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Accepted version. *Environmental Science & Technology*, Vol. 48, No. 13 (June 2014): 7393-7400.
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The Impacts of Triclosan on Anaerobic Community Structures, Function, and Antimicrobial Resistance

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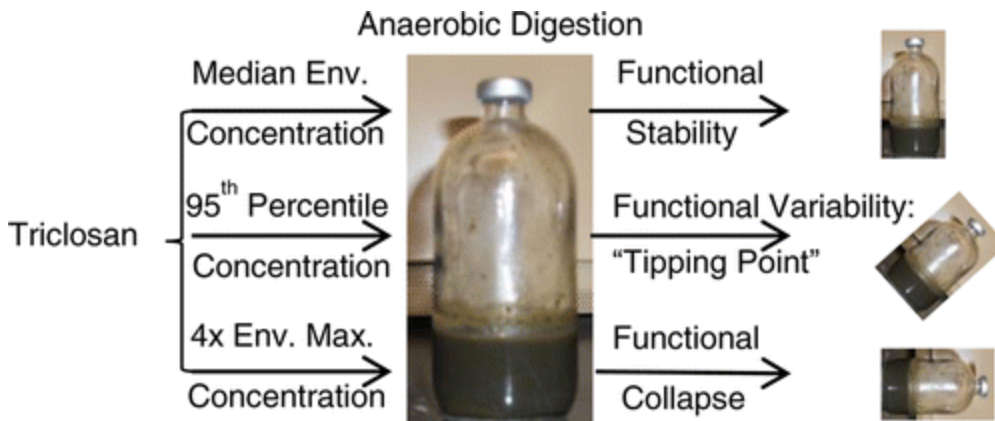
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Abstract



Triclosan is a widespread antimicrobial agent that accumulates in anaerobic digesters used to treat the residual solids generated at municipal wastewater treatment plants; there is very little information, however, about how triclosan impacts microbial communities in anaerobic digesters. We investigated how triclosan impacts the community structure, function and antimicrobial resistance genes in lab-scale anaerobic digesters. Previously exposed (to triclosan) communities were amended with 5, 50, and 500 mg/kg of triclosan, corresponding to the median, 95th percentile, and 4-fold higher than maximum triclosan concentration that has been detected in U.S. biosolids. Triclosan amendment caused all of the *Bacteria* and *Archaea* communities to structurally diverge from that of the control cultures (based on ARISA). At the end of the experiment, all triclosan-amended *Archaea* communities had diverged from the control communities, regardless of the triclosan concentration added. In contrast, over time the *Bacteria* communities that were amended with lower concentrations of triclosan (5 mg/kg and 50 mg/kg) initially diverged and then reconverged with the control community structure. Methane production at 500 mg/kg was nearly half the methane production in control cultures. At 50 mg/kg, a large variability in methane production was observed, suggesting that 50 mg/kg may be a tipping point where function begins to fail in some communities. When previously unexposed communities were exposed to 500 mg triclosan/kg, function was maintained, but the abundance of a gene encoding for triclosan resistance (*mexB*) increased. This research suggests that triclosan could inhibit methane production in anaerobic digesters if concentrations were to increase and may also select for resistant *Bacteria*. In both cases, microbial community composition and exposure history alter the influence of triclosan.

Introduction

Triclosan is a widely used antimicrobial agent that is discharged to the environment from municipal wastewater treatment plants (WWTPs) with both the liquid effluent and the treated residual

biosolids.¹⁻³ Over 100 t of triclosan are estimated to enter WWTPs in the U.S. every year; approximately half or greater of the influent triclosan is sorbed to solid residuals.^{1,3} In addition, triclosan is persistent under anaerobic conditions, resulting in little to no removal during anaerobic digestion.^{2,4} As a result, triclosan concentrations are particularly high in residual biosolids,⁵ up to 133 mg triclosan/kg dry solids in U.S. biosolids.⁶

Triclosan is a micropollutant of concern because it affects a wide variety of organisms. Triclosan can decrease aggression and alter swimming behavior in fathead minnows^{7,8} and decrease strength in mice.⁹ Because triclosan is a biologically active compound that specifically targets *Bacteria*,¹⁰ it can also affect microorganisms in environmental¹¹ and engineered systems.¹² For example, in activated sludge biomass, triclosan can decrease oxygen uptake and inhibit nitrification.¹² The effects of triclosan on the structure and function of anaerobic wastewater communities have not been established, although similar biologically active compounds, such as antibiotics, have been shown to hinder anaerobic digestion¹³ and denitrification.¹⁴ In addition, sublethal exposure to triclosan can increase cross-resistance to other antimicrobials¹⁵ and potentially increase the number of antimicrobial resistance genes (ARGs) in a given environment. Clinical isolates of *Acinetobacter baumannii* from Chinese hospitals developed resistance to triclosan as well as other antimicrobials following triclosan exposure.¹⁶ Triclosan-resistant bacteria exhibiting resistance to other antimicrobials were also found in a wastewater-impacted river.¹⁷ The effect of triclosan on ARGs in anaerobic wastewater communities, where ARGs are abundant,¹⁸⁻²⁰ is poorly understood. Because ARGs are contaminants of concern²¹ with negative public health consequences,²² it is important to understand if triclosan can have an impact on the proliferation of ARGs in anaerobic wastewater communities where triclosan exposure can be high.

