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Biodegradation of an antimicrobial compound triclosan under sulfate reducing condition

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Abstract

Triclosan is an antibacterial and antifungal agent that is present in many detergents and surgical cleaning treatment products. This antimicrobial compound is discharged from households and collected at the local sewage treatment plant. Because triclosan inhibits phospholipid biosynthesis, it affects the microbial population that perform waste degradation. Sewage treatment plants are the major reservoir of triclosan as the wastewater from various households are discharged and treated in the local sewage treatment plant. This study was conducted to determine whether triclosan degradation occurs in the anaerobic digester of the Thibodaux Sewage Treatment Plant. Bacterial enrichment cultures were developed under various electron acceptor conditions including nitrate-reducing, sulfate-reducing, and mixed electron acceptor condition. The results showed the bacterial consortia developed under various conditions were not inhibited by 100 ppm of triclosan. More than 96% of triclosan was removed in both co-metabolic and triclosan as the sole carbon source conditions under sulfate-reducing condition. The molecular analysis of the consortium showed wide biodiversity of bacteria in the consortium.

Keywords :
Anaerobic digester, sulfate reducing bacteria, triclosan, biodegradation, *Desulfovibrio*

1 Introduction

Personal care products (PCPs) are household products used for personal hygiene and disinfection. Personal care products from hospitals and healthcare facilities, domestic and industrial sources and landfill leachates can enter ecosystems via effluents/wastewaters and cause harmful effects on organisms (Miazek Brozek-Pluska et al., 2019). Many PCPs contain antimicrobial agents that can be harmful to organisms and the environment.

In September 2016, the U. S. Food and Drug Administration banned the recreational use of nineteen antimicrobial agents including triclosan. Before the banning, triclosan was used as an antimicrobial in more than 2000 products such as soaps, toothpastes, detergents, clothing, toys, carpets, plastics, and paints (Halden et al., 2017).

Triclosan (5-chloro-2-(2-4-dichlorophenoxy)phenol) is a polychloro phenoxy phenolic compound. It is slightly water soluble (10 mg/L at 20°C) and readily soluble in organic solvents. Triclosan has a log Kow value of 4.76, and it has high sorption potential as a result. Because of its high Kow value, triclosan adsorbs onto settled sewage sludge, which is incorporated into biosolids. It is a weak acid (pKa = 8.1), and it can readily disassociate as a result (Dhillon et al., 2015).

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Developed in the 1960s, triclosan was quickly produced at an increased volume and incorporated into antiseptic personal care products (Halden 2014). Triclosan is widely used in personal care products in concentrations of up to 0.3% (Ribado et al. 2015). Annual global production of TCS was estimated at 1500 tons, and a total of 132 million liters of TCS-containing products was used in a single year in the United States (Alfhili and Lee, 2019).

Phenolic chemicals are an important group of ubiquitous environmental pollutants that include some well-known endocrine disruptors such as triclosan and bisphenol A (BPA) and biotransformation products of aromatic compounds such as hydroxylated benzo(a)pyrene (OH-BaP) (Ashrap et al., 2017). Triclosan itself has been shown to be a weak endocrine disruptor, but it can transform phase I, II, and III drug-process genes by binding to the nuclear receptor pregnane X receptor (PXR) and constitutive androstane receptor (CAR) (Yueh Tukey., 2016). It has also been shown to adversely affect thyroid hormone homeostasis and produce some carcinogenic effects such as liver tumorigenesis in mice (Yueh Tukey., 2016). Because triclosan is lipid-soluble, it can easily cross cell membranes (Ying et al., 2007). When triclosan is exposed to bacteria, it enters the cell and inhibits the enzyme enoyl-acyl carrier protein reductase, which is essential for phospholipid biosynthesis. Triclosan mimics the natural substrate of the enzyme, nicotinamide adenine dinucleotide (NAD^+) and blocks the active site, which terminates the production of fatty acids (Lubarsky et al., 2012). Triclosan can also be bactericidal at higher concentrations by destabilizing cell membranes (Petersen., 2016).

