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Anatoxin-a degradation by using titanium dioxide

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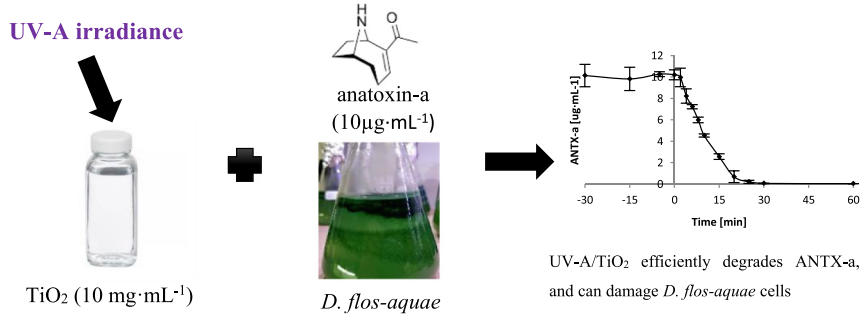
HIGHLIGHTS

- TiO2 photocatalysis efficiently degrades neurotoxic anatoxin-a.
- Several toxin decomposition products were noted with m/z ratio from 156.11 to 216.1.
- Photocatalyst can damage cyanobacterial cells and degrade the released toxin.
- Homogenization of cyanobacterial cells accelerates degradation of toxin.
- Decomposition products were not toxic to *T. platyurus*.

GRAPHICAL ABSTRACT



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ABSTRACT

Advanced oxidation processes, such as using titanium dioxide (TiO_2) photocatalysis, are being developed to reduce or eliminate the toxicity of treated water. In this study, the removal of purified anatoxin-a (ANTX-a), live *Dolichospermum flos-aquae* cells, and a cell extract of this organism under UV-A/TiO_2 photocatalysis, creation of decomposition products and their toxicity were investigated. Total degradation of purified ANTX-a from the initial concentration of 10 mg·L^{-1} with the addition of TiO_2 under UV-A irradiation was achieved in 30 min. Under these conditions several decomposition products were noted with *m/z* ratio from 156.11 to 216.1. Analysis of the completely degraded ANTX-a sample using Thamnotoxkit F™ toxicity test showed that it was no longer toxic. TiO_2 photocatalysis was also efficient in the decomposition of the living cyanobacterial cells. Degradation of their cell structures and degradation of released toxin was also achieved in 30 min. Earlier homogenization of cyanobacteria culture significantly accelerated degradation of ANTX-a to 10 min.

1. Introduction

Freshwater ecosystems have high ecological value and are key life-giving resources for aquatic fauna and flora. However, these ecosystems are fragile and susceptible to continuous eutrophication, which causes the proliferation of cyanobacterial harmful algal blooms (Cyano-HABs) (Steffen et al., 2014). Some cyanobacterial species are able to synthesize toxic secondary metabolites called cyanotoxins. Out of the many described groups of toxins, three of them are the most commonly determined in freshwater reservoirs. These include hepatotoxic microcystins (MC), cytotoxic cylindrospermopsin (CYN), and neurotoxic anatoxin-a (ANTX-a) (Merel et al., 2013). Current studies on cyanobacteria and their harmful secondary metabolites mainly concentrate on three aspects. The first one is reducing the occurrence of Cyano-HABs by identifying cyanobacteria in water and sediment core, reconstructing the present and historical composition of cyanobacterial communities, determining the impact of environmental changes on the formation of Cyano-HABs (Codd et al., 2015; Weckström et al., 2016; Wood et al., 2009). The second is reducing the occurrence of Cyano-

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HABs by searching the effective physical, chemical, technological or biological methods. The last one is removing extracellular toxins dissolved in water by natural or physicochemical methods (Boopathi and Ki, 2014; Rapala et al., 1993; Vlad et al., 2014; Westrick et al., 2010). Examples of natural degradation include e.g. photodegradation, biodegradation, and absorption of toxins by sediments which depends on physio-chemical factors such as water pH, humic content, microbial pre-exposure to cyanotoxins making it difficult to predict degradation (i.e. degradation rate of microcystins by selected microorganisms was determined in the range from 1.5 to 101,520.0 $\mu g \cdot L^{-1} \cdot day^{-1}$) (Dziga et al., 2013; Kaminski et al., 2015; Kormas and Lymperopoulou, 2013; Pflugmacher et al., 2001; Rapala et al., 1994). Conventional water treatment involves coagulation, flocculation or flotation, which are often inefficient for the degradation of dissolved toxins and can even increase their concentration by cell lysis and release of intracellular toxins (Merel et al., 2013; Vlad et al., 2014). The most commonly applied methods like chlorination and ozonation are also insufficient (30 min contact of 20 μg ANTX-a$\cdot L^{-1}$ and chlorine in concentration 15 $mg \cdot L^{-1}$ caused only 16% toxin removal) (Newcombe and Nicholson, 2004). To overcome these limitations, advanced oxidation processes (AOPs) are optimizing and introducing to reduce or eliminate cyanotoxins in treated water in a relatively short time. AOPs are characterized by their capability to produce highly reactive short-lived species such as HO• radicals (Andreozzi et al., 1999). One AOP of interest is titanium dioxide (TiO_2) photocatalysis (Lawton et al., 2003). TiO_2 as a photocatylst is stable, easy to obtain, inexpensive, industrially mass-produced and appears to be an efficient method for the cyanotoxins degradation.

In contrast to other well-known and studied cyanobacterial toxins such as microcystins or cylindrospermopsin, photocatalytical degradation of ANTX-a using TiO_2 was not well recognized. ANTX-a is one of the most common freshwater neurotoxic cyanotoxin. It is naturally occurring secondary metabolite produced by some strains of *Dolichospermum* (particularly *D. flos-aquae*, and *D. mendotae*) and a few other genera of freshwater cyanobacteria including i.a. *Aphanizomenon*, *Microcystis*, *Planktothrix* and *Oscillatoria* (Merel et al., 2013; van Apeldoorn et al., 2007). There are numerous reports on the toxic effects of ANTX-a on various species of animals and plants (i.e., bats, cattle, dogs, lesser flamingos, Mallard ducks, and mice; for review (Ha et al., 2014; Kaminski et al., 2016; Osswald et al., 2007)). *Dolichospermum* blooms are not only undesirable because of their toxicity, but are also responsible for a great losses to the tourism industry (Steffensen, 2008).

TiO_2 photocatalysis activated by UV-A irradiation offers a relatively cheap, and easy to use method for drinking water treatment, which can effectively degrade cyanotoxins and other emerging pollutants to non-toxic products. Since this advanced photocatalytic method was found to be very efficient in nodularin and microcystins degradation (complete removal within 5 min) and is beginning to be explored for use in water treatment plants, it seems reasonable to determine its effectiveness in the degradation of ANTX-a (Pestana et al., 2015).

The aim of this study was to evaluate and compare TiO_2 photocatalysis for destruction of purified ANTX-a, extract containing the toxin and the effect on living –ANTX-a containing cyanobacterial cells, *D. flos-aquae*. Additionally, it was also identified some of the decomposition products by using MS/MS analysis, and their detoxification was verified by using commercial *Thamnocephalus platyurus* test.

2. Material and methods

2.1. