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ABSTRACT

Triclosan is an antimicrobial agent which is widely used in household and personal care products. Widespread use of this compound has led to the elevated concentrations of triclosan in wastewater, wastewater treatment plants (WWTPs) and receiving waters. Removal of triclosan and formation of triclosan-methyl was investigated in activated sludge from a standard activated sludge WWTP equipped with enhanced biological phosphorus removal. The removal was found to occur mainly under aerobic conditions while under anoxic (nitrate reducing) and anaerobic conditions rather low removal rates were determined. In a laboratory-scale activated sludge reactor 75% of the triclosan was removed under aerobic conditions within 150 h, while no removal was observed under anaerobic or anoxic conditions. One percent of the triclosan was converted to triclosan-methyl under aerobic conditions, less under anoxic (nitrate reducing) and none under anaerobic conditions.

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1. Introduction

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenylether) is currently used as a bactericide in personal care products such as toothpaste, shampoos, and soaps. It is additionally used as a stabilizing agent in a multitude of detergents and cosmetics (Adolfson-Erici et al., 2002). Triclosan inhibits bacterial growth by blocking lipid biosynthesis (Schweizer, 2001). Microalgae communities are particularly sensitive to triclosan with effective concentrations around 10 ng L^{-1} (Wilson et al., 2009). A mechanism responsible for this effect has been proposed (Franz et al., 2008). Additionally, triclosan has also been linked to a range of health and environmental effects, such as skin irritation, allergy susceptibility, and also other ecological toxicity to the aquatic and terrestrial environment (Coogan et al., 2007), e.g. it has an effect on earth worms (*Eisenia fetida*) (Lin et al., 2010) and on Japanese medaka fish (Nassef et al., 2010).

After use triclosan ends up in the wastewater with typical concentrations of $1–10 \text{ μg L}^{-1}$ (Adolfson-Erici et al., 2002; Lindström et al., 2002; Singer et al., 2002; Bester, 2005). Removal of about 90% was measured in wastewater treatment plants (WWTP) employing conventional activated sludge process of which 40–60% was due to biodegradation while the remainder was due to sorption to the sludge (Singer et al., 2002; Bester, 2003, 2005; Coogan et al., 2007; Heidler and Halden; 2007; Ying et al., 2007).
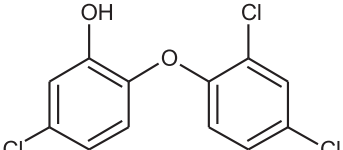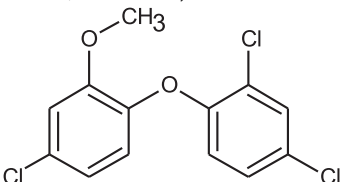
On the other hand, this means that most removal occurs due to biodegradation processes (Singer et al., 2002; Bester, 2003; Heidler and Halden, 2007). However, only little is known about the reaction pathways and conditions (Federle et al., 2002). About 5% of triclosan is biomethylated to triclosan-methyl (2,4,4′-trichloro-2′-methoxy-diphenylether) (Bester, 2003, 2005; Heidler and Halden, 2007). The structural formulas and basic physico-chemical parameters of triclosan and triclosan-methyl are compared in Table 1. Another 5% of triclosan is transformed to bound residues (Bester, 2003). The biochemical pathways and conditions for formation of triclosan-methyl are largely unknown up to now, as most studies focused on the mass flow of triclosan-methyl in the WWTP treatment process (Bester, 2005), its formation in estuarine systems (DeLorenzo et al., 2007) as well as bioaccumulation of triclosan-methyl in fish samples (Lindström et al., 2002; Balmer et al., 2004). It is known, though, that triclosan-methyl is more persistent, lipophilic, bio-accumulative and less sensitive towards photo-degradation in the environment than its parent compound (Lindström et al., 2002; Balmer et al., 2004). Typical concentrations of triclosan in sludge were $2–8 \text{ mg kg}^{-1}$ dry matter in Germany (Bester, 2003) while triclosan-methyl was only detected with $0.004–0.311 \text{ mg kg}^{-1}$ (dry weight) in sewage sludge samples from municipal wastewater treatment plants in Spain (Sánchez-Brunete et al., 2010).