The aim of this work was to understand the effects of triclosan on the structure and function of anaerobic microbial communities, including whether exposure selects for genes encoding resistance to antimicrobials. Anaerobic microbial communities are composed of both *Bacteria* and *Archaea*, with methane production directly driven by a subset of *Archaea*, the methanogens. In anaerobic communities,

complex and synergistic relationships exist between different *Bacteria* and between *Bacteria* and *Archaea*, facilitating the breakdown of organic substrates into methane. Experiments were performed with anaerobic microbial cultures that were either previously exposed to triclosan (taken from a municipal anaerobic digester) or had no known previous exposure to triclosan ("previously unexposed," taken from a manure-fed anaerobic digester). Cultures were amended with triclosan at concentrations similar to those currently detected in anaerobic digesters as well as at concentrations that might be expected in the future (4-fold higher than the current maximum detected concentrations). We hypothesized that, at current concentrations, triclosan would shift the composition of the *Archaea* and *Bacteria*, but that function would remain stable. At higher concentrations it was expected that communities would lose function and/or ARGs would proliferate as communities adapted.

Materials and Methods

Previously Exposed Experiment

This experiment was designed to test the hypothesis that increased exposure to triclosan would impact the community structures, function, and the abundance of antimicrobial resistance genes of anaerobic digester communities previously exposed, and presumably adapted, to triclosan. In this experiment anaerobic enrichment cultures were inoculated with biosolids from a bench-scale anaerobic digester (mean hydraulic residence time (HRT) of 35 days) that was processing blended primary and secondary municipal sludge and was originally inoculated with biosolids from a full-scale municipal anaerobic digester. Background levels of triclosan in this inoculum, as quantified by LC-MS-MS, were 3.96 (± 0.83) mg/kg (concentrations and method details given in the Supporting Information (SI), Section S1 and in Table S1).

Serum bottles were used to simulate anaerobic digesters. Their operation and feed schedule are described in detail in the SI, Section S2. Briefly, 160 mL serum bottles had a liquid volume of 60 mL. Each bottle was fed a synthetic acid-alcohol-glucose mixture at 2 g COD/L-day and some bottles were also fed triclosan at time = 0, as described

below. Bottles were incubated at 37 °C in the dark in a shaking incubator (125 rpm). The HRT was 15 days. After the enrichment cultures had been operating for 45 days (three retention times), periodic triplicate samples were taken from each enrichment culture for microbial analysis. The experiment lasted for 57 days.

Triclosan was added to these anaerobic enrichment cultures (set up in triplicate) at nominal concentrations of 0, 5, 50, or 500 mg/kg (concentrations shown in the SI Section S2, Table S4). A published survey on triclosan concentrations in biosolids throughout wastewater treatment plants in the U.S. ($n = 74$) found the median concentration to be 3.9 mg/kg, the 95th percentile triclosan concentration to be 62.2 mg/kg, and the maximum triclosan concentration to be 133 mg/kg.⁶ Therefore, the triclosan concentrations imposed during this experiment spanned this range and offered an opportunity to investigate community structure and function in the case of a 4-fold increase in the current maximum triclosan concentration.

Previously Unexposed Experiments

Long-Term (245 day) Experiment

This experiment was designed to test the hypothesis that environmentally relevant concentrations of triclosan will shift community structure and increase the abundance of ARGs in previously unexposed communities, but will not inhibit function. Indeed, this experiment was designed to mimic what has happened in the past at the field scale with the initial exposure of anaerobic digesters to slowly increasing triclosan concentrations over time. The current median, 95th percentile, and maximum triclosan concentrations in U.S. biosolids are 3.9 mg/kg, 62 mg/kg, and 133 mg/kg, respectively.⁶ To mimic what might have occurred in the past and avoid shocking the previously unexposed organisms, triclosan was initially added at a low concentration (approximately 10 mg triclosan/kg solids). Triclosan concentrations in the culture were subsequently increased over time. At Day 40 the triclosan concentration was increased stepwise from 10 mg/kg to 40 mg/kg, followed by a second stepwise increase to 62 mg/kg on Day 160. From Day 160 to Day 245 (the end of the experiment), concentrations were

increased linearly to approximately 90 mg/kg. The mass of triclosan in each enrichment culture for the duration of the experiment was calculated from the dilution rate, as shown in eq 1, and this mass was then normalized to total solids to generate the concentration of triclosan (see SI, Section S4, Figure S1).