Bacteria that are exposed to triclosan can develop resistance through mutations in the fabI gene that encodes enoyl-acyl carrier protein reductase, which cause overexpression of the enzyme, or through efflux pumps. Exposure to triclosan can also promote

the distribution of antimicrobial and antibiotic resistance genes through gene transfer (Drury et al., 2013). This is because genes encoding resistance to a broad range of antibiotics have been found to be able to spread among bacteria via horizontal gene transfer (Hong et al., 2018). The incidence of triclosan use has led to more resistant isolates in various geographical locations. Ribado et al. (2017) found that the routine use of triclosan-containing toothpaste increased the abundance of antibiotic-resistant *Proteobacteria* in infants and adults. Triclosan is used as an antimicrobial agent in coated sutures, and the presence of triclosan decreases the incidence of surgical site infections (Ahmed et al., 2019). In clinical practice, TCS is used as a disinfectant and an antiseptic in surgical sutures, scrubs, implants, and medical devices. Although the occurrence of infection is decreased, the use of triclosan in a clinical setting has been shown to promote triclosan resistance. Current isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* are less susceptible to triclosan than similar isolates from 1965 (Skovgaard et al., 2017). Approximately 96% of triclosan-containing products end up down the drain (McAvoy et al., 2002). Because of its widespread use in personal and industrial products, triclosan has been discharged into local sewage systems, where it can spread to local waterways. In 2002, triclosan was detected in 58% of 139 streams in the United States at concentrations of 0.14 to 2.3 µg/L (Lee et al., 2012). Triclosan can persist in soils with a half-life of several months, and degradation depends on the microbial composition of individual soils (Waria et al., 2011). Because of the physical and chemical properties of triclosan, it can quickly absorb into the tissue of organisms. A study by Calafat et al. (2007) reported that triclosan, in concentrations of 2.4 – 3,790 µg/L, was detected in the urine samples of 74.6% of patients above the age of 6. Another study by Bever et al. (2018) detected triclosan in the breast milk of 27% of patients.

Triclosan can be biotransformed into potentially more toxic and persistent compounds, such as chlorinated phenols and biphenyl ethers after chlorination, methyl triclosan after biological methylation, and chlorinated dibenzodioxins after photooxidation. These harmful compounds can be cytotoxic, genotoxic, and disruptive to endocrine processes in aquatic organisms such as fish, crustaceans, and algae (Bedoux et al., 2012). Algae are shown to be especially susceptible to the toxicity of triclosan. In a study by Tatarazako et al. (2004), microalgae were shown to be 30 to 80-fold more sensitive to triclosan than bacteria and fish. Because of these detrimental effects, researchers have conducted experiments to characterize potential techniques of removal of this harmful compound from the environment. Many bacterial consortia of activated sludge and pure cultures have been reported to degrade triclosan and use it as a carbon source (Chen et al., 2011). Biodegradation is the biologically catalyzed reduction in the complexity of an organic compound by microorganisms. The microbial biodegradation of triclosan will tell us how long it persists in nature. Ecological observations suggest that sulfate-reducing, nitrate reducing, and methanogenic bacteria might metabolize nitroaromatic compounds under anaerobic conditions if appropriate electron donors and electron acceptors are present in the environment (Boopathy, 2007). Under anaerobic conditions, the sulfate-reducing bacterium, *Desulfovibrio* sp. (B strain) transformed trinitrotoluene (TNT) to toluene (Boopathy and Kulpa, 1992; Boopathy et al., 1993) by reduction and deamination reactions. Gorontzy et al. (1993) reported that under anaerobic conditions, methanogenic bacteria reduced nitrophenols and nitrobenzoic acids. Preuss et al. (1993) demonstrated conversion of TNT to triaminotoluene by a *Desulfovibrio* sp.

Biodegradation of triclosan has been reported in soil and wastewater treatment systems. In a study on the biodegradation of triclosan in biosolids-amended soils, 80% of [14C] triclosan had been degraded into methyltriclosan after 18 weeks (Waria et al. 2011). In a study on aerobic wastewater treatment conditions, a starting concentration of triclosan of 1-2 mg/L was almost completely removed in only 150 hours, where the final concentration remained at less than 0.01 mg/L until the end of the experiment (Chen et al. 2011). Although triclosan under aerobic condition shows relatively rapid degradation, triclosan is degraded slowly under anaerobic conditions, a process that can take longer than 70 days (Kim et al. 2011). In a study by Lee et al. (2012), a wastewater microorganism, the KCY1 strain of the bacterium *Sphingophyxis*, was found to degrade triclosan into individual metabolites via meta-cleavage and produced various metabolites (Lee et al., 2012).

Sewage treatment plants are the major reservoir of triclosan as the wastewater in the ecosystem from various households are discharged and treated in the local sewage treatment plant. This study was conducted to determine whether triclosan degradation occurs in the anaerobic digester of a Sewage Treatment Plant. Although triclosan biodegradation has been well documented in other studies, there is little research on triclosan biodegradation in the effluent from a small rural sewage treatment plant. The purpose of this study was to determine the triclosan biodegradation potential of native bacteria in a rural sewage treatment plant.

