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Feasibility of Using Freshwater Microalgae to Remove Triclosan from Aqueous Media**Ana Gisell Pazmino-Sosa^a, Jean-François Blais^b, Pascale Champagne^{c*}**

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Abstract

Triclosan, [5-chloro-2-(2,4-dichlorophenoxy)phenol] (TCS), a broad-spectrum antimicrobial agent found in many personal care products, has raised concerns due to its presence in the environment. TCS has been associated to harmful effects, including oxidative damage in golfish cells, increased lipid peroxidation in clams, and disruption of the hypothalamic-pituitary-gonadal axis in Catla fish, and its potential contribution to antimicrobial resistance. This study evaluates the feasibility of using two freshwater microalgae species to remove TCS from aqueous media by 1) determining the toxicity of TCS on algal cultures, 2) evaluating their potential to remove TCS, and 3) identifying the TCS degradation kinetics. The toxicity test assessed various concentrations of TCS (0.06, 0.10, 0.20, 0.30, 1 mg L⁻¹) on *Chlorella vulgaris* and *Scenedesmus obliquus* growth. Results showed that *C. vulgaris* was entirely inhibited by concentrations exceeding 0.10 mg L⁻¹. In comparison, *S. obliquus* tolerated up to 0.30 mg L⁻¹ after six days of lag phase, but 1 mg L⁻¹ was toxic for both species. The removal efficiency achieved by *S. obliquus* was between 79% and 94% across all concentrations tested, while *C.*

vulgaris achieved 70-95% removal only in concentrations lower than 0.10 mg L⁻¹. The degradation kinetics revealed that the TCS half-life in wastewater was 1.3 days when *S. obliquus* was present, highlighting its potential to enhance pollutant removal. This study provides insights into the use of *S. obliquus* for removing contaminants from natural environments, contributing to understanding TCS dynamics in ecosystems with the presence of microalgae.

Keywords: triclosan, microalgae, toxicity, elimination, reaction rate, kinetics

1. Introduction

Triclosan, [5-chloro-2-(2,4-dichlorophenoxy)phenol] (TCS), is a broad-spectrum antimicrobial agent found in a wide range of personal care products, including hand-disinfecting soaps, deodorants, cosmetics, dental hygiene products, and medical creams. It is typically used at concentrations of 0.1 to 0.3% of the product weight [1, 2]. In 2010, TCS was found to be the active ingredient in 93% of liquid, gel, or foam soaps, resulting in global production of approximately 1,500 tons per year [3,4]. The chemical structure of TCS is similar to other environmental pollutants, such as polychlorinated biphenyls (PCBs) and dioxins, characterized by an aromatic nature and chlorine content, which contributes to its resistance and persistence in the environment [5]. The primary sources of TCS in wastewater include rinse-off personal care and cleaning products, human feces and urine excretion [4].

The presence of TCS in the environment has been linked to various detrimental effects, including disruptions in the microsomal detoxification process, nephrotoxicity, hepatotoxicity, and reduced prenatal and postnatal survival in rats [6]. Additionally, TCS has been shown to cause oxidative damage in goldfish *Carassius auratus* cells [7], an increase in lipid peroxidation

in the clam *Ruditapes philippinarum* [8], and the overproduction of reactive oxygen species (ROS) that can interfere with the cellular antioxidant defense system of organisms [9]. Moreover, research has linked TCS to endocrine disruption in fish such as Catla, causing premature stimulation of steroid biosynthesis and interfering with the hypothalamic-pituitary-gonadal (HPG) axis [10]. Concerns regarding the contribution of TCS to antimicrobial resistance have been also raised, representing a significant public health concern [11]. As a result, regulations have been implemented to limit TCS concentration and use. For example, in Canada, TCS acceptable concentration in mouthwashes is limited to 0.03%, non-prescription drugs are limited to 1.0%, and cosmetics and natural health products are limited to 0.3% (w/w) [12]. Similarly, the Food and Drug Administration (FDA) has banned TCS in US over-the-counter consumer antiseptic wash products [13]. These measures aim to mitigate adverse environmental and health impacts due to TCS exposure.

Despite regulatory efforts, TCS continues to be detected in drinking and surface water, as well as wastewater-treated effluents and biosolids [14, 5]. A study conducted in Canada between 2002 and 2013 found that wastewater treatment plant effluents reported TCS concentrations ranging from 12 to 4,160 ng TCS L⁻¹, while surface waters had 844 ng TCS L⁻¹ [15]. Similarly, across the US, TCS was present in 57.6% of water bodies, making it one of the ten most recurrent surface water contaminants [16]. TCS has been detected in wastewater from hospitals, industries and landfills [17], as well as sewage sludge [18]. The removal of TCS from wastewater depends on the treatment processes employed, with 50% of its elimination attributed to sorption to solids in the primary treatment [19].

While aerobic bacteria and fungi have been studied for their ability to biodegrade TCS [20, 21],

limited information is available regarding its biodegradation by microalgae. Microalgae offer several advantages over bacteria for removing contaminants, as they are highly tolerant to pollutants and generate biomass through nutrient uptake, thereby enhancing their capacity for pollutant removal [22]. The aerobic conditions microalgae create as a result of photosynthesis could play a crucial role in TCS removal. Researchers have reported that certain microalgal species can remove TCS from synthetic media, with removal efficiencies ranging from 30-100% [23, 24], depending on the concentration tested. However, there is still a need to understand their efficiency in removing TCS from real wastewater. In our previous work [25], we observed that freshwater microalgal species *C. vulgaris* and *S. obliquus* exhibited tolerance and removal potential for synthetic estrogen (EE2). Therefore, our current research aims to evaluate these same species with the following objectives: 1) assess whether TCS is toxic to microalgae in terms of biomass production and growth rate, 2) investigate the potential for TCS removal by *C. vulgaris* and *S. obliquus*, and 3) determine the degradation kinetics of TCS facilitated by microalgae in wastewater environments. This information will be valuable in identifying efficient TCS biodegradation processes and to provide insights into the applicability of using microalgae in contaminated environments.