$$C_t = C_o e^{t/\tau} \quad (1)$$

Where t = time since triclosan feed, C_t is concentration of triclosan in enrichment culture at time = t , C_o = initial triclosan concentration following feed, τ = HRT.

All enrichment cultures experienced an unintended temperature drop to 5 °C for 72 h on Day 22 and an increase to 42 °C for 24 h on Day 231. The enrichment cultures used in this experiment were inoculated with biosolids from a bench-scale anaerobic digester (described in the SI, Section S3) that was inoculated with dairy farm biosolids from a full-scale manure digester that had not been previously exposed to triclosan. Triclosan concentrations in the extract from the dairy farm biosolids (0.005 ± 0.0004 mg/kg) were not different than the triclosan concentration in the extract from the sand blank (0.005 mg/kg); this source was therefore considered to be unexposed to triclosan. Background measurements for triclosan are described in SI, Section S1).

Serum bottle anaerobic digesters, similar to those described previously, were used in these experiments; the operation and feed schedule are described in detail in the SI, Section S4. As in the previously exposed enrichment cultures, each bottle was fed an acetate-alcohol-glucose mixture. In contrast, the average organic loading rate was lower, at 0.27 g COD/L-day, and the HRT was higher, at 50 days. A 1.5 mL aliquot was periodically taken from each enrichment culture for DNA extraction and subsequent microbial analysis; the experiment lasted for 245 days.

Short-Term (17-Day) Experiment

This experiment was performed as a proof-of-concept experiment to test the hypothesis that, at higher than currently observed concentrations (i.e., 500 mg/kg), triclosan will select for ARGs in previously unexposed communities. Anaerobic enrichment cultures were established in 160 mL serum bottles and operated as described previously (Previously Exposed Experiment), with the exception that these enrichment cultures were inoculated with biosolids from a bench-scale anaerobic digester (HRT = 45 days) processing cow manure; triclosan concentrations in these biosolids ($<0.24 \pm 0.03$ mg/kg) were lower than the triclosan concentration in the extract from the sand blank; this source was therefore considered to be unexposed to triclosan (see the SI, Table S1). Triclosan was added to these enrichment cultures at approximately 0, 5, 50, and 500 mg/kg to mimic the conditions in Previously Exposed Experiment (concentrations shown in the SI, Table S5). The experiment lasted for 17 days.

Microbiological Methods

DNA Extraction

Biomass samples (1.5 mL) from the previously exposed experiment and the short-term previously unexposed experiment were centrifuged for 10 min at 13 200g; samples from the long-term previously unexposed experiment were centrifuged for 1.5 min (due to lower solids content) at 13 200g. Following centrifugation, the supernatant was discarded. Lysis buffer (120 mM phosphate buffer, pH 8.0, 5% sodium dodecyl sulfate) was used to resuspend the pellets and cells were lysed by imposing 3 freeze-thaw cycles followed by incubation at 70 °C for 90 min. The FastDNA Spin Kit (MP Biomedicals) was used to extract the DNA, which was then stored at -20 °C until use.

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Community fingerprints were produced by ARISA using primers ITSF (5'-GTC GTA ACA AGG TAG CCG TA-3') and ITSReub (5'-GCC

AAG GCA TCC ACC-3') to amplify the intergenic spacer region of *Bacteria*²³ and primers 1389F (5'-ACG GGC GGT GTG TGC AAG-3') and 71R (5'-TCG GYG CCG AGC CGA GCC ATC C-3') to amplify the intergenic spacer region of *Archaea*.²⁴ The PCR protocol was as described previously;²⁵ for further detail see the SI, Section S6. Fragments were resolved by denaturing capillary electrophoresis using an ABI 3130xL Genetic Analyzer (Applied Biosystems) at the University of Minnesota Biomedical Genomics Center. Fragments were binned using the R code "interactive binner"²⁶ for which the window size was set to two base pairs and the shifting window was set to 0.1 basepairs. The percent contribution of one operational taxonomic unit (OTU) to the total community (area of one OTU divided by area of all OTUs) was averaged across replicates, and this average contribution was used for statistical analysis. Replicates from previously exposed and short-term (17-day) previously unexposed experiments were from triplicate samples/DNA extracts. Replicates from the long-term previously unexposed experiment were from triplicate PCR reactions on a single sample/DNA extract.

