



Removal of a synthetic broad-spectrum antimicrobial agent, triclosan, in wastewater treatment systems: A short review

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ABSTRACT

Contaminants of emerging concern (CECs) including endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care product chemicals (PPCPs) have recently received more attention because of their occurrence in water bodies and harmful impacts on human health and aquatic organisms. Triclosan is widely used as a synthetic broad-spectrum antimicrobial agent due to its antimicrobial efficacy. However, triclosan detected in aquatic environment has been recently considered as one of CECs, because of the potential for endocrine disruption, the formation of toxic by-products and the development of cross-resistance to antibiotics in aquatic environment. This comprehensive review focuses on the regulations, toxicology, fate and transport, occurrence and removal efficiency of triclosan. Overall, this review aims to provide better understanding of triclosan and insight into application of biological treatment process as an efficient method for triclosan removal.

Keywords: Antimicrobial, Contaminants of emerging concern (CECs), Pharmaceuticals and personal care product chemicals (PPCPs), Removal, Triclosan, Wastewater

1. Introduction

A public health concern about triclosan has recently emerged due to its widespread occurrence in wastewater, surface waters, sediment, soils, and even in urine, human blood, and breast milk [1-10]. Triclosan has been incorporated into numerous personal care, household, and industrial products since 1965, due to its effectiveness as a broad-spectrum antimicrobial agent [11, 12]. Global production of triclosan was estimated to exceed 1,500 tons per year, of which more than 43% of the production was used in the US and Europe [10, 13].

Once triclosan is released into the environment, triclosan can be potentially transformed into more toxic compounds, like chlorodioxins, chlorophenol, chloroform, p-hydroquinone, and 2,4-dichlorophenol when interacted with strong oxidants or under UV light [14]. Furthermore, trace levels of triclosan could promote the development of cross-resistance to antibiotics [15], and cause adverse health effects within the ecosystem [16]. Triclosan is also a weak androgenic and estrogenic compound [17-19].

Through commercial or residential washing of equipment outdoors with triclosan-containing products, triclosan may be transported into the stormwater drain systems; untreated triclosan is released directly into creeks, rivers, and ultimately to the Bay.

Moreover, wastewater is a major source of triclosan to the environment due to incomplete removal of triclosan by conventional wastewater treatment processes. Researchers have been actively seeking effective treatment strategies for triclosan and this task is challenging. Many advanced physical/chemical processes like UV irradiation, ozonation, sorption, advanced oxidation processes [10, 20-28] have shown effectiveness for triclosan removal. Biodegradation of triclosan by many fungi, bacterial consortia, activated sludge and pure strains has also been reported [29-36], suggesting that biological removal of triclosan could be an alternative treatment method for triclosan removal.

2. Chemical Property and Regulations

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol; CAS 3380-34-5) is a nonionic, lipophilic, and chlorinated phenolic compound that has been used as a synthetic broad-spectrum antimicrobial agent, since the Ciba Specialty Chemicals (Basel, Switzerland) first produced triclosan in 1965 [11, 12]. Due to its effective antimicrobial ability, triclosan has been widely incorporated into numerous personal care and household products, and industrial products such as deodorants, soaps, skin cream, toothpastes, laundry detergents, socks, sport



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footwear, toys, fabrics, computer keyboards, countertops, and many different plastic kitchenware all over America, Europe and Asia. Triclosan has been globally produced over 1,500 tons per year, especially, more than 300 tons and 350 tons of triclosan are yearly used in the US and in Europe, respectively [10, 13].