2. Materials and methods

2.1 Chemicals

Triclosan (>98% purity) was purchased from Sigma-Aldrich, and the stock solution (50 mg L⁻¹) was prepared by dissolving the pure powder in chromatographic-grade methanol (MeOH, Sigma Aldrich). The certified reference material TCS-M, 5-chloro-2-(2,4-

dichlorophenoxy)phenol, was obtained from Wellington Laboratories (> 98%, Guelph, ON, Canada). All other chemicals used were of analytical grade.

2.2 Microalgal strains, cultivation, and inoculum preparation

The microalgal strains used in this study were *Chlorella vulgaris* (MCWW-28) and *Scenedesmus obliquus* (Bow-12), obtained from the National Research Council of Canada. The information regarding their cultivation and inoculum preparation has been described in our previous research [25]. Briefly, the microalgal species were individually inoculated in 250-mL Erlenmeyer flasks containing 100 mL Bold's Basal Medium (BBM) at 10% (v/v) microalgae concentration. The recipe followed to prepare BBM was described by Andersen (2005) [26]. These flasks were placed on a shaker at 150 rotation per minute (rpm) at 20°C under day: night light cycles (12:12 h) with white LED light ($300 \mu\text{mol photon} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) (Caron, gBrite™ LED).

2.3 Secondary wastewater (WW) and synthetic wastewater (SW)

The wastewater used in this study was collected from the secondary (biological) treatment process at the East wastewater treatment plant in Quebec City (QC, Canada). The samples were immediately taken to the laboratory, sterilized with a UV lamp, and stored in a dark, cold room at 4°C.

Synthetic wastewater was prepared to simulate the composition of the WW sample. The protocol followed was a modified version of the recipe proposed by Benitez et al. (2018) [27]. It consisted of $37.4 \text{ mg L}^{-1} \text{ CaCl}_2 \cdot 2\text{H}_2\text{O}$, $56.7 \text{ mg L}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, $60.0 \text{ mg L}^{-1} (\text{NH}_4)_2\text{CO}_3$, $5.0 \text{ mg L}^{-1} \text{ NaNO}_3$, $7.1 \text{ mg L}^{-1} \text{ KH}_2\text{PO}_4$, $0.1 \text{ mg L}^{-1} \text{ FeSO}_4 \cdot 7\text{H}_2\text{O}$, $0.1 \text{ mg L}^{-1} \text{ ZnCl}_2$, $0.2 \text{ mg L}^{-1} \text{ CuSO}_4 \cdot 5\text{H}_2\text{O}$, $100 \text{ mg L}^{-1} \text{ C}_6\text{H}_{12}\text{O}_6$ and $0.001 \text{ mg L}^{-1} \text{ H}_3\text{BO}_3$. The solution was filter-sterilized with a $0.22\text{-}\mu\text{m}$ PES

filter and immediately used.

Table 1 summarizes the average values of the principal chemical characteristics of the WW and SW, following the protocols outlined in section 2.9.

Table 1. Characterization of secondary and synthetic wastewater (WW and SW) used in this study

Parameter	Unit	WW	SW
Chemical oxygen demand	mg L ⁻¹	144.9	107
Total suspended solids	mg L ⁻¹	22	< 5
pH		7.5	7.3
Nitrogen total	mg L ⁻¹	16	18
NH ₃ -NH ₄	mg L ⁻¹	15	17
Phosphorus total	mg L ⁻¹	1.6	1.6

2.4 Triclosan toxicity test

Toxicity tests were conducted for twelve days at different TCS concentrations (0.06, 0.10, 0.20, 0.30 and 1 mg L⁻¹). The TCS stock solution was dissolved to achieve the desired concentrations. The solvent used (MeOH) in the medium was less than 1% (v/v), which was proven to be non-toxic for the microalgal species in our previous research [25]. Microalgal cells were obtained from the seed cultures during their exponential growth phase. In 150-mL Erlenmeyer flasks, 100-mL of sterilized BBM was inoculated with 10% microalgal suspension, and the initial cell density was approximately 3×10^6 cells mL⁻¹. Each bioassay was performed in four replicates,

and an algal control (no TCS presence) was included in the set of experiments. Flasks were placed on an orbital shaker at 150 rotation per minute (rpm) at 20°C under day: night light cycles (12:12 h) with white LED light ($300 \mu\text{mol photon. m}^{-2} \text{ s}^{-1}$) (Caron, gBrite™ LED). 10 mL daily aliquots of the microalgal suspension were collected to monitor growth via optical density measurements. Additionally, pH changes were recorded at the beginning and at the end of cultivation period without any adjustments.

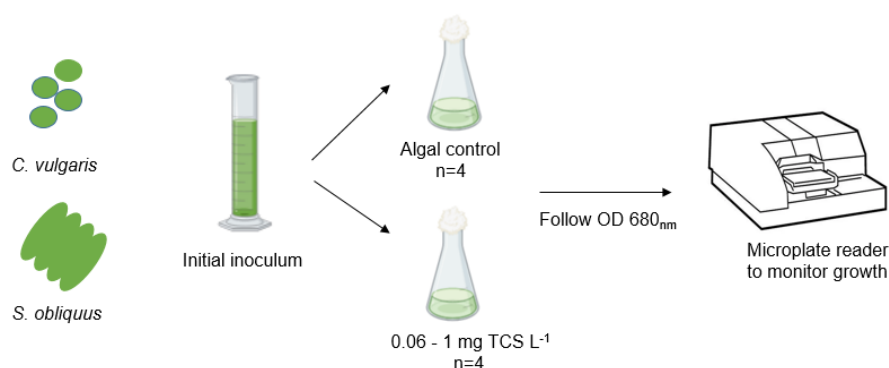


Figure 1. Followed scheme for the toxicity test

2.5 TCS degradation under light/dark conditions in different media

Abiotic tests were conducted without microalgae to investigate the impact of the medium interaction on TCS degradation. These tests added $0.30 \text{ mg TCS L}^{-1}$ to 100 mL of sterile BBM, SW and WW. Flasks were placed on an orbital shaker and exposed to light (24 h) or dark (0 h) conditions (covered with aluminum foil) to assess photolysis effects from light irradiation. TCS concentrations were measured at the start of the experiment and after twelve days of cultivation.