2 Materials and methods

2.1 Sample collection

Anaerobic digester sludge was collected from the Thibodaux Sewage Treatment Plant, Thibodaux, Louisiana, USA. This sludge was placed into a sterile plastic collection bottle, transported to Nicholls State University, and stored at 4°C within 30 minutes of collection.

2.2 Bacterial cultivation

The sludge was used to establish four different consortia of bacteria. All enrichment media were based on Basic Mineral Salt (BMS) Medium, which contained the following components (g/L): K_2HPO_4 (3.5), K_2HPO_4 (1.5), $MgSO_4$ (0.1), NaCl (0.1), $(NH_4)_2SO_4$ (0.25), yeast extract (0.1), glucose (1.0). For the nitrate-reducing condition, 20 millimole of KNO_3 was added electron acceptor. For the sulfate-reducing condition, 20 millimole of Na_2SO_4 was added as electron acceptor. For the mixed condition, 20 millimole each of KNO_3 and 0.25 g/L of Na_2SO_4 were added. A first enrichment was created using 250 mL of media containing 100 ppm of triclosan. One mL of sludge was added after autoclaving the media. The culture bottles were made anaerobic using a gassing manifold system replacing the air in the headspace of the culture bottles with nitrogen gas as shown by Boopathy et al. (1993). After one week, 1 mL of the first enrichment was added to 250 mL of a second enrichment containing 100 ppm of triclosan. The second enrichment was incubated for one week. This second enrichment was used as the inoculum source for all further experiments.

The best results in terms of bacterial growth in the presence of triclosan was observed under sulfate reducing conditions. Further experiments were conducted under sulfate reducing condition under various culture conditions including, triclosan as the sole carbon source, co-metabolic condition with glucose as main substrate, and an abiotic control. The experiments were conducted in triplicates. For the sole carbon source, 1% inoculum of sulfate reducing bacterial consortium was used to start the experiment. The initial triclosan concentration in the culture bottle was 100 ppm, which was dissolved in methanol and the methanol was allowed to evaporate and the residual triclosan was reconstituted with 100 ml BMS medium. In the co-metabolic condition, 1000 ppm of glucose served as main carbon source along with 100 ppm of triclosan. For the abiotic control, after the 1% inoculum was added to the medium, the culture bottles were autoclaved to have killed control. Bacterial growth, organic carbon, triclosan, and ammonia were monitored as per the methods given below in the analytical methods.

2.3 Analytical methods

Bacterial growth in the culture samples was monitored daily by optical density method. Optical density was measured daily with a HACH DR6000 spectrophotometer using the method given by HACH (1999). Optical density was measured at a wavelength of 600 nm. The amount of chemically oxidizable carbon (COD) was measured on days zero and fourteen using colorimetric Reactor Digestion Method using HACH HR COD following the method given by HACH (1999). A quantification of ammonia was recorded using the colorimetric Nessler method (HACH, 1999). Three drops of mineral stabilizer, three drops of polyvinyl alcohol, and one mL of Nessler reagent were added to a cell containing 25 mL of the sample. Cells were manually inverted and left undisturbed for one minute to allow completion of the reaction. The HACH DR6000 was used to quantify the sample in mg/L at a wavelength of 425 nm.

Triclosan concentration was measured according to the methods of Mahitha et al. (2014). Triclosan and its metabolites were monitored using a Hitachi HPLC-system (Hitachi LaChrome Elite L-2310; Dallas, TX, USA) with a UV-Vis detector L-2420, a column oven (Hitachi model L-2350), an L-4000 autosampler injector, and a C-18 ZORBAX Eclipse PAH, 5 µm, 4.6 x 150 mm (Agilent, Santa Clara CA, USA). The mobile phase was acetonitrile:water (70:30) with 0.02% trifluoroacetic acid solution at a flow rate of 1.0 ml/min with an injection volume of 20 µl at 23°C. Standards of 0.5, 1.0, 3.0, 5.0, 10.0, and 15 ppm triclosan were used to establish the standard curve. Frozen enrichment culture samples were prepared and shipped to the Mr. DNA laboratory in Shallowater, TX, USA for bacterial diversity analysis. Results were used to determine the major bacterial groups present in the consortium.

2.4 Statistical analysis

Analysis of variance (ANOVA) was performed using R software (version 4.0.4) according to the methods of Valiela (2009) to detect whether there is a significant difference between treatments. Results were presented as average ± standard deviation from triplicate cultures used in the experiment.