qPCR

The 16S rRNA, *intI1*, and *mexB* genes were enumerated via qPCR using primers and reaction conditions described previously;²⁷⁻³⁰ further details are provided in the SI, Section S7. Melting curve analysis was used to detect nonspecific amplification. The presence of inhibitory compounds in the DNA extract was tested via qPCR on serially diluted samples; these samples exhibited no indication of PCR inhibition. The number of gene copies in a sample was determined from an external standard curve (nine standards) containing known quantities of template. All standard curves had $R^2 > 0.99$, and PCR amplification efficiencies were between 90% and 110%. Quantification limits for the 16S rRNA, *intI1*, and *mexB* genes were 8600, 1410, and 170 gene copies/ μ L DNA extract, respectively.

Analytical Methods

Gas production was measured by injecting a wetted glass syringe into the serum bottles and measuring the displacement volume. Methane was quantified in 100 μ L gas samples on a gas

chromatograph (GC) equipped with a thermal conductivity detector (TCD) as described previously;³¹ the detection limit was 0.95 nmoles of methane per μL of gas sample injected. The pH was measured on an Orion pH meter (8103BNUWP). Total solids were quantified gravimetrically by drying samples overnight in a 105 °C oven. Volatile solids were determined by difference by quantifying the mass remaining after heating the dried sample for 2 h in a 550 °C furnace.

Statistics

Nonmetric multidimensional scaling (nMDS) was used to analyze the ARISA data. The *vegan* package in R was used for nMDS analysis. Function *vegdist* was used to calculate the Bray-Curtis dissimilarity.³² Dissimilarity between samples corresponds to distance between samples in nMDS plots.³³ Student's *t* tests and F-tests were performed using Microsoft Excel, 2007. One-way ANOVA, two-way ANOVA, and Tukey's multiple comparisons tests were performed using GraphPad Prism 6.0. Statistical significance was set at $p < 0.05$ unless stated otherwise.

Results and Discussion

Effects of Triclosan on the Function and Structure of Previously Exposed Communities

Methane production was impacted by triclosan in previously exposed communities (Figure 1). At the conclusion of the experiment, low concentrations of triclosan (5 mg/kg) caused the average cumulative methane production to be 123% of the average cumulative methane production in the control enrichment cultures. At 50 mg/kg, a large variability in methane production was observed. The relative standard deviation (RSD) between triplicate enrichment cultures was dramatically higher, at 48%, compared to the RSD in the enrichment cultures exposed to 0, 5, or 500 mg/kg, which were less than 10%. Indeed, the variance in the 50 mg/kg enrichment cultures was significantly different from the variance in the 0 and 500 mg/kg enrichment cultures (F-test: p -values = 0.031 and 0.008 for 0 and 500 mg/kg, respectively), and different (with 94% confidence) from the variance in the 5 mg/kg enrichment culture (F-test: p -value = 0.053).

Fifty mg/kg triclosan, or potentially an even lower concentration, may therefore be a tipping point where function is extremely variable and exposure to increased concentrations of triclosan are likely to result in functional declines in anaerobic digester communities. This type of functional decline was clearly evident when anaerobic digester communities were exposed to 500 mg/kg triclosan (Figure 1). Indeed, high concentrations of triclosan (500 mg/kg) resulted in less cumulative methane production (only 56%) compared to the cumulative methane produced in the control enrichment cultures. As a result of the large variability in the 50 mg/kg data set, the cumulative methane production between enrichment cultures was not significantly different based on one-way ANOVA (p -value = 0.07). If the 50 mg/kg enrichment cultures were to be excluded from analysis, the resulting ANOVA test on the 0, 5, and 500 mg/kg enrichment cultures would result in a p -value of 0.0003 with all three data sets being statistically different from each other (posthoc Tukey's multiple comparisons test, p -value = 0.04 for 0 vs 5; p -value = 0.002 for 5 vs 500; and p -value = 0.0002 for 0 vs 500).