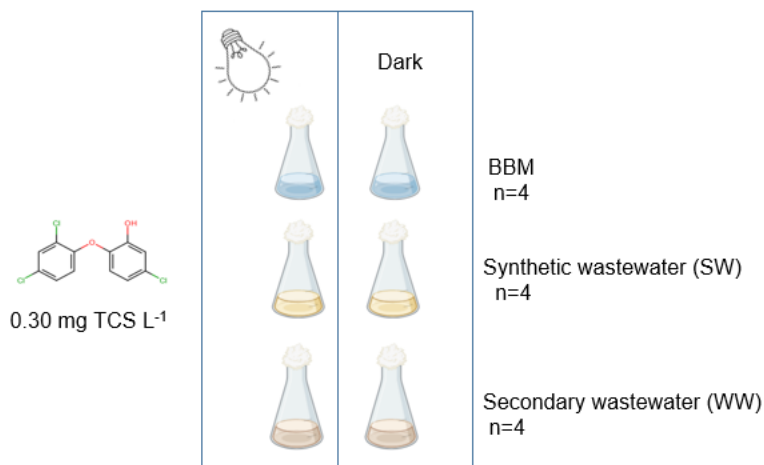


Figure 2. Triclosan abiotic degradation in different testing media

2.6 Triclosan degradation kinetics

S. obliquus was the most tolerant species to TCS concentrations, so it was selected to assess the degradation kinetics of 0.30 mg TCS L⁻¹ over time in three media (BBM, SW, and WW). These tests evaluated the TCS removal efficiency by *S. obliquus* and its dependence on the medium. The algal medium (BBM) provided an ideal environment for algal growth due to its balanced nutrient concentration. Synthetic wastewater (SW) ensured consistency in experimental conditions due to its abiotic nature, while mimicking real wastewater conditions by providing nutrients and organic carbon. Secondary wastewater (WW) was employed to evaluate the treatment efficiency of microalgae in real-world scenarios. The nutrient composition of WW can vary based on the treatment facility, often resulting in higher nitrogen concentrations compared to phosphorus and the presence of microorganisms, which can affect algal growth rates [28]. The wastewater was filtered using a 0.22- μ m PES filter to remove microorganisms and ensure a homogeneous medium. The experimental set up was similar to the one described in Section 2.4, with an initial microalgal density of 3×10^6 cells mL⁻¹, cultivation time of 7 days,

and sampling performed at 0, 2, 4 and 7 days to quantify TCS concentration. The degradation kinetics were assessed based on the disappearance of TCS by fitting the residual concentrations to a pseudo-first-order reaction as follows:

$$\ln C_t = -kt + \ln C_0 \quad (1)$$

where, C_0 and C_t are TCS concentrations (mg L^{-1}) at time zero and time t (d), respectively. The removal rate constant, k (d^{-1}) and half-life ($t_{1/2}$, d) were calculated as followed:

$$k = \ln \left(\frac{C_0}{C_t} \right) \quad (2)$$

$$t_{1/2} = \frac{\ln(2)}{k} \quad (3)$$

2.7 Measurement of microalgal growth

The growth of the microalgal cultures was measured following the protocol described in our previous study [25]. The optical density (OD) at 680 nm and dry cell weight were quantified, and a linear regression was fitted (Equations 4 and 5). Since *S. obliquus* is often found in four-celled groups, each group was counted as one as per Lüring, 2003 [29].

$$\text{Biomass concentration } C.vulgaris \left(\text{mg mL}^{-1} \right) = 0.7338 \text{ OD} - 0.0655 \quad (R^2 > 0.988) \quad (4)$$

$$\text{Biomass concentration } S.obliquus \left(\text{mg mL}^{-1} \right) = 0.4349 \text{ OD} - 0.0959 \quad (R^2 > 0.982) \quad (5)$$

Additionally, the specific growth rate, μ (d^{-1}), was determined using the equation previously reported by Xiong et al. (2016) [30] for cell growth in the exponential phase over time:

$$\mu \left(\text{d}^{-1} \right) = \frac{\ln N_t - \ln N_1}{t - t_1} \quad (6)$$

where N_t is the number of cells at time t , and N_1 is the number of cells at time t_1 . The number of cells was transformed into a natural log, and the slope of the exponential phase was used to calculate the value of μ .

2.8 Measurement of residual triclosan

TCS concentrations were analyzed chromatographically at the beginning (t_0) and end (t) of the toxicity tests. Briefly, 0.5 mL samples were collected from each bioassay at both time points, mixed with chromatographic-grade methanol (MeOH) (1:1 v/v), vortexed, and centrifuged at 5,000 rpm for 10 minutes. A 0.5 mL aliquot of the supernatant was then transferred directly into an HPLC amber-glass vial for subsequent analysis. These measurements represented the residual TCS concentration in the medium, and the percentage removal was calculated as follows:

$$\% \text{ removal} = \frac{C_0 - C_f}{C_0} * 100 \quad (7)$$

where C_f is the concentration (mg L^{-1}) at the end of the cultivation period (t), and C_0 is the initial measured concentration (mg L^{-1}).

TCS analysis was performed using ultra-high-pressure liquid chromatography (UPLC) (Agilent Technologies 1260 Infinity II) with ultraviolet detection wavelength at 210 and 280 nm. Chromatographic separation was achieved using a Poroshell EC- C18 column ($2.7 \mu\text{m}$, $3.0 \times 150 \text{ mm}$) at a column temperature of 32°C . Sample injections were performed by an autosampler with an injection volume of $10 \mu\text{L}$.