3 Results and discussion

The Thibodaux sewage treatment plant serves the city of Thibodaux in treating domestic and commercial wastewater. Thibodaux is a small town of approximately 15000 people. Triclosan concentration was analyzed in the activated sludge and the anaerobic digester twice during the study period to find the concentration of triclosan the bacteria are exposed to during the sewage treatment process.

Table 1 Concentration of triclosan in sewage samples collected from the Thibodaux sewage treatment plant

	January, 2019		May, 2019	
Antibiotic	Aerobic ponds	Anaerobic digester	Aerobic ponds	Anaerobic digester
Triclosan (µg/L)	1.35	4.79	1.44	5.61

Table 1 shows the concentration of triclosan in the sewage treatment plant. The concentration of triclosan was high in the anaerobic digester compared to the activated sludge aerobic pond. The concentration was almost four times higher in the anaerobic digester sludge compared to the aerobic pond. This concentration is comparable to the triclosan value reported in the literature in microgram levels. Lee et al. (2012) reported the triclosan concentration of 0.14 to 2.3 µg/L in various streams in the US. Triclosan is a bacteriostatic biocide that interrupts the synthesis of phospholipids in bacteria. It acts as a xenobiotic and enoyl ACP reductase inhibitor. Because of its persistence, it is imperative to determine efficient and innocuous methods of neutralization. Biodegradation by bacteria is the efficient way to reduce the concentration of triclosan in the environment.

3.1 Bacterial growth under various electron acceptor conditions

Initial screening of the anaerobic digester sludge from the Thibodaux sewage treatment plant indicated the bacteria were able grow in all electron acceptor conditions and there was no severe inhibition of triclosan at 100 ppm concentration. The best bacterial growth was observed under sulfate reducing conditions followed by mixed electron acceptor conditions and nitrate reducing conditions (Figure 1). The maximum growth of 0.98 optical density (OD) unit was observed under sulfate reducing conditions on day 7. There was no lag phase in the cultures in all conditions and this is due to the presence of triclosan in the anaerobic digester as shown in Table 1. The bacteria are constantly exposed to triclosan in microgram level and the bacteria in the sewage treatment plant are adapted to this antimicrobial and in the presence of 100 ppm, the organisms did not show any lag phase and any severe inhibition of growth. Based on these results, further experiments were carried out under various growth conditions using sulfate reducing bacterial (SRB) consortium.

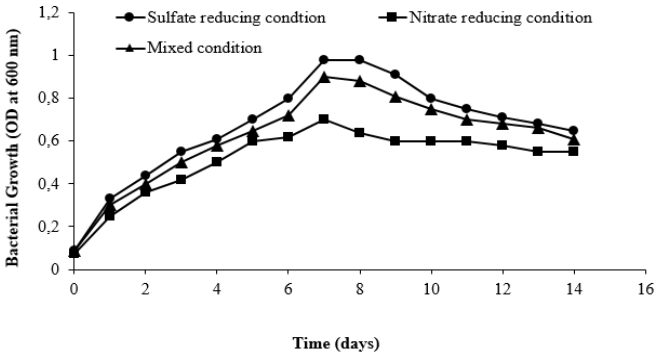

Figure 1 Growth of bacterial enrichment cultures grown under co-metabolic conditions with various electron acceptors in the presence of 100 ppm triclosan. Data represent average of three cultures for each condition and the standard deviation (SD) was less than 0.5%

3.2 Growth of SRB and metabolism of triclosan under various growth conditions

The SRB consortium was grown with triclosan as the sole carbon source and also under co-metabolic condition with glucose as the main substrate. An abiotic control was also maintained in triplicates as described in the methods section. The results are given in Figure 2. The best bacterial growth was observed under co-metabolic condition as the bacteria in the consortium had more carbon in the culture with 1000 ppm glucose plus 100 ppm of triclosan. The SRB consortium was able to use triclosan as the sole carbon source and the growth in this condition was nine times less due to limited carbon availability for bacterial growth. In abiotic control, there was no growth. Bacterial growth in co-metabolic condition was significantly higher compared to triclosan as the sole carbon source condition with a p-value of 0.012.

The concentration of triclosan in the culture medium was removed by 99% in the sole carbon source compared to co-metabolic condition in which the triclosan removal was 97% within 14 days of incubation (Table 2). There was virtually no removal of triclosan in the control indicating there was no physical and chemical removal of triclosan in the cultures. There was no COD re-

moval in the control. Maximum removal of COD was observed in the cultures that received Triclosan as the sole carbon source (97.4%) and in the co-metabolic condition, the COD removal was 94.1%. The nitrogen removal showed similar trend in the cultures indicating the SRB's ability to grow on triclosan and remove carbon and nitrogen in the culture medium. The ability of SRB to degrade triclosan in the anaerobic digester might have been evolved due to constant exposure to triclosan. In the environment, there are multiple substrates available for bacteria and the co-metabolic conditions show even with the simple carbon source like glucose used in this study, the SRB consortium was able to degrade triclosan and this kind of microbial activities might be happening in the sewage treatment plant.

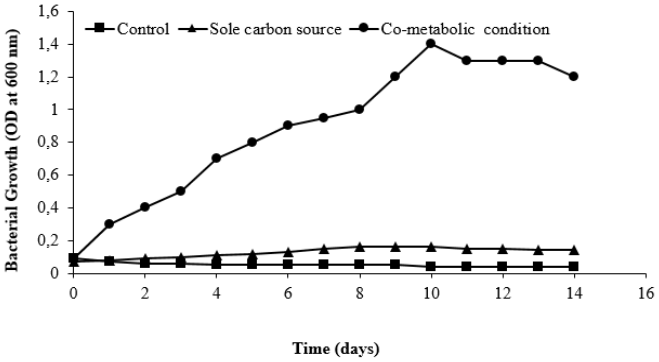

Figure 2 Growth of sulfate reducing bacterial consortium under various growth conditions. Data represent average of three cultures for each condition and the standard deviation (SD) was less than 0.5%

Table 2 Carbon, nitrogen, and triclosan removal in sulfate reducing bacterium consortium

Culture conditions	Removal percentage (%)		
	COD	Nitrogen	Triclosan
Abiotic control	0.3 ± 0.1	0.0	0.5 ± 0.15
Triclosan as sole carbon source	97.4 ± 1.1	96.8 ± 2.3	99.1 ± 2.3
Co-metabolic condition	94.1 ± 1.8	95.7 ± 1.7	97.0 ± 2.1

The highest growth was observed in sulfate-reducing conditions under co-metabolic conditions when glucose acted as a co-substrate. When triclosan was used as the sole source of carbon, bacterial growth was weak, which was likely due to the difference in carbon concentration between the co-metabolic and sole carbon source conditions. Because of triclosan's low solubility in water, we only used 100 ppm in the culture to circumvent toxicity. As a result, the carbon concentration in the co-metabolic condition was 10 times more (1000 ppm glucose in the medium) than in the sole carbon source. The growth and carbon removal after solubilizing triclosan in methanol and reconstituting it in aqueous media ensured an even distribution of triclosan. This study provided evidence that triclosan is not toxic to SRB consortium at a concentration of 100 ppm as bacteria used triclosan as the sole carbon source. Carbon and nitrogen are essential for bacterial growth. The bacterial cell has the C:N ratio of 10:1, and organic carbon and nitrogen are required at this C:N ratio for successful growth of heterotrophic bacteria. Boopathy (2017) showed anaerobic degradation of atrazine in which the bacterial consortium was able to use the nitro group present in the atrazine as nitrogen source for growth. Boopathy (2017) reported that the ammonia concentration in the culture medium increased gradually at the beginning and later it decreased because the bacteria used the ammonia that is generated from atrazine metabolism for growth and cellular functions.

Table 2 shows the percent removal of COD, nitrogen and triclosan under various culture conditions. Triclosan was removed 99.1% under the culture conditions where triclosan was the only carbon source in the medium compared to 97% removal under co-metabolic condition. Similarly, the carbon removal in the form of COD was high in the culture where Triclosan served as the sole carbon source. Nitrogen removal also followed the same trend with a maximum nitrogen removal of 94.1% in the Triclosan as the sole carbon source condition. There was no significant difference between co-metabolic and sole carbon source conditions in relation to removal efficiencies of carbon, nitrogen, and triclosan.

3.3 Biodegradation of triclosan

The HPLC analysis of SRB cultures showed the presence of metabolites. The metabolites were present only in conditions in which both bacteria and triclosan were present and not in the abiotic control. Because the triclosan in the media decreases as this unknown metabolite increased, it is assumed that triclosan was degraded. According to Lee et al. (2012), triclosan can be degraded into monohydroxy-triclosan, 6-chloro-3-(2,4-dichlorophenoxy)-4-hydroxycyclohexa-3,5-diene-1,2-dione, 3-chloro-4-(5,7-dichloro-3-oxo-2,43- dihydrobenzo[1,4]dioxin-2-yl)-2-oxobut-3-enal, 3,5-dichloro-4,6-dihydroxycyclohexa-3,5-diene-1,2-dione, and 2,4-dichlorophenol (Figure 3). All metabolites were observed during the 5 days, but only 2-4-dichlorophenol remained after five days. It is likely that all other metabolites were degraded after five days of incubation. Based on the literature, the unknown metabolite is likely 2-4-dichlorophenol. Future studies should focus on identifying the metabolites and detailing the complete metabolic pathway for triclosan.

3.4 Bacterial biodiversity analysis in the SRB consortium

The molecular analysis was used to construct operational taxonomic units (OTUs) of the SRB cultures. According to the taxonomic annotation results, the top 10 taxa sample at each taxonomic rank (Phylum, Class, Order, Family, Genus) were selected to form the distribution histogram of relative abundance of taxa, which is used to visually see the taxa with higher relative abundance and their proportion in different classification levels of each sample. The results showed the presence of the following major groups of bacteria: *Saccharibacteria, Ternicutes, Actinobacteria, Acidobacteria, Aminicenantes, Firmicutes, Caldiserica, Bacteroidetes, Euryarchaeota,* and *Proteobacteria.* The dominant group representing the consortium was Proteobacteria. According to OTUs clustering the presence of following genera specific to sulfate reducing group were identified, *Desuflovibrio*, *Desulfomonas*, *Desulfobacterium*, *Desulfobacter*, and *Desulfococcus.* This results suggest that the sulfate reducing bacteria in the anaerobic digester is degrading triclosan and play a key role in reducing the toxicity of triclosan to other bacteria in the digester to complete the anaerobic digestion process.

In this study, we did not isolate pure cultures for triclosan degradation. We monitored the bacterial consortia at various electron acceptor conditions and more specifically under sulfate reducing condition. According to the biodiversity analysis, the dominant group of anaerobic bacteria was *Proteobacteria.* Several of this phylum of bacteria have been identified to degrade triclosan. A study by Kim et al. (2011) discovered that the PH-07 strain of *Sphingomonas* could degrade 25% of triclosan in 8 days under co-metabolic conditions. Roh et al. (2009) found that *Nitrosomonas europaea* degraded 45% of 1 mg/L triclosan in 24 hours. Lee et al. (2012) discovered that the KCY1 strain of *Sphingopyxis* could completely degrade triclosan into chlorides and unchlorinated end products. Future studies should focus on isolating pure cultures from the SRB consortium from the anaerobic digester sludge and investigate for complete mineralization of triclosan.

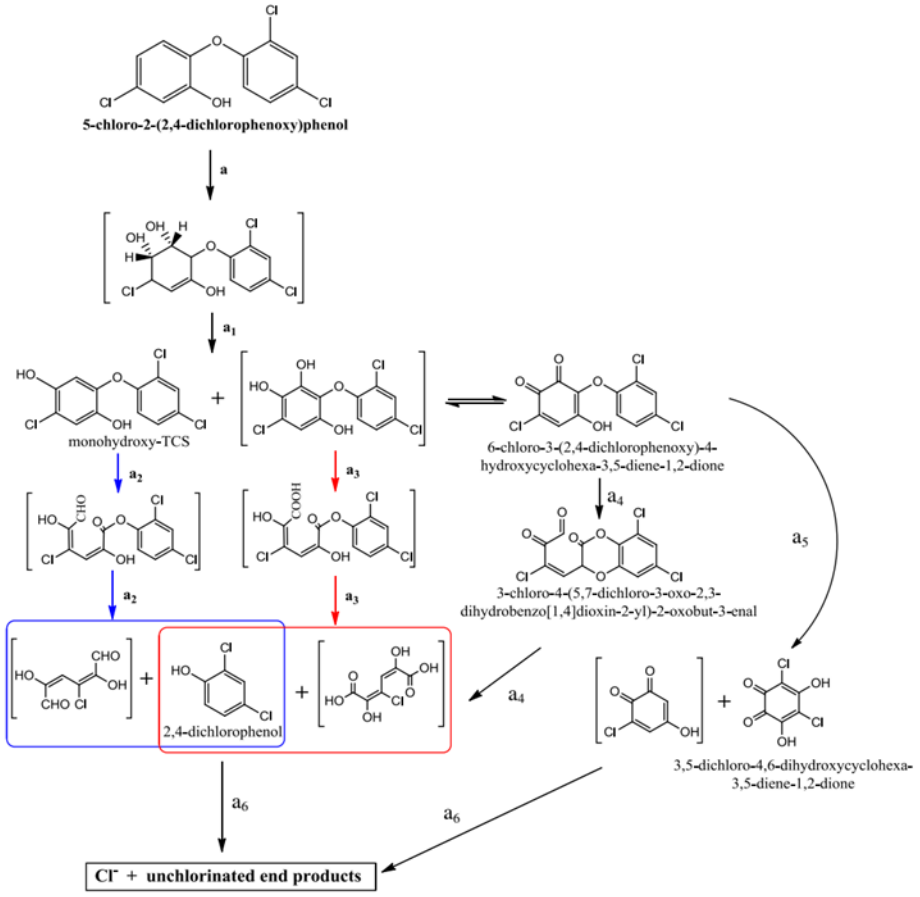

Figure 3 Proposed triclosan metabolic pathway modified from Lee et al. (2012)