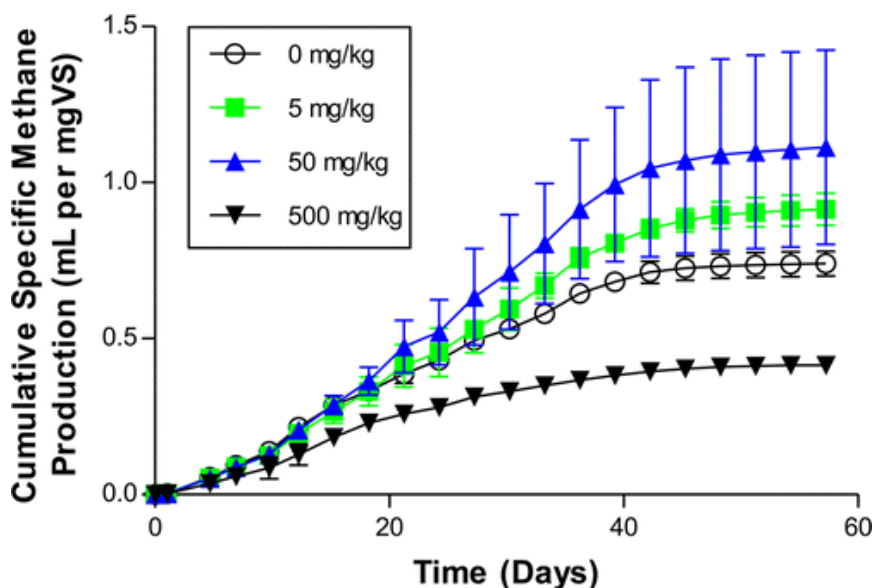


Figure 1. Impact of triclosan on specific methane production in previously exposed communities; 0, 5, 50, and 500 mg/kg are depicted by circles, squares, triangles, and inverted triangles, respectively. Error bars represent standard error of the mean, $n = 3$ for triplicate reactors.

Triclosan addition to the previously exposed anaerobic communities also altered the community composition relative to control communities of both *Bacteria* and *Archaea*, as assessed by

ARISA. Upon triclosan addition, the communities of *Bacteria* diverged from the control communities, and, depending on triclosan concentration, reconverged with the control communities over time. Interestingly, the relative length of time until the reconvergence of triclosan-amended *Bacteria* communities and control *Bacteria* communities corresponded to the concentration of triclosan added (Figure 2), suggesting that the communities may adapt to triclosan, but only if the concentrations were less than a threshold concentration. Indeed, at 500 mg/kg triclosan, the exposed and control *Bacteria* communities did not reconverge before the end of the 57-day experiment. In general the impacts of triclosan on *Archaea* communities were more delayed than the impacts seen on *Bacteria* communities (Figure 3). The 5 mg/kg triclosan dose did not cause separation between triclosan-amended *Archaea* communities and control communities until Day 33 (as opposed to separation in *Bacteria* communities at Day 15), and this separation persisted through Day 57. The 50 mg/kg triclosan dose caused separation at Day 15, sooner than the separation seen in the *Bacteria* communities, but the *Archaea* communities reconverged at Day 33 before separating again at Day 57. The highest triclosan dose caused separation at Day 57. Triclosan rendered *Archaea* communities different from the control communities at Day 57 at all three amended concentrations, but from these results it cannot be determined if the changes in the *Archaea* communities are from direct effects of triclosan or from indirect effects stemming from the altered *Bacteria* communities that are critical for supplying substrate to the methanogenic *Archaea*.

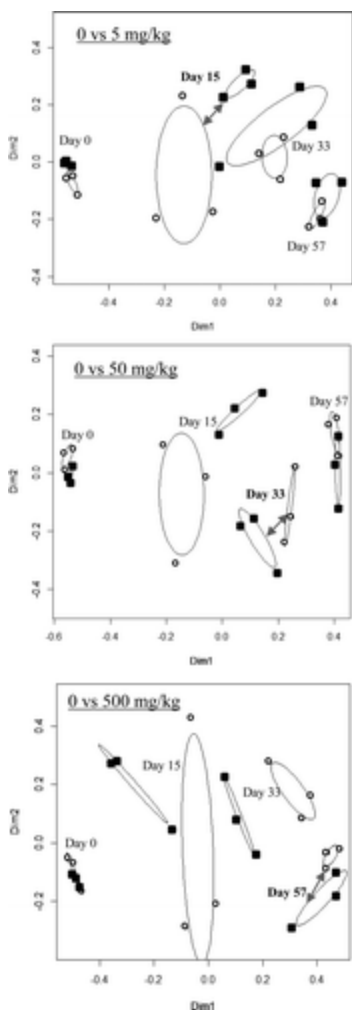


Figure 2. Impact of increased triclosan loading (5 mg/kg on top, 50 mg/kg in middle, 500 mg/kg on bottom) on previously exposed *Bacteria* communities. Samples from days 0, 15, 33, and 57 are shown. The open circles represent control "0 mg/kg" communities in all plots, while the black squares represent triclosan-amended communities for each treatment. Arrows indicate latest time point that triclosan and control communities did not overlap for each treatment.