Triclosan is referred to as halogenated biphenyl ether due to functional groups of ether and phenol. Alternative names for triclosan include 2,4,4'-trichloro-2'-hydroxydiphenyl ether, 5-chloro-2-(2,4-dichlorophenoxy)phenol or trichloro-2'-hydroxydiphenyl ether. In addition, triclosan has commercial names such as Irgasan DP-300, Lexol 300, Ster-Zac, Cloxifenolum, Microban in plastics and clothing, and Biofresh in acrylic fibers [7]. The chemical formula for triclosan is $C_{12}H_7Cl_3O_2$ and its molecular weight is 289.55 g/mol. Triclosan is white to off-white crystalline powder with a hardly detectable phenolic odor. Triclosan is thermally stable; it has a melting point between 56-60°C and a boiling point between 280-290°C [37]. Triclosan is relatively non-volatile and non-soluble in water (solubility: 10 mg/L at 20°C; log K_{ow} = 4.76), but readily soluble in a wide range of organic solvents [37, 38]. The chemical structure of triclosan (Fig. 1) is similar to several emerging contaminants such as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), dioxine, biphenol A, diethylstilbestrol (synthetic nonsteroidal estrogen), and thyroxine (thyroid hormone) [39-41]. Triclosan has been banned in Canada and Japan. In the European Union, triclosan is categorized as a dangerous irritant to the environment and aquatic life [42]. The use of triclosan in food contact plastics was banned in September 2009 in Germany. It has been advised to consumers not to use antibacterial products in Finland and Denmark [43]. In the U.S., the non-pesticide use of triclosan is regulated by the U.S. Food and Drug Administration (FDA), while the pesticide use of triclosan is regulated by the Environmental Protection Agency (EPA). However, the regulation for triclosan in surface waters has not been established.

3. Toxicology of Triclosan

Triclosan has a low level of toxicity to humans and other mammals [38, 44]. Triclosan showed a low acute toxicity in animal studies that the dermal LD_{50} (median lethal dose) is 5,000 mg/kg for rats, and the oral LD_{50} are 4,000 mg/kg for mice, 4,500-5,000 mg/kg for rats, and over 5,000 mg/kg for dogs [37]. One study in male mice also reported that triclosan showed a low acute toxicity (LD_{50} >

1,000 mg/kg) [45]. However, several studies reported that triclosan is acutely and chronically toxic to several different types of aquatic life such as fish and algae in the range of 34.2-200 $\mu\text{g/L}$ [46-49]. Ricart et al. [50] reported that triclosan caused an increase in bacterial mortality with a no effect concentration (NEC) of 0.21 $\mu\text{g/L}$ and it was more toxic to bacteria than algae. As shown in two fish studies, the acute toxicity of triclosan depended on the different life stages of species, especially, showing more toxic effects on juveniles [51, 52].

3.1. Triclosan as a Class of Endocrine Disrupting Compounds (EDCs)

Several studies reported that triclosan is a thyroid disruptor in animals due to the structural similarity of triclosan to thyroid hormones such as thyroxine [39-41, 53]. Damage caused by disrupting thyroid systems may affect normal growth and development of humans, especially, the development of children's brain. Using frogs that have a primary thyroid hormone-signaling mechanism which is almost identical to human's, several studies showed that low levels of triclosan interfered with thyroid-mediated developmental processes of tadpoles into frogs [39, 54, 55]. Further, triclosan may disrupt other critical hormone systems in mammals due to the structural similarity to anthropogenic estrogens (bisphenol A and diethylstilbestrol), and the anti-estrogen (2,3,7,8 tetrachloro-*p*-dioxin) [56]. An *in-vitro* study showed that triclosan exerted androgenic and estrogenic activities in human breast cancer cells [17]. *In-vivo* fish studies suggested that triclosan act as anti-estrogen [57] or weak androgen [18]. Several male rat studies demonstrated that triclosan lowered serum levels of testosterone and several steroidogenic enzyme activities [58, 59]. One study also reported that a triclosan metabolite may have a weak estrogenic activity [47].

3.2. Development of Antibiotic Cross-resistance

Several studies raised a concern that triclosan may promote the development of cross-resistance to antibiotics in microorganisms and lead to the emergence of bacteria resistant to antibiotics in the environment. While antimicrobial agents are generally not intended to destroy particular cellular constituents in bacteria, antibiotics attack specific cellular target to inhibit the growth of cell and the synthesis of cell wall or cell contents [60]. Because triclosan kills bacteria in a similar way as antibiotics by inhibiting the active sites of the specific bacterial fatty acid biosynthetic enzyme, *enoyl-acyl* carrier protein reductase, which is necessary

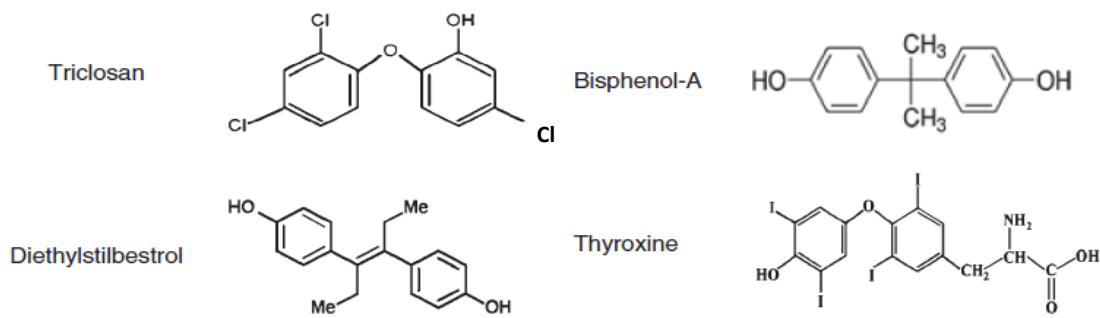


Fig. 1. Chemical structure of triclosan, bisphenol A, diethylstilbestrol, and thyroxine.

to build and reproduce cell membranes [61], bacteria that become resistant to triclosan may have the potential of antibiotic resistance. A laboratory study found the cross-resistance between triclosan and ciprofloxacin (oral antibiotic) in *Pseudomonas aeruginosa* [62]. There is no evidence that triclosan can cause a mutation in bacteria. However, since triclosan kills normal bacteria that are susceptible to triclosan in the environment, mutated bacteria that are resistant to triclosan are more likely to survive and be reproduced in the triclosan-contaminated environment. A laboratory study found that a number of different strains of mutant bacteria that are resistant to triclosan also showed the resistance to a number of clinical-use antibiotics [63].

4. Source, Occurrence, Fate and Transport in the Environment

Urban waters are vulnerable to anthropogenic contamination by human activities and could be polluted with a long-term impact on the urban aquatic ecosystem. Urban wastewater stream can be one of major pathways for emerging organic contaminants including triclosan and these contaminants could be carried in surface and subsurface in urban catchments in association with migration, sorption, transformation processes such as photo-degradation, chemical or biological transformation in urban water systems (Fig. 2) [64].

Since triclosan is primarily a water-borne contaminant, it is detected ubiquitously in aquatic environments at some of the highest concentrations among 95 organic wastewater pollutants [3]. Disposal and usage of triclosan-laden products resulted in triclosan-containing wastewater. About 96% of household and consumer products containing triclosan flows down the drain [65]. Of the triclosan eventually entering wastewater treatment plants (WWTPs), 79% is biodegraded, 15% is adsorbed onto biosolids, and the remaining 6% is discharged continuously into the receiving surface water [1, 10]. Triclosan can be also released into the environment via the application of biosolids to an agricultural field and irrigation of treated wastewater contaminated with triclosan [10, 66, 67]. Consumer product wastes containing triclosan, and sludge from wastewater treatment plants are mostly sent to landfill for disposal, and triclosan can be released into the environment

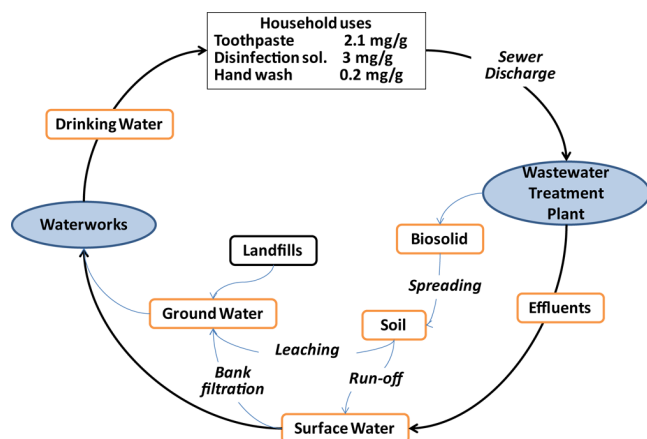


Fig. 2. Life cycle of triclosan in the aquatic environment [64].

through landfill leachate [68]. In addition, through commercial or residential washing of equipment outdoors with triclosan-containing products, runoff containing triclosan goes into stormwater drain systems without treatment, and flows directly into creeks, rivers and eventually to the bay or oceans.