4 Conclusions

The results of this study indicate that bacterial consortia in the anaerobic digester sewage sludge collected from the Thibodaux Sewage Treatment Plant are not inhibited by the presence of triclosan. In sulfate reducing condition, triclosan was degraded under co-metabolic and sole carbon source conditions. The consortium was able to produce many metabolites with a major metabolite of 2,4-dichlorophenol. The molecular analysis of the consortium showed wide biodiversity of bacteria including many genera of sulfate reducing bacteria. Further research is needed to isolate a pure culture of bacteria that can completely metabolize triclosan.

Declaration of competing interest

The authors declare no known competing interests that could have influenced the work reported in this paper.

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References

Ahmed, I., Boulton, A. J., Rizvi, S., Carlos, W., Dickenson, E., Smith , N. A. and Reed, M., 2019. The use of triclosan-coated sutures to prevent surgical site infections: a systematic review and meta-analysis of the literature. BMJ Open. 9, e029727

Alfhili, M. A. and Lee, M.-H., 2019. Triclosan: An Update on Biochemical and Molecular Mechanisms. Oxidative Medicine and Cellular Longevity. 2019, 1607304

Ashrap, P., Zheng, G., Wan, Y., Li, T., Hu, W., Li, W., Zhang, H., Zha ng, Z. and Hu, J., 2017. Discovery of a widespread metabolic pathway within and among phenolic xenobiotics. PNAS. 114, 6062-6067

Bedoux, G., Roig, B., Thomas, O., Dupont, V. and Le Bot, B., 2012. Occurrence and toxicity of antimicrobial triclosan and by-products in the environment. Environ. Sci. Pollut. Res. 19, 1044-1065

Bever, C. S., Rand, A. A., Nording, M., Taft, D., Kalanetra, K. M., Mi lls, D. A., Breck, M. A., Smilowitz, J. T., German, J. B. and Hammock, B. D., 2018. Effects of triclosan in breast milk on the infant fecal microbiome. Chemosphere. 203, 467-473

Boopathy, R., 2007. Anaerobic metabolism of nitroaromatic comp ounds and bioremediation of explosives by sulfate-reducing bacteria. Environmental and engineered systems. Cambridge University Press New York Boopathy, R., 2017. Anaerobic degradation of atrazine. Int. Biodeterior. Biodegradation. 119, 626-630

Boopathy, R. and Kulpa, C. F., 1992. Trinitrotoluene (TNT) as a sole nitrogen source for a sulfate-reducing bacteriumDesulfovibrio sp. (B strain) isolated from an anaerobic digester. Current Microbiology. 25, 235-241

Boopathy, R., Kulpa, C. F. and Wilson, M., 1993. Metabolism of 2,4,6 -trinitrotoluene (TNT) by Desulfovibrio sp. (B strain). Applied Microbiology and Biotechnology. 39, 270-275

Calafat, A. M., Ye, X., Wong, L.-Y., Reidy, J. A. and Needham, L. L., 2008. Urinary Concentrations of Triclosan in the U.S. Population: 2003-2004. Environ. Health Perspect. 116, 303-307

Chen, X., Nielsen, J. L., Furgal, K., Liu, Y., Lolas, I. B. and Bester, K., 2011. Biodegradation of triclosan and formation of methyltriclosan in activated sludge under aerobic conditions. Chemosphere. 84, 452-456

Dhillon, G. S., Kaur, S., Pulicharla, R., Brar, S. K., Cledón, M., Verma, M. and Surampalli, R. Y., 2015. Triclosan: Current Status, Occurrence, Environmental Risks and Bioaccumulation Potential. Int J. Environ Res Public Health. 12, 5657-5684

Drury, B., Scott, J., Rosi-Marshall, E. J. and Kelly, J. J., 2013. Triclosan ance and Influences Taxonomic Composition of Benthic Bacterial Communities. Environ. Sci. Technol. 47, 8923-8930