To maximize the biodegradation of compounds like triclosan and triclosan-methyl it is crucial to understand by which process and in which part of the treatment plants triclosan is eliminated and by which process triclosan-methyl is generated. There are

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Table 1
Structural formulas and other environmental parameters of triclosan and triclosan-methyl.

Triclosan (Bester, 2005; Lindström et al., 2002, EPI Suite 4.0)	Name: 2,4,4'-trichloro, 2'-hydroxy-diphenylether Water solubility: 4.621 mg L^{-1} Vapor pressure: 0.00062 Pa log Kow: 4.2–4.8 log Koc: 4.3 pKa: 7.9
Triclosan-methyl (Bester, 2005; Balmer et al., 2004, EPI Suite 4.0)	Name: 2,4,4'-Trichloro, 2'-methoxy-diphenylether Water solubility: 0.4 mg L^{-1} Vapor pressure: 0.00093 Pa log Kow: 5 log Koc: 4.1

three basic processes in biological treatment in the WWTP: aerobic, anaerobic and anoxic. Aerobic (oxygen present) biological treatment is generally used removal of BOD (biochemical oxygen demand) as well as for nitrification (ammonia to nitrate). Anoxic conditions (no oxygen but nitrate present) are used for denitrification (nitrate to nitrogen gas). True anaerobic conditions (neither oxygen nor nitrate present) are limited to sludge digestion processes such as methane production. These three individual types of biological treatment processes can be run in one tank with different operating regimes in time or in separate tanks to offer better treatment. The current study compared the degradation of triclosan and formation of methyl-triclosan under the different conditions in laboratory-scale experiments to determine which of the processes were important for the biodegradation of triclosan in waste water treatment.

2. Materials and methods

2.1. Activated sludge sampling

Activated sludge samples for the preliminary experiments were sampled from Aalborg East wastewater treatment plant (WWTP), which processes 6×10^6 m³ wastewater (100 000 population equivalents, PE) annually. Samples for the detailed aerobic experiments were from Aalborg West WWTP, which processes 22×10^6 m³ wastewater (330 000 PE) annually.

The other key parameters of the plants are similar. Both receive about 80% municipal wastewater and 20% from local industries. They run with a hydraulic retention time of 24–30 h and sludge retention time of 25–30 d, and the process configurations include a screen chamber, primary sedimentation basins, activated sludge treatment basins and a final clarifier before the treated water is released into the Limfjord. Nitrification and denitrification are performed as alternating denitrification. Phosphorous removal is performed mostly by biological means. The suspended solids (SS) content of the activated sludge was 4 g L^{-1} and its volatile solids content was 2.5 g L^{-1} during the sampling period.

2.2. Degradation experiments

Biodegradation experiments were carried out in 5 L glass bioreactors. During the experiments, all reactors were maintained at 17 ± 2 °C. The reactors were completely covered by aluminium foil to prevent photolytic degradation. They were monitored daily for loss of water by weighing, eventual loss of water was compensated

by adding tap water. No action was undertaken to prevent volatilization of triclosan, as the vapor pressure of triclosan and triclosan-methyl, both are very low (Table 1). The reactors were stirred by means of teflonized magnetic stir bars to keep the sludge homogeneous. No additional carbon source was added to the system, thus they were run as static reactors. Duplicated sludge samples were taken every day from each reactor.

The incubation conditions were established as:

(1) *Aerobic conditions* by supplying air through a diffuser stone with a flow rate of 1.3 L h^{-1}.
(2) *Anaerobic conditions* were maintained by constantly flushing the respective bioreactor with nitrogen gas.
(3) *Anoxic* (nitrate reducing) conditions were maintained by constant addition of potassium nitrate (KNO$_3$)(44 gN d^{-1} L^{-1}).