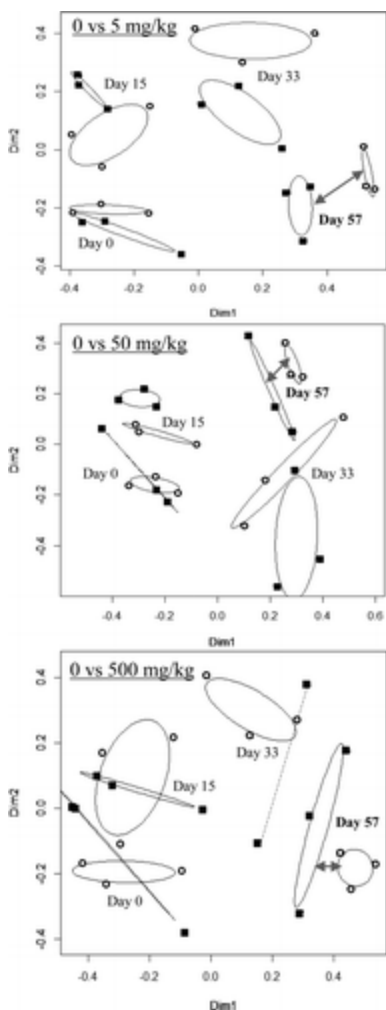


Figure 3. Impact of increased triclosan loading (5 mg/kg on top, 50 mg/kg in middle, 500 mg/kg on bottom) on previously exposed *Archaea* communities. Samples from days 0, 15, 33, and 57 are shown. The open circles represent control “0 mg/kg” communities in all plots, while the black squares represent triclosan-amended communities for each treatment. Dashed line in “0 vs 500 mg/kg” at Day 33 is used to depict distance between duplicate samples. Arrows indicate latest time point that triclosan and control communities did not overlap for each treatment.

Neither the *intI1* gene (Figure 4), which allows for adaptation³⁴ and the acquisition of exogenous ARGs,³⁵ nor the *mexB* gene, a constituent of MexAB-OprM efflux pump that confers resistance to triclosan in *Pseudomonas aeruginosa*²⁹ and *Rhodospirillum rubrum* *S1H*,³⁶ increased in abundance with triclosan exposure, with *mexB* below the detection limit in all enrichment cultures (data not shown). In the 500 mg/kg enrichment cultures, the abundance of *intI1* genes normalized to 16S rRNA genes actually decreased relative to the control by Day 57 (Tukey’s multiple comparisons test p -value = 0.001

following two-way ANOVA where $p < 0.001$ for time and $p = 0.22$ for triclosan; see SI Section S8 for statistical results). These results corroborate both the ARISA and functional results and the seeming inability of this community to adapt to increasing triclosan concentrations under the time conditions of this experiment.

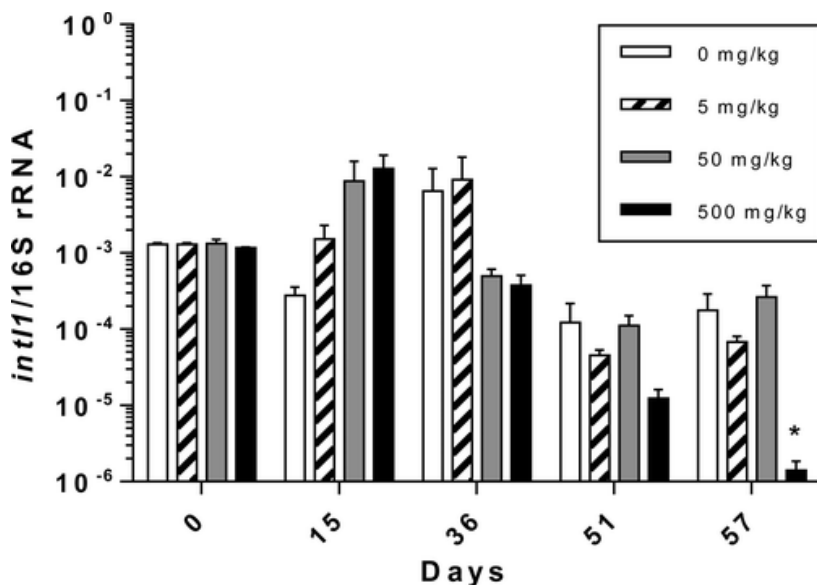


Figure 4. Impact of triclosan on abundance of *intI1* (normalized to 16S rRNA gene copies) in previously exposed communities. Error bars represent standard error of the mean, $n = 3$ for triplicate reactors. The (*) denotes statistical significance at $p < 0.05$ (using Tukey's multiple comparisons test) between 500 mg/kg and 0, 5, and 50 mg/kg enrichment cultures at Day 57 based on log of ratio between *intI1* and 16S rRNA copies.

Effects of Triclosan on the Function and Structure of Previously Unexposed Communities

Unlike the trends observed above, triclosan addition did not substantially impact methane production in previously unexposed communities (Figure 5). A subtle increase in methane production was observed in the triclosan-amended enrichment cultures over the first 71 days of operation; nevertheless, this increase was not statistically significant (t test p -value = 0.16). From days 71 to 245, the average methane produced in the triclosan-amended enrichment cultures was only 6% greater than in the control enrichment cultures (Figure 5). These results demonstrate that previously unexposed anaerobic communities can maintain function under the stress of triclosan exposure at concentrations of 90 mg/kg. Indeed, full-scale anaerobic

digesters are still performing adequately at current triclosan levels (median concentration: 3.9 mg/kg⁶).

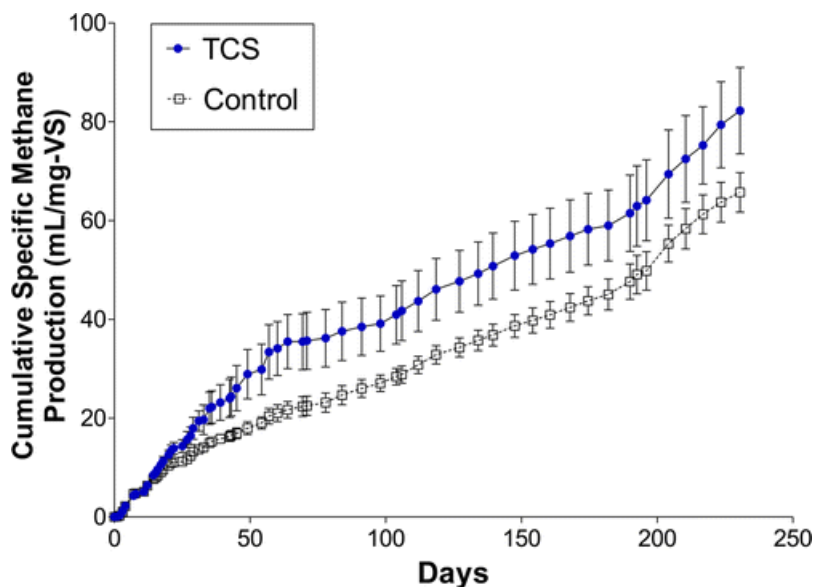


Figure 5. Impact of triclosan (15 mg/kg to 90 mg/kg from Day 0 to Day 245) on cumulative specific methane production in previously unexposed communities. Error bars represent standard error of the mean, $n = 3$ for triplicate reactors.

With respect to community structure, triclosan exposure resulted in an initial community shift followed by structural divergence in *Bacteria* at triclosan concentrations ≥ 60 mg/kg. A *Bacteria* community shift away from the control communities was observed after just 20 days, but by Day 71, the communities had reconverged (SI: Figure S2). When triclosan concentrations increased again, to approximately 70 mg/kg by Day 196, the exposed and control communities again separated, and they remained separated for the remainder of the experiment through an additional increase in triclosan to 90 mg/kg. Similar to the previously exposed experiments at 50 mg/kg, in these experiments no functional decline was observed, but a greater variability of methane production was apparent (Figure 5), as was a seemingly permanent *Bacteria* community shift after Day 71. These results again suggest that triclosan concentrations around 50 mg/kg may be close to a tipping point where community structure is substantially altered, with possible ramifications in terms of decreased function. The impacts of triclosan on *Archaea* were unclear as the initial communities were different (SI Section S9: Figure S2).

No significant difference in copies of *mexB* or *intI1* was observed between triclosan and control cultures in these long-term experiments (see SI Section S10, Figures S3–S4). Additional short-term experiments were therefore performed to determine if exposure to higher triclosan concentrations selected for *mexB* or *intI1*. Although *intI1* did not increase in abundance upon triclosan exposure relative to control cultures (see SI Section S11: Figure S5), *mexB* was detected only in the enrichment cultures amended with 500 mg/kg triclosan (Figure 6). In contrast to the observations made with the previously exposed anaerobic microbial communities, methane production was not significantly different between the 500 mg/kg enrichment cultures and control enrichment cultures (t test p -value = 0.172). It is possible that *Bacteria*, which play a key role in overall anaerobic digestion, were not susceptible to negative effects of triclosan after acquiring ARGs. This result suggests that, perhaps depending on the specific community investigated, increased levels of triclosan could either cause functional collapse, as observed with the previously exposed communities, or force anaerobic digester communities to adapt through the proliferation of genes that provide resistance to triclosan.