Gorontzy, T., Küver, J. and Blotevogel, K.-H., 1993. Microbial trans formation of nitroaromatic compounds under anaerobic conditions. Microbiology. 139, 1331-1336

Hach, D., 1999. Hach DR/2000 spectrophotometer handbook. Ha ch, Loveland, CO, USA. 350-390

Halden, R. U., 2014. On the Need and Speed of Regulating Triclosan and Triclocarban in the United States. Environ. Sci. Technol. 48, 3603-3611

Halden, R. U., Lindeman, A. E., Aiello, A. E., Andrews, D., Arnold, W. A., Fair, P., Fuoco, R. E., Geer, L. A., Johnson, P. I., Lohmann, R., McNeill, K., Sacks, V. P., Schettler, T., Weber, R., Zoeller, R. T. and Blum, A., 2017. The Florence Statement on Triclosan and Triclocarban. Environ. Health Perspect. 125, 064501

Hong, B., Ba, Y., Niu, L., Lou, F., Zhang, Z., Liu, H., Pan, Y. and Zhao, Y., 2018. A Comprehensive Research on Antibiotic Resistance Genes in Microbiota of Aquatic Animals. Front. Microbiol. 9, 1-10

Kim, Y.-M., Murugesan, K., Schmidt, S., Bokare, V., Jeon, J.-R., Kim, E.-J. and Chang, Y.-S., 2011. Triclosan susceptibility and co-metabolism – A comparison for three aerobic pollutant-degrading bacteria. Bioresour. Technol. 102, 2206-2212

Lee, D. G., Zhao, F., Rezenom, Y. H., Russell, D. H. and Chu, K.-H., 2012. Biodegradation of triclosan by a wastewater microorganism. Water Res. 46, 4226-4234

Lubarsky, H. V., Gerbersdorf, S. U., Hubas, C., Behrens, S., Riccia rdi, F. and Paterson, D. M., 2012. Impairment of the Bacterial Biofilm Stability by Triclosan. PLOS ONE. 7, e31183

Mahitha, P., Gurupadayya, B. and Chandan, R., 2014. Analytical method development and validation of triclosan in dental formulations. Int. J. Pharm. Sci. Res. 5, 3810

McAvoy, D. C., Schatowitz, B., Jacob, M., Hauk, A. and Eckhoff, W. S., 2002. Measurement of triclosan in wastewater treatment systems. Environ. Toxicol. Chem. 21, 1323-1329

Miazek, K. and Brozek-Pluska, B., 2019. Effect of PHRs and PCPs on Microalgal Growth, Metabolism and Microalgae-Based Bioremediation Processes: A Review. Int. J. Mol. Sci. 20, 2492

Petersen, R. C., 2016. Triclosan antimicrobial polymers. AIMS Mol Sci. 3, 88-103

Preuss, A., Fimpel, J. and Diekert, G., 1993. Anaerobic transforma tion of 2,4,6-trinitrotoluene (TNT). Arch. Microbiol. 159, 345-353

Ribado, J. V., Ley, C., Haggerty, T. D., Tkachenko, E., Bhatt, A. S. and Parsonnet, J., 2017. Household triclosan and triclocarban effects on the infant and maternal microbiome. EMBO Mol. Med. 9, 1732-1741

Roh, H., Subramanya, N., Zhao, F., Yu, C.-P., Sandt, J. and Chu, K.-H., 2009. Biodegradation potential of wastewater micropollutants by ammonia-oxidizing bacteria. Chemosphere. 77, 1084-1089

Skovgaard, S., Nielsen, L. N., Larsen, M. H., Skov, R. L., Ingmer, H. and Westh, H., 2013. Staphylococcus epidermidis Isolated in 1965 Are More Susceptible to Triclosan than Current Isolates. PLOS ONE. 8, e62197

Tatarazako, N., Ishibashi, H., Teshima, K., Kishi, K. and Arizono, K., 2004. Effects of triclosan on various aquatic organisms. Environ Sci. 11, 133-140

Valiela, I., 2001. Doing science: design, analysis, and communicati on of scientific research. Greenwood Publishing Group

Waria, M., O'Connor, G. A. and Toor, G. S., 2011. Biodegradation of triclosan in biosolids-amended soils. Environ. Toxicol. Chem. 30, 2488-2496

Ying, G.-G., Yu, X.-Y. and Kookana, R. S., 2007. Biological degradati on of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling. Env. Pol. 150, 300-305

Yueh, M.-F. and Tukey, R. H., 2016. Triclosan: A Widespread Enviro nmental Toxicant with Many Biological Effects. Annual Review of Pharmacology and Toxicology. 56, 251-272