CaCl_2.2H_2O$	50
$(NH_4)_2HPO_4$	80
$FeCl_2.4H_2O$	*40
$CoCl_2.6H_2O$	*10
KI	10
$(NaPO_3)_6$	10
$NiCl_2.6H_2O$	1
$ZnCl_2$	1
$MnCl_2.4H_2O$	0.5
NH_4VO_3	0.5
$CuCl_2.2H_2O$	0.5
$AlCl_3.6H_2O$	0.5
$NaMoO_4.2H_2O$	0.5
H_3BO_3	0.5
$NaWO_4.2H_2O$	0.5
Na_2SeO_3	0.5
$NaHCO_3$	6000
$Na_2S.9H_20$	300
L-Cysteine	10
*Yeast Extract	*10
*Dog Food (seived >0.4 um)	*30000

*indicate deviations from Speece[2]

Section S3. Triclocarban Anaerobic Toxicity Tests

A dose response curve was constructed for TCC. Reactors (160-mL) were maintained with a 50 mL working volume. Triplicate reactors were given 7 distinct doses of TCC (Sigma-Aldrich, St. Louis, MO) based on previous observations (0, 1, 500, 1000, 2000, 5000, 10000, and 30000 mg/kg based on total solids) and 3.8 g/L_r of calcium propionate to ensure that substrate was not limiting. TCC was added to reactors in 50 µL of Dimethyl Sulfoxide. Biogas production rate was measured over 10 days. The maximum rate of biogas production was calculated for each dose of TCC. Dose response curves were constructed with these data and the concentrations which inhibit 50% of methane production (IC_{50}= 850 mg/kg) and 10% of methane production (IC_{10} = 450 mg/kg) were interpolated from the data using GraphPad Prism.

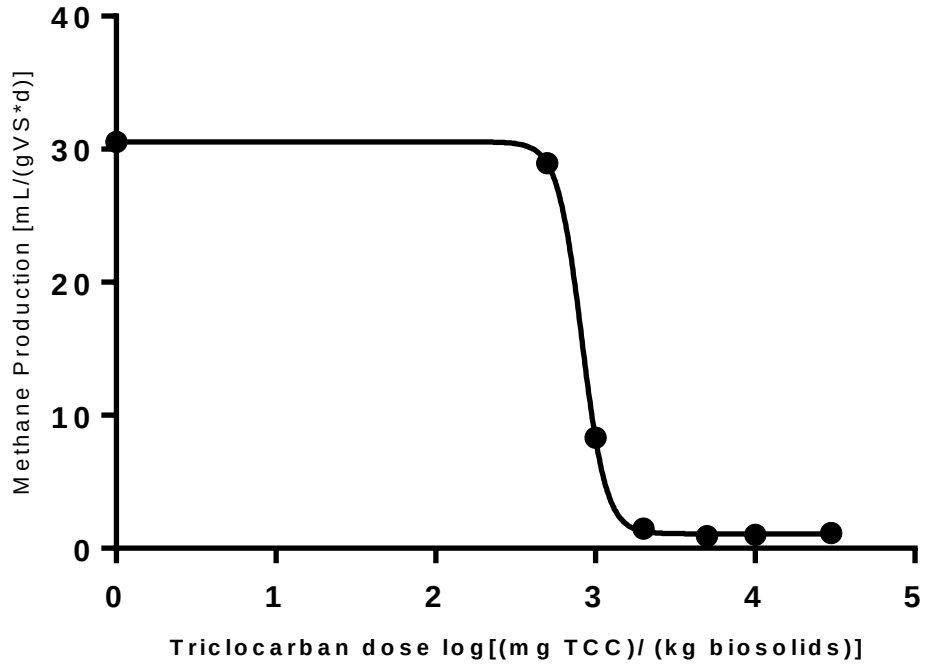

Figure S3. Methane production at a given TCC dose (n=3). Error bars representing standard deviation of the mean are included, however they are occluded by the data points.