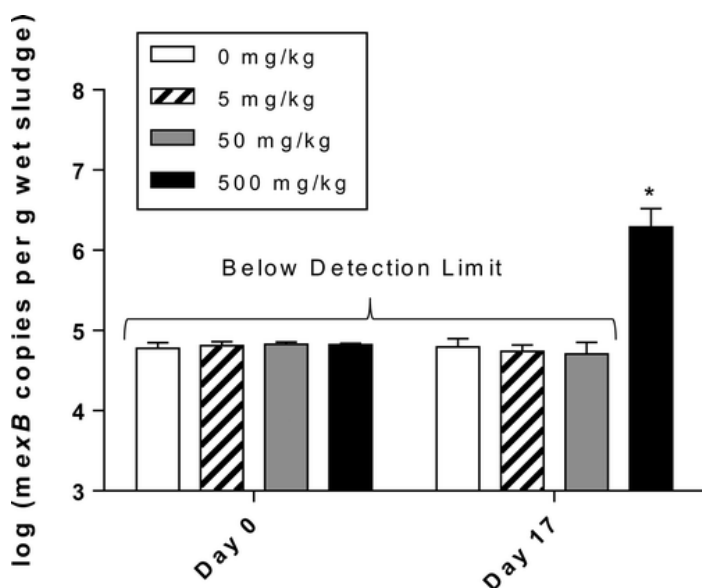


Figure 6. Impact of triclosan on abundance of *mexB* (normalized to grams of wet sludge) in previously unexposed communities (17-day experiment). Error bars represent standard error of the mean, $n = 3$ for triplicate reactors. Only 500 mg/kg at Day 17 (three enrichment cultures) yielded results above detection limit; *mexB* was not present in any other samples above detection limit. The variability in samples below detection limit stems from variability in grams of wet sludge extracted for each sample, that is, the detection limit was the same for each sample.

Implications

Very little research has been performed to address the impacts of triclosan on anaerobic communities, which are critical for the function of municipal anaerobic digesters in which high triclosan exposure occurs. Depending on the concentration of triclosan and the exposure history of the anaerobic digester community, we observed that triclosan impacted community structures, overall digester function (as quantified by the functional end-point, methane production), and the proliferation of an ARG. Indeed, our research suggests that environmental concentrations of triclosan may already be at, or may be approaching, a tipping point in anaerobic digesters where performance becomes highly variable (Figure 1). In addition, concentrations only 4-fold higher than the currently detected maximum (133 mg/kg⁶), decreased methane production to approximately 50% of control levels in previously exposed communities (Figure 1). This result is another indication that we may be closer than previously thought to functional declines in anaerobic digester performance as a result of triclosan exposure. The selection of antimicrobial resistance genes is one adaptation mechanism to antimicrobials;³⁷ interestingly, we observed that, when function was not negatively affected by high (500 mg/kg) triclosan exposure, *mexB* increased in abundance. The increase in *mexB* with exposure to 500 mg/kg triclosan in the previously unexposed communities implies that triclosan preferentially selected for resistant bacteria. This research suggests that the microbial community composition and exposure history alter the influence triclosan has on function and the selection of ARGs. More research is needed to determine what role, if any, resistance genes play in maintaining the functional health of anaerobic digesters exposed to triclosan. Other researchers have recently shown that triclosan alters the community structure and selects for resistant bacteria in aerobic sediments³⁸ and also selects for ARGs in aerobic activated sludge microcosms,³⁹ which is consistent with our observations. The proliferation of triclosan-resistant bacteria in anaerobic digesters could also introduce more triclosan-resistant bacteria into the environment following the land application of biosolids.^{40,41}

A limitation to this research is that previously exposed methanogenic communities were amended with high concentrations of triclosan at Day 0, as opposed to being exposed to a slow increase in triclosan concentration over time, as would be expected in full-scale anaerobic digesters. This loading regime may have impacted the community's ability to adapt, as microbes can have increased toxicity tolerance following ramp-up periods (e.g., ref 42). Nevertheless, the digesters in the United States are in some cases at, or very close to, the triclosan concentrations at which a functional tipping point could be breached with subsequent functional implications.

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

The authors declare no competing financial interest.

Acknowledgment

PJ.M. was supported by the NSF Integrative Graduate Education and Research Traineeship (IGERT) under Grant No. DGE-0504195 and the Water Environment Federation Canham Graduate Studies Scholarship.

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