The mobile phase (isocratic) was a mixture of 80:20 MeOH and water, with a total run time of 8 min and the retention time of 5.2 min. The detection limit for TCS was $1.86 \mu\text{g L}^{-1}$, and the quantification limit was $5.78 \mu\text{g L}^{-1}$.

Additionally, TCS adsorption on microalgal cells was assessed following the protocol described by Xiong et al. (2016) [30]. At the end of cultivation, 10 mL of microalgal suspension was collected and centrifuged at 10,000 rpm for 15 min. The supernatant was discarded, and the cell pellet was washed three times with distilled water, then resuspended in 5 mL MeOH, vortexed, and centrifuged again. Finally, 0.5 mL of the supernatant was mixed with 0.5 mL MeOH in an amber-glass vial for analysis to determine the amount of TCS sorbed onto microalgal cells.

2.9 Physico-chemical parameters

- a. pH: Measurement of pH values were performed with a pH-meter Orion Versa Star Pro™ Multiparameter Benchtop Meter. The electrode used was Thermo Scientific™ Orion™ ROSS Ultra™ Glass Triode™ that was filled and calibrated before analysis.
- b. Ammoniacal Nitrogen ($\text{NH}_3\text{-NH}_4$): Measurements were conducted using a Technicon Autoanalyzer (Lachat Instruments, Loveland, CO, USA) following the QuickChem 10-107-06-2-B method (NH_4 ,TKN) [31]. The calibration curve was established with standard concentrations of 0, 0.5, 1, 3, 5 and 10 mg N- NH_4 L⁻¹. Dilutions were prepared using ultrapure water containing 0.2% H_2SO_4 . A 10 mL sample was filtered and acidified with concentrated H_2SO_4 to lower the pH below 2. If necessary, samples were diluted to fit within the calibration curve range.
- c. Total phosphorus: These analyses were performed using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) Varian 725-ES (Littleton, CO, USA) following the QuickChem 10-115-01-1-B (PO_4) method [31]. The calibration curve was established with standard concentrations of 0, 0.5, 1, 3, 5 and 10 mg PO_4 L⁻¹. A 5 mL sample was filtered and acidified with 0.25 mL HNO_3 . If necessary, samples were diluted to fit within the calibration curve range.
- d. Chemical oxygen demand: COD measurements were performed using a UV spectrophotometer following the MA 315-DCO 1.1 analytical method [31]. The calibration curve was established using standard concentrations of 0, 100, 250, 500 and 1000 mg L⁻¹. For sample or standard preparations, 2.5 mL of filtered sample/standard, 1.5 mL of digestion solution, and 3.5 mL of acid reagent were added to digestion tubes. The tubes were vortexed and subjected to digestion at 150°C for two hours. After cooling, the absorbance of the digested sample was measured at 600 nm.

e. Total suspended solids (TSS): TSS were determined following method 1684 [32]. Briefly, pre-weighed and pre-dried glass fiber filters with a pore size of 0.45 μm were used. A 20 mL of sample was filtered using a vacuum filtration system and then dried overnight at 105°C. The filter weight after filtration was recorded, and TSS were calculated using the following equation:

$$\text{TSS (mg L}^{-1}\text{)} = \frac{W_2 - W_1 \times 1000}{\text{volume (mL)}} \quad (8)$$

where, W_2 is the final weight of the filter with dried solids (mg) and W_1 is the initial weight of the filter (mg).

2.10 Statistical analysis

For each analysis, the mean and standard deviation were calculated for the four replicates of each bioassay. The results were analyzed for normal distribution and homogenous variance ($n = 4$). SigmaPlot 2.0 software was used to test significant differences between the growth rate, final biomass, and removal percentage using one-way analysis of variance (ANOVA) once normal distribution was proved.

3. Results

3.1 Triclosan toxicity tests

In this study, the growth behavior of two species of algae, *C. vulgaris* and *S. obliquus*, was examined in the presence of different concentrations of TCS. The results indicated a difference in TCS tolerance among the microalgal species (Figure 1). For instance, *C. vulgaris* required a

lag phase of 6 days to initiate growth at concentrations of 0.06 and 0.10 mg TCS L⁻¹, followed by an exponential phase from day 6 to day 12, resulting in calculated growth rates (GR) of 0.35 d⁻¹ and 0.11 d⁻¹, respectively (Table 2). These results suggested the importance of the adaptation phase for *C. vulgaris* in environments containing TCS, which allowed algae to grow despite the presence of TCS. The final biomass of *C. vulgaris* was 419 mg L⁻¹ and 186 mg L⁻¹, respectively. The biomass concentration (BC) of this species was significantly lower compared to the control ($p < 0.05$), and it decreased as TCS concentrations increased, which is consistent with the study conducted by Taştan et al. (2017) [33] that reported decreasing biomass production of *Chlorella* sp. due to higher TCS concentrations (from 3 to 12 mg L⁻¹).