Triclosan concentrations in wastewater influents in Korea, United States, Europe, China, and Japan range from 148 to 785, 245 to 86,200, 142 to 2,301 and 55 to 1,000 ng/L, respectively [64, 69, 70]. Triclosan in wastewater effluents in Korea, United States, Europe, China, and Japan are detected at the concentrations between 0-127, 50-5,037, 10-2,210, 10.9-1,023, and 30-360 ng/L, respectively [1, 2, 10, 64, 69, 71-74]. In surface river water, triclosan was detected in Korea, United States, Europe, China, and Japan with reported values varying from 0 to 149, 3.5 to 34.9, < 0.2 to 285, 2.5 to 478 and 11 to 31, respectively [64, 69, 70, 75]. A recent USGS study also reported that approximately 58% of 139 U.S. streams were contaminated with triclosan at the concentrations ranging from 0.14 to 2.3 $\mu\text{g/L}$ [3]. Triclosan was found in lake-, marine-, and river-sediments at the concentrations in the range from 37 to 53 ng/g, from 0.27 to 130.7 ng/g, and from 4.4 to 35.7 ng/g, respectively [10, 76, 77]. Lapworth et al. [78] reported triclosan concentrations found in groundwater ranging between 7-2,110 ng/L. The ranges of triclosan concentrations detected in WWTP influents, WWTP effluents, and surface river water in the different countries are briefly summarized in Table 1.

Triclosan concentrations in activated sludge have been detected in the range of 500 to 15,600 $\mu\text{g/kg}$ of dry weight [71, 79]. Triclosan in biosolids have been detected at the concentrations between 90 and 30,000 $\mu\text{g/kg}$ [4, 79-82]. Triclosan was also detected at concentrations ranging from 69 to 833 ng/g in agricultural soil amended with biosolids [4]. Furthermore, biosolid application to an agricultural field and the irrigation of treated wastewater containing triclosan contributed to triclosan detection in vegetation contributed from [66, 67]. One field study reported that triclosan in soybean plants was detected at the concentrations between 36 and 80 ng/g of dry weight from irrigation treatment samples, and between 13 and 136 ng/g of dry weight from biosolids application treatment samples [83].

In addition to triclosan detection in the environment, triclosan was detected in 75% of 2,517 human urine samples at concentrations of 2.4-3,790 $\mu\text{g/L}$ [84] and in the 61% of 90 urine samples from age 6-8 year-old girls [85]. Triclosan was also found in human blood [6, 8, 41, 86]; for example, triclosan was detected in the range between 4.1-19 ng/g in blood serum samples [41]. Triclosan in the range of 100-2,100 $\mu\text{g/kg}$ of lipid was detected in the 96.8% of 62 samples of breast milk [9] and concentrations of triclosan in breast milk ranged from 0.018 to 0.95 ng/g [8]. Moreover, triclosan was found in indoor dusts ($\sim 1.1 \mu\text{g/g}$) [87], and foods (0.02-0.15 ng/g) such as dairy products, meat, fish and egg [88].

Triclosan released into the environment can be transformed into more toxic products through photodegradation and/or biodegradation. Under anaerobic conditions, methylation of triclosan into methyl-triclosan in surface waters and wastewater has been reported [89]. Due to the lipophilic property of methyl-triclosan ($K_{ow} = 5.2$), it may bioaccumulate in wildlife and human. A fish study found the concentrated methyl-triclosan in fatty tissue [90] and another study reported that methyl-triclosan has acute toxicities in the marine bacterium *Vibrio fischeri* at low

Table 1. Occurrence and Concentrations of Triclosan in WWTP Influent, WWTP Effluent and Surface water in the Different Countries

	Location	Concentration (ng/L)	Reference
WWTP influent	Korea	148-460	[69]
	Korea	280-745	[69]
	Korea	247-785	[70]
	United States	245-86,200	[64]
	Europe	52-21,900	[64]
	China	142-2,301	[64]
	Japan	55-1,000	[64]
	WWTP effluent	Korea	0-29.6
Korea		0-127	[69]
Korea		1.3-32	[73]
Korea		5.6-16	[74]
United States		50-5,370	[64]
Europe		10-2,210	[64]
China		10.9-1,023	[64]
Japan		30-360	[64]
Surface river water		Korea	0-46.1
	Korea	1-29	[75]
	Korea	16-82	[75]
	Korea	79-149	[70]
	United States	3.5-34.9	[64]
	Europe	< 0.2-285	[64]
	China	2.5-478	[64]
	Japan	11-31	[64]