Section S4. Primers and qPCR data

Table S3: qPCR details

	Forward & Reverse Primer	Annealing Temp (°C)	Average Efficiency (%)	Limit of Quantification (copies/µL)	Ref.
16S	F (5'-CCTACG GGAGGCAGCAG-3') R (5'-ATTACCGCGGCTGCTGG-3')	60	101.5%	10^4	[3]
mex(B)	F (5'-GTGTTCGGCTCGCAGTACTC-3') R (5'-AACCGTCGGGATTGACCTTG-3')	63	103.0%	$5x10^2$	[4]
intI1	F (5'-CCTCCCGCACGATGATC-3') R (5'-TCCACGCATCGTCAGGC-3')	60	94.9%	$5x10^2$	[5]
tet(L)	F (5'-TCGTTAGCGTGCTGTCATTC-3') R (5'-GTATCCCACCAATGTAGCCG-3')	60	88.2%	$5x10^2$	[6]
erm(F)	F (5'-CAACCAAAGCTGTGTCGTTT-3') R (5'-TCGTTTTACGGGTCAGCACTT-3')	60	86.6%	$5x10^2$	[7]

qPCR was performed on a BioRad CFX Connect Real Time System (Hercules, CA). Assays began with a 10 min initial denaturation at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s and combined annealing and extension at the primer-specific for 30 s. Reaction volumes of 20 µL consisted of 10 µL of BioRad iTaq SYBR Green Supermix (Life Science Research, Hercules, CA), 5 uL of diluted DNA and 5 uL of Ultrapure water with optimized quantities of forward and reverse primers (1 nM for resistance genes and intI1 and 2 nM for 16S rRNA gene). Approximately 50 ng and 0.25 ng of template DNA were required for resistance gene quantification and 16S rRNA quantification respectively.

Samples were diluted to be within the linear range of the standard curve and remove inhibitor substances. Data were only used if the the R^2 value was greater than 0.95. Resistance genes in the feed were below detection limits in all cases. Positive standards for PCR were generated as described elsewhere [8,9].