The preliminary experiments were incubated for 80 h under aerobic, anaerobic and anoxic conditions with starting concentrations of 0.1 mg L^{-1} triclosan, which is exceeding typical wastewater concentrations by a factor of 10 but it is in the same range as expected in activated sludge in municipal WWTPs (Bester, 2005).

Detailed aerobic experiments were performed for 10 d at five different initial triclosan concentrations to determine the rate of triclosan-methyl formed from triclosan under aerobic conditions. Triclosan concentrations of 0.02, 0.5, 1, 2 and 3 mg L^{-1} were used in order to investigate whether the degradation of triclosan and formation of triclosan-methyl were concentration related. The 3 mg L^{-1} is towards the very high end of the concentration that can still be found in rare cases in sludge (Stasinakis et al., 2007). The high concentrations were chosen, to be able to discriminate between triclosan an triclosan-methyl already present in the sludge and those freshly spiked for the experiments. In these experiments oxygen concentrations were measured and continuously kept above 4.0 mg L^{-1}.

2.3. Extraction and instrumental analysis

2.3.1. Liquid sludge

Ten millilitre sludge samples from the experiments were diluted by tap water to 1 L and extracted successively for 20 min with 10 mL toluene by means of vigorous stirring with a teflonized magnetic stir bar after adding an aliquot of 100 µL of internal standard solution (musk xylene D$_{15}$). The organic phase was separated from the aqueous one and the residual water was removed from

Table 2
Quality assurance data including the MS data (analytical and verifier ions) as well as limit of quantification (LOQ) of the experiments for the liquid samples. Data for recovery rate (RR) and relative standard deviation (RSD) for liquid sludge were from Bester (2003), while those for solid sludge were from Chen and Bester (2009).

Analyte	Analytical ion (amu)	Verifier ion (amu)	Retention time (min)	LOQ (ng L^{-1})	(RR) for liquid sludge (%)	(RSD) for liquid sludge (%)	(RR) for solid sludge (%)	(RSD) for solid sludge (%)
Triclosan	288	290	6.11	10	88	11	114	12
Triclosan-methyl	302	304	6.04	0.3	102	11	55	10

the organic phase by freezing the samples overnight at -20 °C. The organic extracts were concentrated to 1 mL with a nitrogen flow condensator at 55 °C.

2.3.2. Solid sludge

To determine sorption of triclosan into the solid phase, another 10 mL sludge samples were taken every day from each reactor. The samples were filtered through GC-50 glass fiber filters (Advantec, Tokyo, Japan) with pore size of 0.2 µm. Filter residues (sludge solid matter) were immediately stored in a refrigerating room at −27 °C overnight and then lyophilized at 2 mbar and −46 °C. The lyophilized samples were extracted by means of accelerated solvent extraction (ASE) with ethyl acetate at 90 °C and 150 bar (Chen and Bester, 2009). The resulting extracts were then concentrated by using a Büchi multiport concentrator at 80 °C and 70 mbar (Büchi, Essen, Germany) after adding 10 mL toluene and 100 µL internal standard solution.

2.3.3. Instrumental analysis

Triclosan extracts from the liquid and solid sludge samples were both finally analysed by gas chromatography with mass spectrometric detection (GC-MS, Thermo-Trace GC-MS) equipped with a splitless injector and A200S autosampler. Samples (1 µL) were injected into the injector in splitless (1.5 min) mode held at a temperature of 240 °C. The GC separation was performed with a Rxi-5Sil MS column (Restek, Bellefonte, USA), L: 10 m; ID: 0.18 mm; film: 0.18 µm and a temperature programme of: 90 °C (hold: 1 min) ramped with 50 °C min^{-1} to 135 °C and then with 10 °C min^{-1} to 220 °C. Finally, the baking temperature was reached by ramping the column with 40 °C min^{-1} to 260 °C which was held for 6 min. Helium (5.0) was used as carrier gas with a flow rate of 1.3 mL min^{-1}. The transfer line of the mass spectrometer (Trace MS, Thermo Finnigan, Dreieich, Germany) was held at 250 °C. The ion source was operated at 160 °C. The mass spectrometer was operated in selected ion monitoring (SIM) utilizing 31–61 ms dwell time. The detector of the mass spectrometer was operated at 450 V. Table 2 lists the retention times of triclosan and triclosan-methyl and the mass fragments used for the detection.