levels [91]. A great concern about the rapid transformation of triclosan into 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD) during the photo-degradation in natural water and wastewater has been raised [14, 92-96] because dioxin is highly carcinogenic and can cause such severe health problems as affecting reproductive abilities and weakening the immune system [97]. Triclosan can be transformed into chlorophenols (2,4-dichlorophenol and 2,4,6-trichlorophenol) in the presence of chlorine [98-100] or chloramines [101]. These chlorophenols are listed as the priority pollutants by EPA, and are potentially toxic and stable in the environment [98]. Recent studies have shown that triclosan in tap/surface water or in common triclosan-containing hygienic products reacts with residual chlorine to form potentially carcinogenic chloroform [99-101].

5. Treatment Technologies for Triclosan Removal

5.1. Physical/Chemical Removal of Triclosan

A granular activated carbon (GAC) treatment system was effective by decreasing 2,032 ng/L of triclosan concentration in influent to 102 ng/L in effluent [102]. Powdered activated carbon achieved 95% of triclosan reduction during drinking water treatment [103]. Different sorbents such as kaolinite and montmorillonite are effective for triclosan removal [28]. Advanced membrane technologies such as nanofiltration/reverse osmosis membranes are effective

Table 2. Triclosan Removal Efficiencies by Different Treatments

Treatment	Removal efficiency (%)	Reference
Granular activated carbon	95%	[102]
Powdered activated carbon	95%	[103]
Ultrasonication	60%	[105]
Conventional Sand filtration	0%	[106]
UV irradiation	93%	[26]
Ozonation	58-100%	[23]
UV/H ₂ O ₂	> 95%	[107]
UV/TiO ₂	75-82%	[108]
TiO ₂	30%	[108]
UV/Fe (II)	100%	[109]
Electro-oxidation	> 90%	[110]
Activated sludge	95-98%	[111]
Trickling filter	86-97%	[111]
Rotating biological contactors	58-96%	[111]
Primary treatment	32%	[71]

for triclosan removal, but more expensive than other treatment options [104]. 60% of initial triclosan in domestic wastewater was removed by ultrasonication [105]. However, conventional sand filtration showed no removal of triclosan [106].

Triclosan can be effectively removed by chemical oxidation processes including UV irradiation, ozonation, UV/hydrogen peroxide-advanced oxidation process and chlorination. One study reported that 93% of initial triclosan was removed by UV irradiation in wastewater [26]. Ozonation can rapidly oxidize triclosan during wastewater treatment [23]. UV/hydrogen peroxide-advanced oxidation process showed high removal efficiency of triclosan [107]. Triclosan was degraded during chlorination at neutral pH (7.3-8.3) [98]. UV light in combination with TiO₂ achieved a 75-82% reduction in triclosan, whereas 30% of triclosan was removed by TiO₂ as a sole oxidant [108]. Triclosan was almost completely removed within 60 min by UV/Fe (II) [109]. Oxidants such as manganese oxides (δ -MnO₂ and MnOOH), titanium dioxide, permanganate (Mn (VII)), and Fe (III)-saturated montmorillonite have shown to oxidize triclosan effectively [20-24, 105]. A recent study demonstrated > 99.9% of degradation efficiency via electrochemical degradation at Ti/SnO₂-Sb/Ce-PbO₂ anodes [110].

5.2. Biological Removal of Triclosan

Several studies reported triclosan removal in wastewater treatment plants (WWTPs) by biological treatment process [10, 71, 111, 112]. The removal efficiency of triclosan is dependent on the operations and configurations of WWTPs. Thompson et al. reported that overall removal of triclosan ranged between 95-98% (activated sludge), 86-97% (trickling filter), and 58-96% (rotating biological contactors) [111]. McAvoy et al. also found that triclosan was removed with rates of 96%, 71%, and 32% for activated sludge plants, trickling filter plants, and primary treatment plants, respectively [71]. In general, activated sludge treatment enables the greatest removal of triclosan. One study estimated that 79% and 15% of triclosan

was removed by biodegradation and adsorption onto biosolids, respectively, and then the remaining 6% of triclosan was discharged into the receiving water [10].