Section S5. Reactor pH

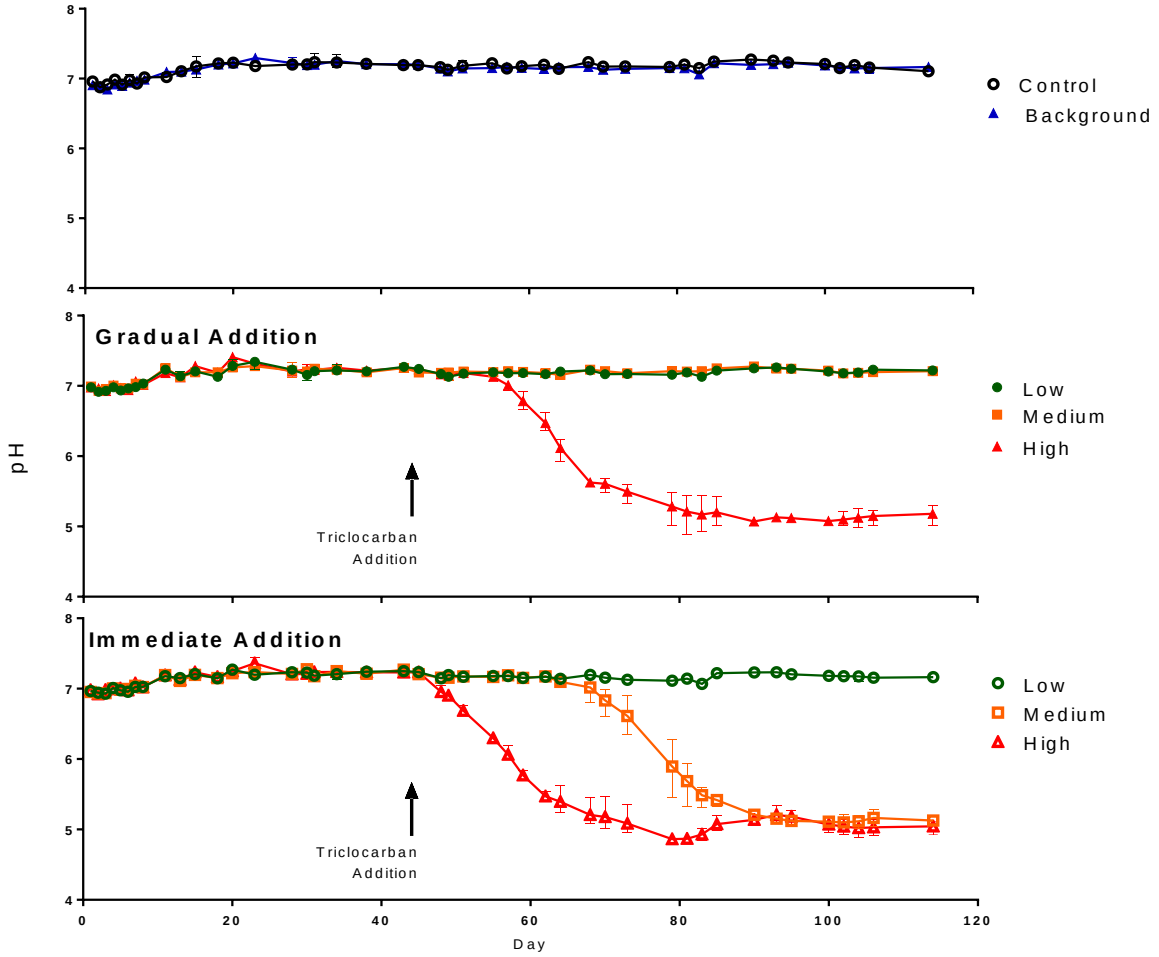

Figure S5: Average digester pH over the duration of the study. Error bars represent the range of the data points.

Section S6. Digester VFA concentrations

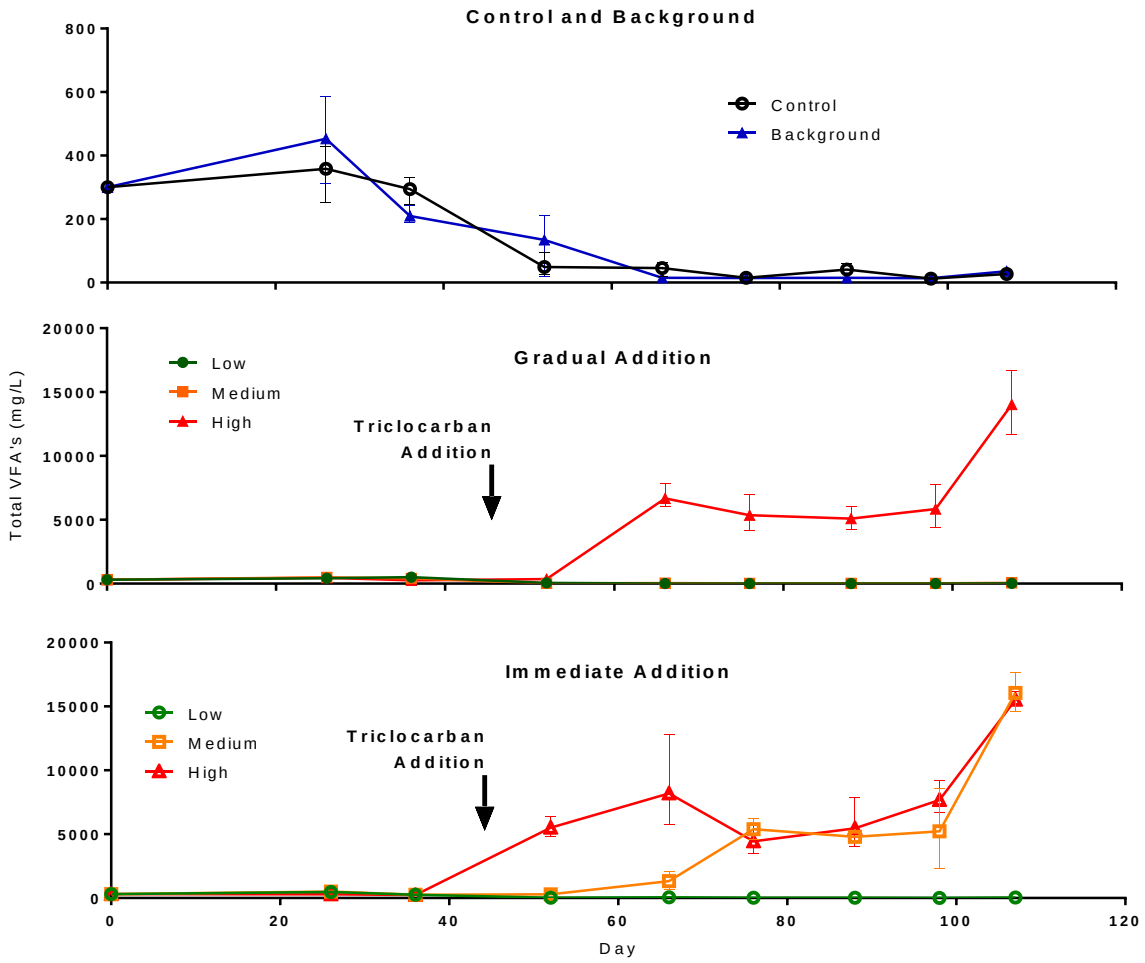

Figure S6: Total VFA concentration in the bioreactors including acetic acid, proprionic acid, butyric acid, iso-butyric acid, valeric acid, and iso-valeric acid. Note the top graph is on a different Y-axis. Error bars represent the range of the data points.