2.3.4. Data treatment

The average of the duplicate extractions measured by duplicate injections was used for further data processing. The calibrations were performed as a multi-step internal standard calibration (10–10 000 ng mL^{-1}). The full method and validation data for triclosan and triclosan-methyl for liquid samples were described in Bester (2005), while those for the solids were described by Chen and Bester (2009). Both are shown in Table 2. To additionally validate this method for recovery of triclosan from liquid sludge, it was tested by extracting several activated sludge samples spiked with this biocide. Five different concentrations (between 20 µg g^{-1} and 3000 µg g^{-1}) were dosed and for each concentration two samples were extracted; thus 10 extractions were performed. The recovery rate of triclosan was 82% with 10% relative standard deviation, which is consistent with previous measurements (Bester, 2005).

2.4. Materials

Triclosan was purchased from Ehrenstorfer (Augsburg, Germany) with a purity of ⩾99% according to the supplier. Triclosan-methyl was synthesized from triclosan by methylation with trimethylsulfonium hydroxide solution (Macherey–Nagel, Dueren, Germany) at 40 °C (Bester, 2003). Toluene was used in residue grade (z.R.) quality and purchased from Merck (Darmstadt, Germany). The internal standard musk xylene D$_{15}$ (Ehrenstorfer, Augsburg, Germany) was used to quantify triclosan and triclosan-methyl (Andresen and Bester, 2006).

3. Results and discussions

3.1. Preliminary experiments

In this experiment the fate of triclosan was investigated in reactor experiments under aerobic, anaerobic and anoxic conditions with sludge from Aalborg East WWTP. After 80 h the concentration of the parent compound was reduced from 30 to 15 µg L^{-1} (49%, i.e. significantly) under aerobic conditions, but only from 32 to 28 µg L^{-1} (11%) and from 32 to 29 µg L^{-1} (16%) under anaerobic and anoxic conditions, respectively, which is very close to the method standard deviation, i.e., 11% (Bester, 2005).

Opposite to the triclosan concentrations, those of triclosan-methyl concordantly increased from 4.2 to 5.0 µg L^{-1} (16%) during the aerobic incubation and from 4.1 to 4.8 µg L^{-1} (17%) during the anoxic incubation. Considering the analytical standard deviation, this increase is significant. Additionally, no change of concentrations was detected under anaerobic condition.

In summary, the fastest removal triclosan removal and its highest transformation rate to triclosan-methyl were determined under aerobic conditions. Therefore, the more detailed experiments on
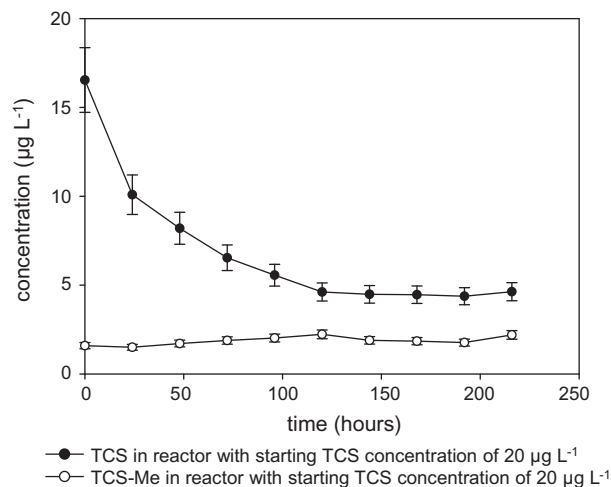

Fig. 1. Concentrations of triclosan and triclosan-methyl in aerated reactors. Starting concentration 20 µg L^{-1} triclosan (unspiked). Error bars indicate standard error of 11% (Bester, 2005).
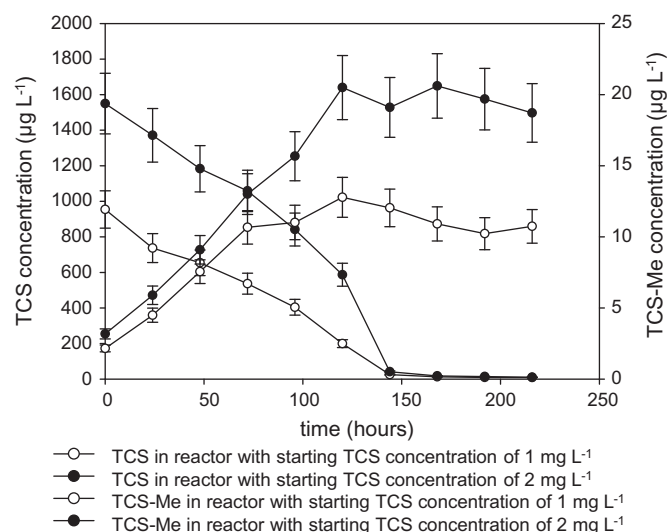
Fig. 2. Concentrations of triclosan and triclosan-methyl in aerated reactors. Starting concentration of 1 and 2 mg L^{-1} triclosan (spiked). Error bars indicate standard error of 11% (Bester, 2005).

degradation and methylation of triclosan were carried out in activated sludge under the aerobic conditions.

3.2. Detailed aerobic kinetic experiments

To make sure the elevated concentrations of triclosan-methyl at the end of the experiment really originated from the freshly added triclosan and not from an old and eventually unknown pool of triclosan in the sludge, several experiments were performed with different triclosan concentrations in aerobic experiments. Triclosan concentrations were rapidly reduced in all reactors while triclosan-methyl concentrations increased concomitantly. Fig. 1 shows these data for the reactors with 0.02 mg L^{-1} starting triclosan concentrations (unspiked), while in Fig 2 the data for starting triclosan concentrations of 1 and 2 mg L^{-1} (spiked) are shown, respectively. The major part of removal was achieved within 150 h (Figs. 1 and 2), after which the triclosan concentrations remained almost constant at less than 0.01 mg L^{-1} to the end of the experiment (220 h) (Fig. 1).

The production of triclosan-methyl occurred in all experiments. The concentrations of triclosan-methyl increased according to the starting levels of the parent compound, though the concentrations of the metabolite remained significantly lower than the initial parent concentrations (Fig. 2). It is assessed that in these reactors 1% of triclosan was transformed into triclosan-methyl during the experiment under aerobic conditions. However, in the reactor with triclosan starting concentration of 0.02 mg L^{-1} (Fig. 1), the production of

triclosan-methyl was mostly obscured by the background concentrations (from the sludge from the waste water treatment plant). In the reactors fed with 1 and 2 mg L^{-1} triclosan, the concentrations of triclosan-methyl increased (Fig. 2), reaching the highest concentrations after 120 h, at which they remained until the end of the experiment. The concentration increase of the metabolite coincided with the concentration decrease of the parent compound. Though no strict mathematical equations could be established, it is clear, that the higher the starting concentration of triclosan was, the higher was the metabolite concentration at the end of the experiment, thus proving the triclosan-methyl was really formed from the added triclosan. The experiment thus indicates that the biomethylation of triclosan can occur in aerobic reactors. As the concentrations of triclosan-methyl are unchanged even after more than 100 h after the main pool of triclosan is consumed, it is obvious that the metabolite cannot be degraded within timeframes relevant for wastewater treatment.

To quantify the possible sorption of triclosan, solid samples were analysed. Consistently 10% of the triclosan found in the experiment medium (liquid sludge) was sorbed to the solids throughout the experiment. The triclosan concentrations in the solid phase show thus decrease in parallel to those in the liquid phase. The partitioning of triclosan between the solid and liquid phase remains constant, thus exchange processes are quick in comparison to the degradation. Additionally, the pH value of the sludge was measured as triclosan adsorption and extraction are pH dependent (Lindström et al., 2002). The pH value remained constant (6.9 ± 0.5) during the experiment indicating that the concentration changes measured are not influenced by pH.

At low concentrations (normal WWTP levels, up to 20 μg L^{-1}) the biological degradation of triclosan followed the first-order kinetics (Fig. 1), while the reaction kinetics is more complex at higher concentrations (>500 μg L^{-1}). Thus, the pseudo-first-order rates and half-lives from reactors were calculated to give an overview of the performance of the system (Table 3). The estimated half-lives ($t_{1/2}$) were found to be 54–86 h, and the elimination rates considering a 10-d period were 75% and 86% for the reactors with initial triclosan concentration of 0.02 and 0.5 mg L^{-1}, and 99% for reactors with the initial triclosan concentration of 1, 2 and 3 mg L^{-1}.

The half-life of triclosan in this experiment was not dependent on the concentration. However, the elimination rates were relatively lower when the starting concentration was low (0.02 mg L^{-1}), and reached higher values (>99%) when the starting concentration was high (>1 mg L^{-1}). These data are from steady state lab scale experiment, thus should be extrapolated to full-scale WWTPs (which are flow through systems) with caution, as external carbon sources, temperature, interference of other organic compounds etc. may lead to different rates.

The rate constants of triclosan-methyl generation increased concordantly with the starting concentration of triclosan as shown in Table 3. With the initial triclosan concentrations of 0.5, 1, 2 and

Table 3
Degradation rate constant (k) of triclosan and generation rate of triclosan-methyl in different concentrations in aerobic activated sludge systems. Starting concentration (C_o) and final concentrations (C_f) are given. The final concentrations were measured after 220 h. Half-lives and pseudo-first-order rate constants for the degradation of triclosan were calculated from the data between lag phase (24 h) and end of reaction (120 h), whereas generation rates of triclosan-methyl were calculated by using the data between 0–120 h.

Removal of TCS					Formation of TCS-Me		
C_o (μg L^{-1})	C_f (μg L^{-1})	k (s^{-1})	R^2	$t_{1/2}$ (h)	C_o (μg L^{-1})	C_f (μg L^{-1})	k (s^{-1})
20	5	0.0081	0.9969	86	1.6	2.2	0.004
500[*]	55	0.0095	0.9961	73	2.8	6.4	0.0054
1000	10	0.0129	0.8832	54	2.2	10.7	0.0103
2000	10	0.0085	0.9451	82	3.2	18.7	0.0127
3000	10	0.0123	0.7094	56	3.2	19.8	0.0129

[*] This experiment was ended after 168 h.

3 mg L^{-1}, the rate constants were 0.0054, 0.0103, 0.0127 and 0.0129 s^{-1}, respectively.

Biomethylation of triclosan under aerobic conditions was surprising as methylation of pollutants such as mercury (Gray et al., 2004, 2006; Barringer and Szabo, 2006), antimony, arsenic (Duster et al., 2008), bismuth (Michalke et al., 2002) and phenols (Pfeifer et al., 2001) is usually associated with anaerobic, anoxic (no oxygen but nitrate present), methanogenic or sulfate reducing regimes. However, biomethylation, e.g., by cobalamin (Vitamin B12) (Wehmeier et al., 2004) is not restricted to anaerobic conditions. Older literature reported the conditions that induced methylation processes were rather "organic-rich" (Compeau and Bartha, 1985), while others have reported that polychlorinated phenoxy phenols (PCPP) were biomethylated in contaminated soil and in several pure and mixed bacterial cultures under aerobic conditions (Valo and Salkinoja-Salonen, 1986). Additionally, biomethylation of chlorinated phenolic compounds (Häggblom et al., 1988) and tetrabromobisphenol-A (George and Häggblom, 2008) has been detected under aerobic conditions.