Several studies demonstrated the aerobic biodegradation of triclosan by wastewater enrichment consortium [29, 30], nitrifying activated sludge [32], wastewater microorganisms (*Sphingomonas* sp. Rd1 and *Sphingomonas* sp. PH-07) [29, 34], soil microorganisms [31], and two white rot fungi (*Trametes versicolor* and *Pycnoporus cinnabarinus*) [33]. A recent study demonstrated the 72% of maximum triclosan biodegradation yield at 7.5 mg/L of triclosan concentration by *Aspergillus versicolor* [113]. 0.15-0.18 $\mu\text{g/mL}$ of triclosan was degraded cometabolically by all these known strains and cultures. The two soil bacteria, *Pseudomonas putida* TriRY and *Alcaligenes xylooxidans* subsp. *denitrificans* TR1 utilized triclosan as a carbon source and degraded it within 4 days and 10 days, respectively [31]. One study suggested that a diphenyl-ether degrad-

ing bacterium, *Sphingomonas* sp. PH-07 used 2,3-dioxygenase enzyme to cometabolically degrade 25% of 10 mg/L triclosan in 8 days and proposed a possible degradation pathway for triclosan [34]. The degradation pathways of triclosan by a new wastewater isolate, *Sphingopyxis* strain KCY1, and several oxygenase-expressing bacteria have elucidated (Fig. 3) [35, 36]. The phylogenetic diversity of triclosan-utilizing bacteria was investigated in activated sludge systems using DNA-based stable-isotope probing technique [114]. An ammonia-oxidizing bacterium (AOB), *Nitrosomonas europaea* were found to cometabolically degrade 45% of 1 mg/L triclosan within 24 hours [32]. This study also demonstrated that ammonia monooxygenase in *Nitrosomonas europaea* was involved in triclosan biodegradation.

While triclosan in aerobic condition shows relatively rapid degradation; triclosan is slowly degraded or persistent under anaerobic condition. Anaerobic degradation of triclosan was not observed