Section S7. Abundance of genes normalized to digester volume

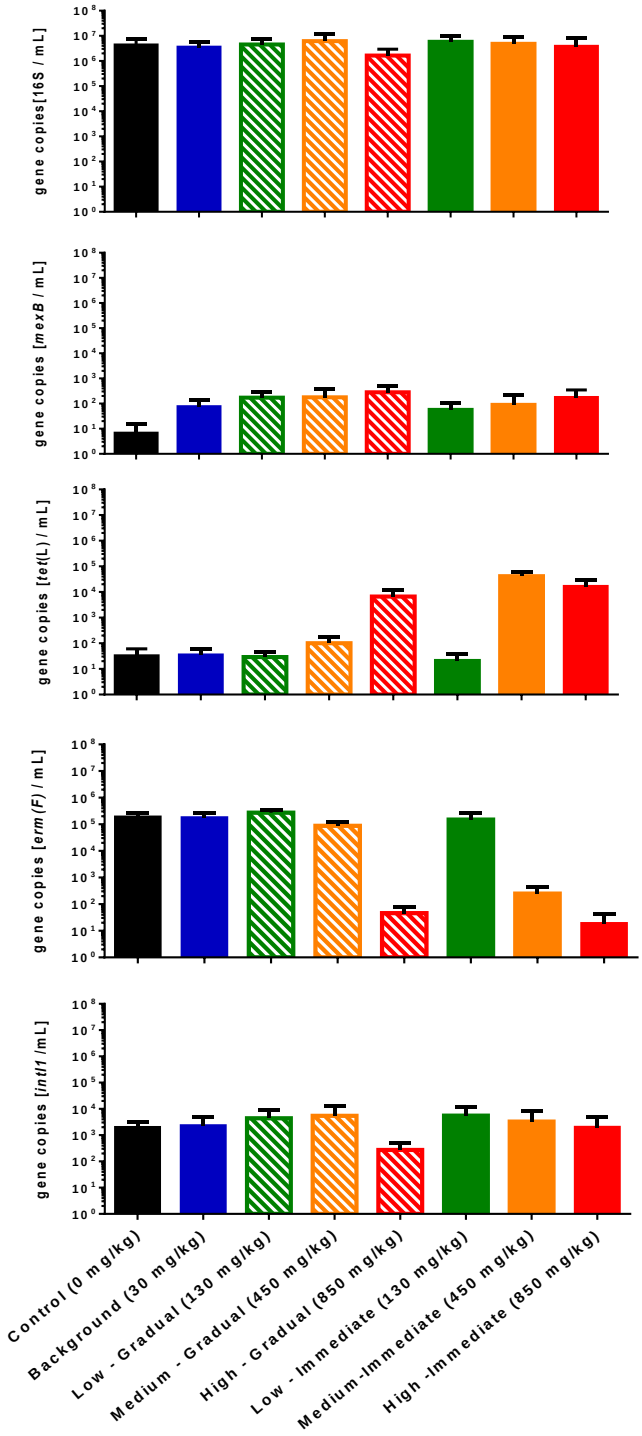

Figure S7**:** Gene abundances normalized to mL of reactor volume. Note no significant differences were found between concentrations of 16S rRNA with ANOVA testing (ANOVA, $p = 0.21$).