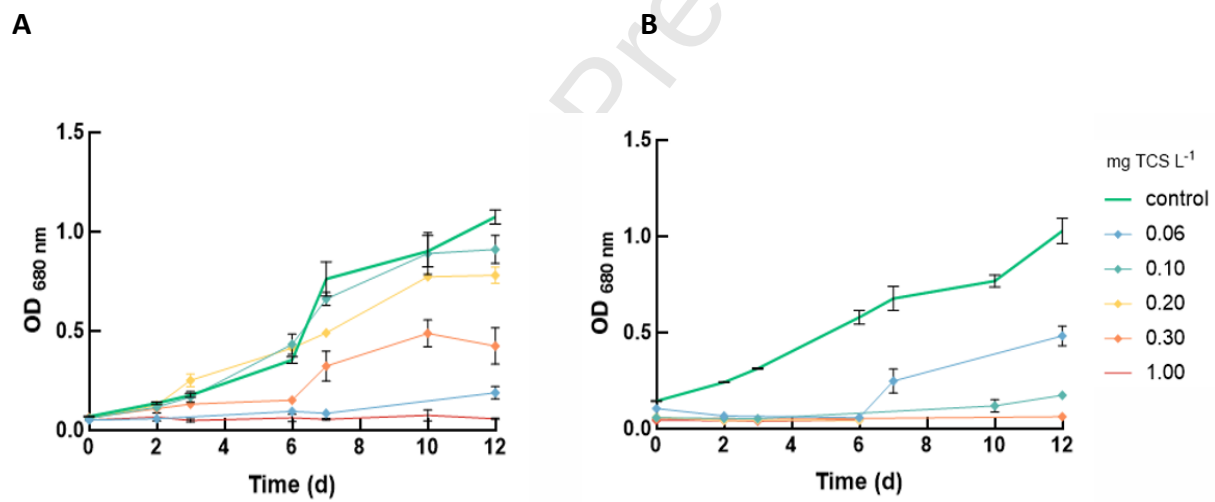


Figure 3. Growth curves for A) *S. obliquus* and B) *C. vulgaris* over a twelve-day cultivation period for different TCS concentrations tested. Error bars represent standard deviations ($n=4$)

Table 2. Growth rates (μ , d^{-1}) and pH values obtained for *S. obliquus* and *C. vulgaris* cultivated in different TCS concentrations

Microalgae	TCS ($mg\ L^{-1}$)	Initial pH	Final pH	Growth rate (μ) (d^{-1})
<i>S. obliquus</i>	control	6.90	8.11	0.32 ± 0.02
	0.06	6.81	9.53	0.12 ± 0.01
	0.10	7.20	9.23	0.26 ± 0.02
	0.20	6.73	9.43	0.23 ± 0.01
	0.30	6.70	9.43	0.18 ± 0.01
	1.00	7.34	6.92	0.02 ± 0.01
<i>C. vulgaris</i>	control	7.01	8.90	0.24 ± 0.01
	0.06	6.82	9.81	0.35 ± 0.01
	0.10	7.23	7.30	0.11 ± 0.01
	0.20	6.92	7.71	
	0.30	6.90	7.70	
	1.00	7.42	6.93	

Conversely, results for *S. obliquus* culture showed similar behavior between the tested concentrations of 0.06 and 0.30 $mg\ L^{-1}$, where a lag phase of 6 days was observed, while an exponential phase occurred between day 6 to day 10, resulting in a GR of $0.12\ d^{-1}$ and $0.18\ d^{-1}$, respectively. The BC for these concentrations were 178 $mg\ L^{-1}$ and 373 $mg\ L^{-1}$, respectively. However, at concentrations of 0.10 and 0.20 $mg\ L^{-1}$, an immediate growth was observed, and GR was $0.26\ d^{-1}$ and $0.23\ d^{-1}$, while the final BC was 492 $mg\ L^{-1}$ and 435 $mg\ L^{-1}$, respectively. As a result, the BC formed two significantly different groups based on TCS concentrations tested:

0.06 and 0.30 mg L⁻¹, and 0.10 and 0.20 mg L⁻¹. Toxicity generally increases linearly with concentration. However, other factors, such as TCS availability and threshold effects, could explain the difference in algal growth results obtained for the two groups. TCS inhibits fatty acid biosynthesis even at sublethal concentrations [11], which could lead to the observed effects across the range of concentrations tested.

Finally, it was found that the highest concentration tested, 1 mg TCS L⁻¹, resulted in a complete inhibition of cell development and growth for both species. This finding was consistent with the results reported by Xin et al. (2019) [34], which observed the highest growth suppression in six green algal species exposed to 1 mg TCS L⁻¹. However, in this study, lower concentrations of 0.20 mg L⁻¹ led to algae growth inhibition in *C. vulgaris*. Similar results were observed by Atengueño-Reyes et al. (2023) [35], who identified a growth inhibitory effect starting at 0.32 mg L⁻¹ on a microalgal consortium consisting of *S. obliquus* and *Desmosdesmus* spp. The observed difference in TCS tolerance could be attributed to the specific algal species and whether it was cultivated as an isolated inoculum or as part of a consortium [36].

Limited information on the cultivation of *C. vulgaris* in media containing TCS is available. The study conducted by Dai et al. (2021) [37] observed that when the TCS concentration exceeded 1.05 mg L⁻¹, it completely inhibited the growth of *C. vulgaris* due to cell membrane damage caused by an increase in reactive oxygen species (ROS). On the other hand, concentrations below 0.75 mg L⁻¹ did not hinder growth and even had a positive effect on cell density during the 10-day cultivation period [37]. The difference observed in our results could be due to the light conditions (16 h vs 12 h in this study), as longer or continuous illumination stimulates the growth of microalgal species and helps them overcome pollutant toxicity [38]. When

investigating the effects of TCS on *S. obliquus*, the study performed by Wang et al. (2018) [24] found that concentrations ranging from 0.05 – 0.80 mg L⁻¹ did not lead to a lag phase or growth inhibition in the first four days of cultivation. In contrast, our study showed a lag phase lasting six days at concentrations of 0.06 and 0.30 mg L⁻¹. This difference could be associated with the higher initial algal cell concentration (1.0 x 10⁷ cells mL⁻¹ vs 3.5 x 10⁶ cells mL⁻¹ in this study), which helped the culture adapt to the TCS-containing medium more rapidly.

Our research has shown that *S. obliquus* can tolerate higher concentrations of TCS compared to *C. vulgaris*, as reported by Wang et al. (2018) [24]. Some studies have suggested that cell size may correlate with pollutant concentration tolerance. This is because smaller-sized cells may be more susceptible to toxicity due to differences in intracellular diffusion rates [39]. *C. vulgaris* cell size ranges between 2 to 10 µm diameter and they exist as single cells [40], while *S. obliquus* cells have a similar size range, but can exist in unicellular form or colonies comprising 4 or 8 cells [41]. The configuration of *S. obliquus* as multicellular colonies leads to lower nutrient adsorption from surroundings, resulting in a higher tolerance to environmental pollutants [42]. Therefore, the observed differences in toxicity between the two algal species could be attributed to culture conditions affecting the configuration of the cells in the medium, giving them different tolerances to environmental concentrations [35].