4. Conclusions

Triclosan-methyl was formed concomitantly with the removal of triclosan in activated sludge under aerobic conditions. Triclosan-methyl was also formed under anoxic (nitrate reducing) conditions although at lower rates but was not formed under anaerobic conditions in laboratory experiments. According to these laboratory experiments, the emissions of triclosan-methyl will thus be affected mostly by the management of the BOD removal and nitrification tanks but not during anaerobic digestion.

Acknowledgement

References

Adolfsson-Erici, M., Pettersson, M., Parkkonen, J., Sturve, J., 2002. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. Chemosphere 46, 1485–1489.

Andresen, J., Bester, K., 2006. Elimination of organophosphate ester flame retardants and plasticizers in drinking water purification. Water Res. 40, 621–629.

Balmer, M.E., Poiger, T., Droz, C., Romanin, K., Bergqvist, P.A., Müller, M.D., Buser, H.R., 2004. Occurrence of methyl triclosan, a transformation product of the bactericide triclosan, in fish from various lakes in Switzerland. Environ. Sci. Technol. 38, 390–395.

Barringer, J.L., Szabo, Z., 2006. Overview of investigations into mercury in ground water, soils, and septage, New Jersey coastal plain. Water Air Soil Pollut. 175, 193–221.

Bester, K., 2003. Triclosan in a sewage treatment process—balances and monitoring data. Water Res. 37, 3891–3896.

Bester, K., 2005. Fate of triclosan and triclosan-methyl in sewage treatment plants and surface waters. Arch. Environ. Contam. Toxicol. 49, 9–17.

Chen, X.J., Bester, K., 2009. Determination of organic micro-pollutants such as personal care products, plasticizers and flame retardants in sludge. Anal. Bioanal. Chem. 395, 1877–1884.

Compeau, G.C., Bartha, R., 1985. Sulfate-reducing bacteria-principal methylators of mercury in anoxic estuarine sediment. Appl. Environ. Microbiol. 50, 498–502.

Coogan, M.A., Edziyie, R.E., La Point, T.W., Venables, B.J., 2007. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater treatment plant receiving stream. Chemosphere 67, 1911–1918.

DeLorenzo, M.E., Keller, J.M., Arthur, C.D., Finnegan, M.C., Harper, H.E., Winder, V.L., Zdankiewicz, D.L., 2007. Toxicity of the antimicrobial compound triclosan and formation of the metabolite methyl-triclosan in estuarine systems. Environ. Toxicol. 23, 224–232.

Duster, L., Vink, J.M., Hirner, A.V., 2008. Methylantimony and –arsenic species in sediment pore water tested with the sediment or fauna incubation experiment. Environ. Sci. Technol. 42, 5866–5871.

Federle, T.W., Kaiser, S.K., Nuck, B.A., 2002. Fate and effects of triclosan in activated sludge. Environ. Toxicol. Chem. 21, 1330–1337.

Franz, S., Altenburger, R., Heilmeier, H., Schmitt-Jansen, M., 2008. What contributes to the sensitivity of microalgae to triclosan? Aquat. Toxicol. 90, 102–108.

George, K.W., Häggblom, M.M., 2008. Microbial O-methylation of the flame retardant tetrabromobisphenol-A. Environ. Sci. Technol. 42, 5555–5561.

Gray, J.E., Hines, M.E., Biester, H., 2006. Mercury methylation influenced by areas of past mercury mining in the Terlingua district, Southwest Texas USA. Appl. Geochem. 21, 1940–1954.