Section S8. Total nMDS

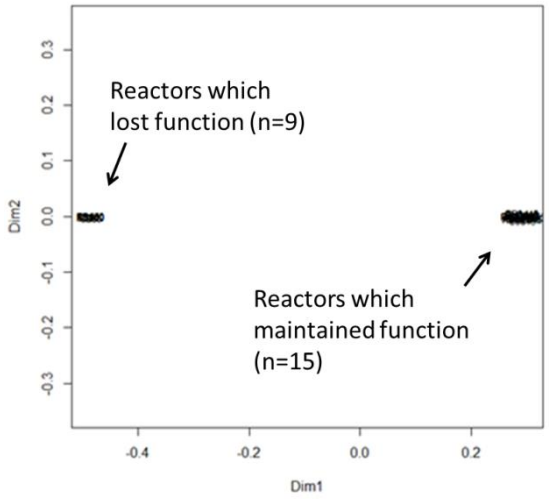

Figure S8: nMDS plot of all reactors at Day 110. Differences between functioning and non-functioning reactors is at a level such that differences cannot be observed within these groups.

Section S9. Digester Biogas Production

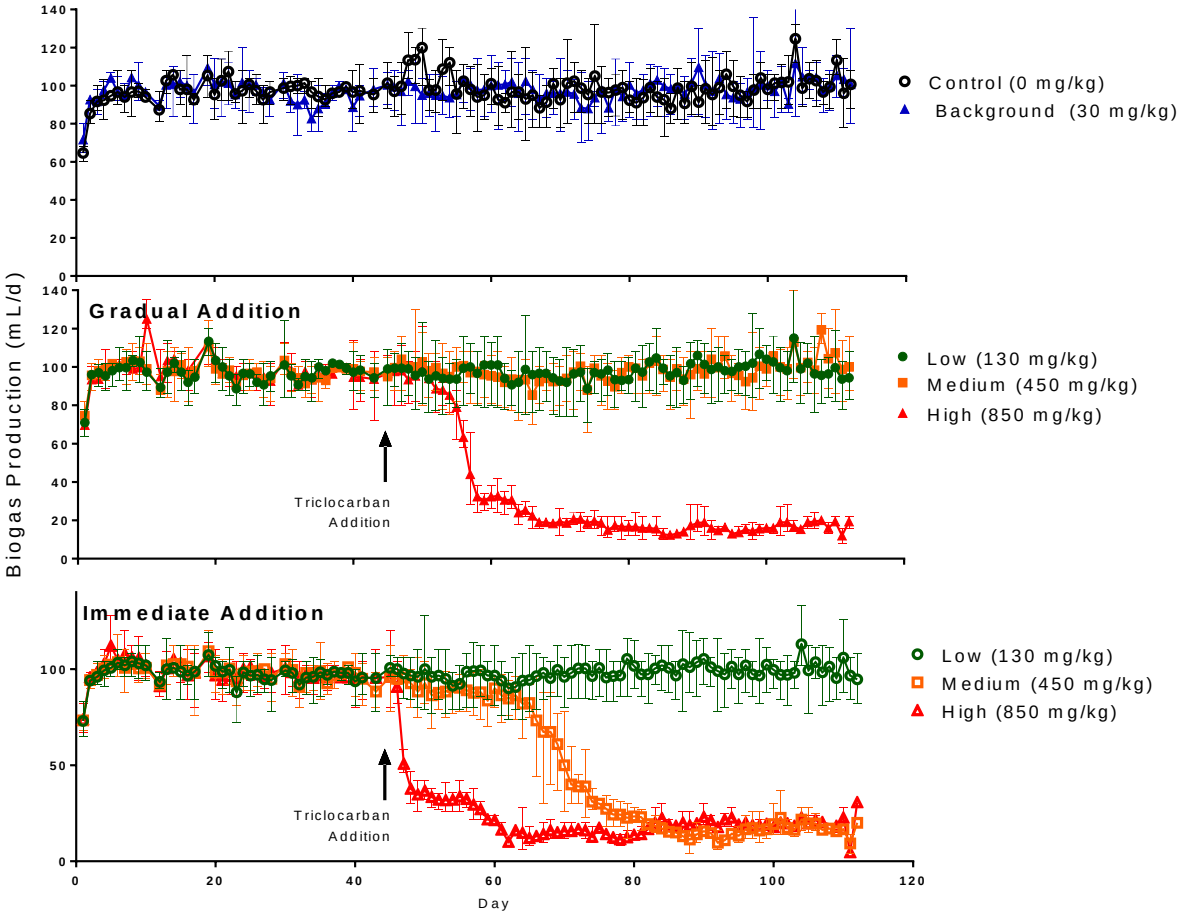

Figure S9: Total biogas produced over the duration of the study. Error bars represent the range of the data.

Section S10. References

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