Another factor involved in TCS toxicity in microalgal cultures is pH. TCS has a dissociation constant (pKa) of 8.1, making it a weak acid that is entirely neutral at pH 6 and fully ionized at pH 10 [14]. TCS is more toxic at lower pH levels due to its lipophilic characteristic, which enables the neutral form to easily traverse phospholipid membranes [43]. Overall, the pH variability during this research was from 6.92 to 9.23, mainly in *S. obliquus* cultures where

growth was observed. The increase in pH is a consequence of algal photosynthesis, where the uptake of CO_2 from the medium and release of OH^- to the environment occurs [44]. Therefore, during the lag phase of the cultures, TCS was mainly in its more toxic form, inhibiting algal development. However, as microalgal biomass concentration increased, pH increased, leading to lower toxicity in the algal cultures after six days. Similar conclusions were drawn by Roberts et al. (2014) [43], who observed higher toxicity at pH 7.0 compared to 8.5 in a culture of *S. subspicatus*. Moreover, when 1 mg L^{-1} TCS was tested in our study, pH culture remained at 7 for both species, where TCS is mainly in neutral form, contributing to cell death.

In general, the detrimental effect of TCS on bacterial cells is associated with the inhibition of fatty acid synthesis [4]. The same toxic pathway could be observed in microalgal cells [45]. Furthermore, TCS is predicted to accumulate in mitochondria and chloroplasts, where lipid synthesis occurs [43]. However, the sensitivity and tolerance to TCS by microalgae is dependent on species tested and exposure concentration, as concluded by Bi et al. (2018) [46]. In this research, *C. vulgaris* growth was completely inhibited at concentrations higher than 0.10 mg L^{-1} . At the same time, for *S. obliquus*, the lag phase allowed the culture to grow in $0.30 \text{ mg TCS L}^{-1}$, but 1 mg L^{-1} was toxic for both species, inhibiting cell development.

3.2 Triclosan degradation under light/dark conditions in different media

In this research, a TCS concentration of 0.30 mg L^{-1} was tested in BBM, SW and WW under both light and dark conditions without algae. Conducting abiotic tests allowed for the assessment of the natural degradation of TCS in the medium due to chemical and physical interactions, particularly with light. Furthermore, each medium was selected for specific purpose: BBM was used as a control medium, providing a controlled environment without additional variables. SW

ensured consistent composition, allowing for controlled comparisons of TCS degradation. And, WW represented real environmental conditions, containing naturally occurring organic matter, microorganisms, and other contaminants that could influence TCS degradation.

The results indicated that TCS degradation was negligible in BBM and SW, with removal ranging between 7% and 14% (Table 3). These findings are consistent with previous research by Roberts et al. (2014) [43], which showed little to no degradation ($< 10\%$) of TCS in algal medium after ten days. Moreover, no significant difference was observed between light and dark conditions in these media ($p > 0.05$), suggesting minimal interactions with TCS. In contrast, the elimination of 39% and 18% was observed in WW in light and dark conditions, respectively. These results demonstrated that the medium used significantly influences TCS percentage removal ($p < 0.05$). In WW, TCS degradation was likely due to a pH increase and indirect photodegradation. The pH of the medium increased from 7.54 to 8.30, possibly due to interactions between light, organic matter, and agitation, leading to TCS dissociation, where at least 50% of TCS became negatively charged. In this condition, TCS photodegradation and adsorption occurred more quickly than in its neutral form [47]. Indirect photodegradation occurred when a photosensitizer, such as dissolved organic matter, was present in WW. Light absorption generates free radicals that cause photooxidation of organic compounds [48]. Moreover, organic matter has been found to enhance the photodegradation rate of phenolic compounds [49].

Overall, the results of this study suggested that TCS degradation did not occur significantly under abiotic conditions, except in WW while exposed to light, which could likely be attributed to compound photolysis, medium pH, and the potential presence of photosensitizers. These results established a baseline of TCS degradation during biotic processes.

Table 1. Abiotic degradation of 0.30 mg TCS L⁻¹ spiked on algae medium (BBM), synthetic wastewater (SW) and wastewater (WW) exposed to light or dark for seven days

Medium	Removal (%)		Final pH
	Light	Dark	
BBM	7.19 ± 2.62	9.27 ± 1.97	7.04
SW	14.20 ± 2.49	11.49 ± 0.17	7.65
WW	39.03 ± 1.23	18.50 ± 0.69	8.30

3.3 Triclosan removal by microalgae

To assess the feasibility of using *C. vulgaris* and *S. obliquus* for further experiments, their potential to remove TCS was initially evaluated in Bold's Basal medium (BBM). The results shown that these microalgal species had different abilities to remove TCS at various concentrations. For instance, *C. vulgaris* removed 95% of TCS at the lowest concentration tested (0.06 mg L⁻¹), whereas 76% was removed at 0.10 mg L⁻¹. As TCS concentrations increased, the removal efficiency of *C. vulgaris* significantly decreased ($p < 0.05$) (Figure 2A). Additionally, the biomass produced by *C. vulgaris* influenced the removal efficiency of TCS, with higher biomass resulting in greater TCS removal. This suggested that TCS concentration significantly influenced both the final microalgal biomass produced and the removal efficiency of *C. vulgaris*. Other studies have also observed a relationship between algal growth and pollutant removal. For example, Taştan et al. (2017) [33] found that higher biomass of *Chlorella* sp. resulted in higher TCS removal. Specifically, a 19% increase in removal was observed when

biomass increased from 300 mg L⁻¹ to 400 mg L⁻¹. Similarly, Bano et al. (2021) [23] noted a negative correlation between the remaining TCS concentration in the medium and microalgal growth. *C. vulgaris* biomass is necessary for TCS removal, and concentrations leading to toxicity will inevitably impact removal efficiency.

On the other hand, *S. obliquus* showed removal percentages between 78% (at 0.06 mg L⁻¹) to over 94% (at 0.10 and 0.30 mg L⁻¹). However, only 38% was removed at the highest concentration tested (1 mg L⁻¹) (Figure 2B). The results showed no significant relationship between the concentrations tested and biomass produced ($p > 0.05$), with the exception of 1 mg L⁻¹ tested. For *S. obliquus*, the highest algal growth was observed at 0.10 - 0.30 mg L⁻¹, with an increase in pH from 6.92 to 9.23. This suggests that the algal growth contributed to the degradation of TCS and its dissociation. Even at the lowest concentration tested (0.06 mg L⁻¹), 78% removal was observed despite low algal growth, which was attributed to a pH change in the medium, increasing from 6.90 to 8.11. This increase in pH is expected due to photosynthesis, where inorganic carbon is fixed, and OH⁻ or H⁺ maintains electroneutrality inside the cell [50].

Various microalgal species demonstrate different capacities for removing TCS from algal medium. For example, *Nannochloris* sp. completely removed 10 µg TCS L⁻¹ within 7 days, primarily through cell sorption and biodegradation during the exponential phase [51]. In contrast, *Chlorococcum* sp. rapidly removed 1,000 µg TCS L⁻¹ within the first six days of cultivation, but the removal percentage gradually decreased over 30 days, ultimately reaching 78% [34]. However, in the same study, at a lower concentration of 154 µg TCS L⁻¹, removal was slower, with 50% of the initial concentration still present in the medium after 30 days. These

findings suggest that lower TCS concentrations do not necessarily result in higher removal efficiencies, as corroborated in this research at the low concentration tested using *S. obliquus*.

In this study, *S. obliquus* efficiently removed TCS from the medium (>78%) at initial concentrations ranging from 0.06 to 0.30 mg TCS L⁻¹, regardless of the biomass produced. However, no TCS was detected on *S. obliquus* biomass, while 12% was adsorbed onto *C. vulgaris* cells. These findings align with those of Larsen et al. (2019) [52], who reported that only a small fraction of TCS (7%) was associated with the algal biomass of *C. vulgaris* and *S. obliquus*.

At lower pH values, the neutral form of TCS is expected to be more readily absorbed onto cell surfaces [53]. The pH values observed in the cultures (Table 2) explain these results, as only bioassays with no biomass growth maintained a pH that allowed the neutral form of TCS to persist and be sorbed.

Overall, the presence of *S. obliquus* increased the removal efficiency of TCS across all concentrations investigated compared to the abiotic controls.

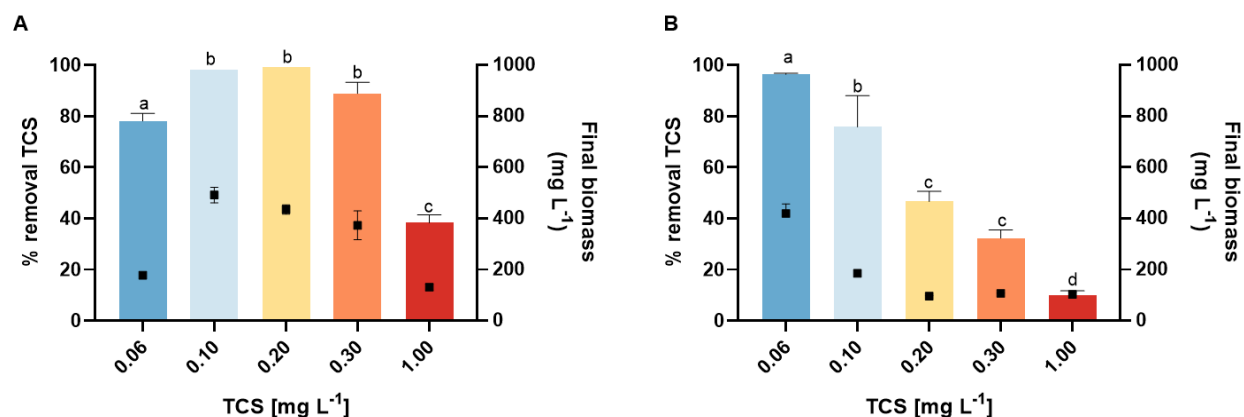


Figure 4. TCS removal percentage - filled bars (left axis) and final biomass produced – black square ■ (right axis) for *S. obliquus* (A) and *C. vulgaris* (B) after twelve days of cultivation at TCS concentrations tested (Mean and standard deviation shown, $n=4$). The lowercase a-e in each bar represents a significant difference ($p \leq 0.05$)

3.4 Degradation kinetics of TCS by microalga

The TCS degradation (0.30 mg L⁻¹) mediated by *S. obliquus* over time was fitted to a first-order kinetic model for the three media tested: BBM, synthetic wastewater (SW) and wastewater (WW) (Figure 3). The trend revealed a steady decrease in TCS over seven days, with WW demonstrating notably faster degradation after the second day of cultivation, while for BBM, the degradation was faster from day 4. The final percent removals achieved were 98% in WW, 80% in SW and 70% in BBM, with the degradation rate (k) and half-lives summarized in Table 4. The degradation rates were 0.06, 0.26 and 0.60 d⁻¹ for BBM, SW and WW; corresponding to half-life ($t_{1/2}$) values of 10.94, 2.64 and 1.30 days. The degradation fit a first-order kinetic model, with R^2 values ranging from 0.85 to 0.98, indicating a good fit. Kinetic parameters of TCS in

microalgae cultures in the same media tested are not available in the literature, making data comparison not possible.

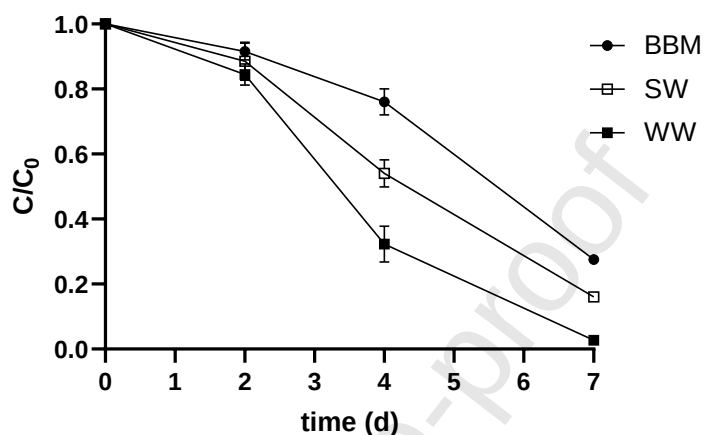


Figure 5. Degradation kinetics of TCS by *S. obliquus* tested in BBM (●), synthetic wastewater (SW, □) and wastewater (WW, ■)

Table 4. Kinetic parameters of TCS degradation by *S. obliquus* tested in BBM, synthetic wastewater (SW) and wastewater (WW)

Medium	k (d ⁻¹)	t _{1/2} (d)	R ²
BBM	0.06 ± 0.01	10.94	0.856
SW	0.26 ± 0.01	2.64	0.975
WW	0.60 ± 0.02	1.30	0.988

Studies evaluating the removal of TCS in algal media are more common; however, testing natural wastewater is infrequent due to the significant variability of this matrix. In this case, surface water might be used, and considered as a complex matrix due to the presence of

microorganisms and other contaminants. Bai and Acharya (2017) [54] evaluated lake water samples for TCS ($10 \mu\text{g L}^{-1}$) degradation by *Nannochloris sp.* They reported 95% removal that followed a first-order decay model from day 0 to 7, with a half-life of 1.3 d and k of 0.53 d^{-1} . These values are consistent with our results obtained using WW, half-life (1.3 d) and k (0.60 d^{-1}). This similarity could be associated with the rapid photolysis and TCS uptake by algae and other microorganisms that could occur in the environment. Notably, our research evaluated a concentration 30-fold higher ($300 \mu\text{g L}^{-1}$ vs $10 \mu\text{g L}^{-1}$), corroborating the feasibility of using *S. obliquus* for effective TCS removal. In contrast, Larsen et al. (2019) [52] reported degradation constants of 0.052 and 0.011 d^{-1} for $10 \mu\text{g TCS L}^{-1}$ by *C. vulgaris* and *S. obliquus*, and removal efficiencies of 100% and 76%, respectively. Contrary to our results, they observed better performance for TCS elimination by *C. vulgaris*, which could be attributed to different cultivation conditions, such as a more extended photoperiod (16:8 light: dark), lower concentration tested and cultivation medium used [52]. Moreover, the authors noted that phototransformation was the primary removal mechanism.

When *Euglena gracilis* strain Z was tested for $10 \mu\text{g TCS L}^{-1}$ degradation by Lam et al. (2022) [44], three different media were tested under similar experimental conditions to those employed in the present study. However, the media consisted of autoclaved wetland water, miliQ water and BBM. Their results showed degradation constants of 0.076 d^{-1} , 0.095 d^{-1} and 0.196 d^{-1} . Taking the k values for BBM, our results were 3-fold lower, probably explained by the different algal species and the initial TCS concentration tested. Notably, *E. gracilis* strain Z showed a faster degradation rate in BBM than the other media tested, contrary to our results, where algae in WW showed a faster degradation rate. The different algal species tested, the

TCS concentration, and indirect photolysis occurring in WW could explain this difference. Moreover, TCS dissociation due to pH variation and the presence of dissolved oxygen concentrations produced by algae could have enhanced the degradation rate. Therefore, overall, WW showed faster degradation than SW and BBM.

To our knowledge, this is the first study to provide insights into the potential use of *S. obliquus* for removing contaminants from natural environments such as WW. Moreover, the k and half-life values contribute to understanding TCS in ecosystems where microalgae are present.

4. Conclusions

In this work, two common freshwater algal species were exposed to different concentrations of TCS to evaluate its toxicity. Results showed that *C. vulgaris* required a lag phase of six days to grow in concentrations lower than 0.10 mg L^{-1} , while higher concentrations are toxic for this species. In the case of *S. obliquus* cultures, the species showed tolerance to TCS up to 0.30 mg L^{-1} , while 1 mg L^{-1} completely inhibited their growth. The removal efficiency decreased as the TCS concentrations increased, which was more noticeable in *C. vulgaris* culture. Additionally, degradation kinetics varied depending on the media tested, with a higher degradation rate and lower half-life observed in WW than in BBM. Despite variations in experimental conditions and media compositions, the effectiveness of *S. obliquus* in degrading TCS remains consistent throughout the research. These findings support the potential use of *S. obliquus* for removing contaminants like TCS from natural environments, highlighting its ability for wastewater treatment and environmental remediation.

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CRedit authorship contribution statement

AGPS: wrote the manuscript, designed and conducted the experiments, obtained and analyzed the results. PC and JFB supervised the project and reviewed the manuscript.

Conflict of interest:

We declare that we have no conflicts of interest that could influence the research, analysis, or interpretation of the results presented in this manuscript.

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Thank you for considering our submission. We believe that our research aligns with the scope and objectives of *Algal Research* journal.

Highlights:

- Triclosan (TCS) is an endocrine disruptor harmful to ecosystems and human health.
- TCS inhibits microalgal growth and biomass production, with species-specific effects.
- TCS degradation without algae in BBM and synthetic media was under 14%.
- TCS removal in wastewater without algae reached 39%, indicating matrix interactions.
- *S. obliquus* enhanced TCS removal in BBM, synthetic, and wastewater to 70-98%.
- TCS degradation by *S. obliquus* had half-lives of 10.94 to 1.30 days in various media.