Gray, J.E., Hines, M.E., Higueras, P.L., Adatto, I., Lasorsa, B.K., 2004. Mercury speciation and microbial transformations in mine wastes, stream sediments, and surface waters at the Almadén mining district Spain. Environ. Sci. Technol. 38, 4285–4292.

Häggblom, M.M., Nohynek, L.J., Salkinoja-Salonen, M.S., 1988. Degradation and O-methylation of chlorinated phenolic compounds by rhodococcus and mycobacterium strains. Appl. Environ. Microbiol. 54, 3043–3052.

Heidler, J., Halden, R.U., 2007. Mass balance assessment of triclosan removal during conventional sewage treatment. Chemosphere 66, 362–369.

Lin, D., Zhou, Q., Xie, X., Liu, Y., 2010. Potential biochemical and genetic toxicity of triclosan as an emerging pollutant on earthworms (Eisenia fetida). Chemosphere 81, 1328–1333.

Lindström, A., Bürge, I.J., Poiger, T., Bergqvist, P.A., Müller, M.D., Buser, H.R., 2002. Occurrence and environmental behavior of the bactericide triclosan and its methyl derivative in surface waters and in wastewater. Environ. Sci. Technol. 36, 2322–2329.

Michalke, K., Meyer, J., Hirner, A.V., Hensel, R., 2002. Biomethylation of bismuth by the methanogen methanobacterium formicicum. Appl. Organomet. Chem. 16, 221–227.

Nassef, M., Matsumoto, S., Seki, M., Khalil, F., Kang, I.J., Shimasaki, Y., Oshima, Y., Honjo, T., 2010. Acute effects of triclosan, diclofenac and carbamazepine on feeding performance of Japanese medaka fish (Oryzias latipes). Chemosphere 80, 1095–1100.

Pfeifer, O., Lohmann, U., Ballschmiter, K., 2001. Halogenated methyl-phenyl ethers (anisoles) in the environment: Determination of vapor pressures, aqueous solubilities, Henry's law constants, and gas/water- (Kgw), n-octanol/water- (Kow) and gas/n-octanol (Kgo) partition coefficients. Fresenius J. Anal. Chem. 371, 598–606.

Sánchez-Brunete, C., Miguel, E., Albero, B., Tadeo, J.L., 2010. Determination of triclosan and methyl triclosan in environmental solid samples by matrix solid-phase dispersion and gas chromatography–mass spectrometry. J. Sep. Sci. 33, 2768–2775.

Schweizer, H.P., 2001. Triclosan: a widely used biocide and its link to antibiotics. FEMS Microbiol. Lett. 202, 1–7.

Singer, H., Müller, S., Tixier, C., Pillonel, L., 2002. Triclosan: occurrence and fate of a widely used biocide in the aquatic environment: field measurements in wastewater treatment plants, surface waters, and lake sediments. Environ. Sci. Technol. 36, 4998–5004.

Stasinakis, A.S., Petalas, A.V., Mamais, D., Thomaidis, N.S., Gatidou, G., Lekkas, T.D., 2007. Investigation of triclosan fate and toxicity in continuous-flow activated sludge systems. Chemosphere 68, 375–381.

Valo, R., Salkinoja-Salonen, M., 1986. Microbial transformation of polychlorinated phenoxy phenols. J. Gen. Appl. Microbiol. 32, 505–517.

Wehmeier, S., Raab, A., Feldmann, J., 2004. Investigations into the role of methylcobalamin and glutathione for the methylation of antimony using isotopically enriched antimony(V). Appl. Organomet. Chem. 18, 631–639.

Wilson, B., Chen, R.F., Cantwell, M., Gontz, A., Zhu, J., Olsen, C.R., 2009. The partitioning of triclosan between aqueous and particulate bound phases in the Hudson River Estuary. Mar. Pollut. Bull. 59, 207–212.

Ying, G.G., Yu, X.Y., Kookana, R.S., 2007. Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling. Environ. Pollut. 150, 300–305.