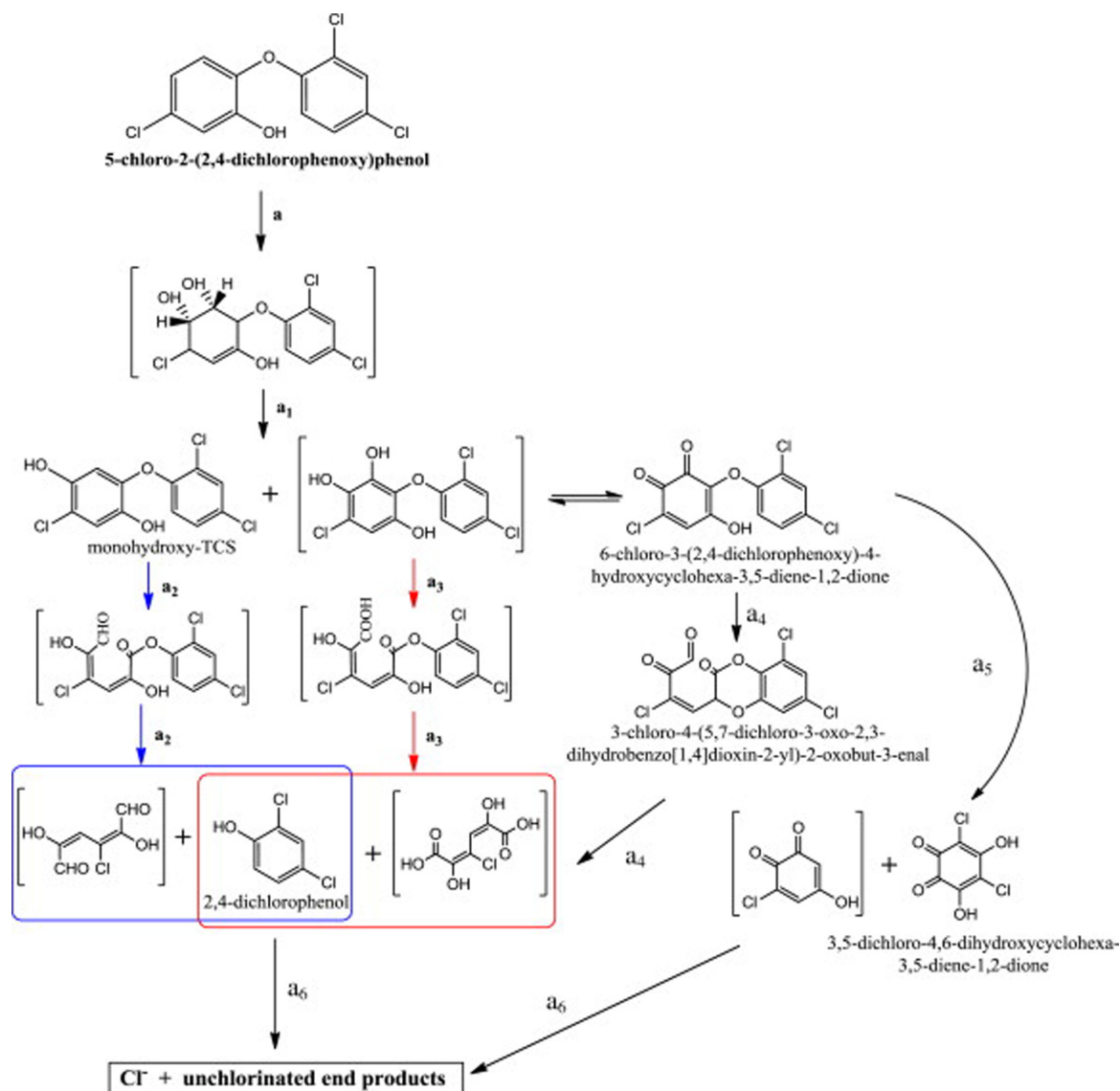


Fig. 3. Triclosan biodegradation pathway by a wastewater microorganism [35].

in 70 days [115]. Similarly, a study reported remaining of 91% of ^{14}C -triclosan after incubation under anaerobic conditions for 147 days [106]. One study reported that anaerobic triclosan biodegradation was not observed, but 75% of triclosan removal by aerobic activated sludge after 150-hr incubation [116].

6. Conclusion and Recommendation

Triclosan is a common synthetic antimicrobial agent incorporated in numerous industrial and personal care products. However, the detection of triclosan in the environment has raised a public health concern, because the exposure of triclosan may promote the development of the cross-resistance to antibiotics, cause endocrine disruption to aquatic microorganisms, and potentially form more toxic by-products. Wastewater is one major source for environmental triclosan due to its incomplete removal by conventional wastewater treatment processes. Many advanced physical/chemical processes have shown effective removal for triclosan. However, these methods may be costly and potentially generate more toxic by-products. Approximately 80% of triclosan loading in wastewater treatment plants (WWTPs) can be rapidly biodegraded by microorganisms [29-34], suggesting that the biological removal of triclosan could be an inexpensive and effective alternative for triclosan removal.

Therefore, the knowledge of the microbiology with respect to triclosan biodegradation by investigating microorganisms capable of degrading triclosan, their degradative enzymes, and degradation pathways for triclosan is essential for developing an effective treatment strategy for triclosan removal in wastewater. Additional research about microorganisms in term of their degradation ability toward triclosan is required. Known microorganisms that are capable of degrading other xenobiotics with the similar chemical structure to triclosan, and that exhibit the metabolism or cometabolism of a wide variety of these toxic pollutants could be potential candidates as a bioaugmentation agent for biological treatment systems.

Furthermore, sequencing the complete genome of microorganisms capable of degrading triclosan will provide insights into factors regulating the unique enzymatic system for either metabolic or cometabolic degradation of triclosan. The availability of the genome sequence will significantly accelerate the analysis of the genes and enzymes involved in triclosan degradation. Eventually, the information obtained from the genome sequences of triclosan-degrading microorganisms would be useful to develop advanced biological treatment processes for triclosan removal.

It is also important to note that microcosm studies of triclosan biodegradation in true environment is needed to provide comprehensive information of degradation kinetics at ambient concentrations by indigenous microorganisms present in natural conditions for the representative of the true environment such as contaminated hazard waste sites, wastewater or receiving surface waters by considering the interactions with other species in indigenous microbial community, oxygen, nitrogen sources, cell densities, inducing substrates and nutrient conditions, pH and temperature.

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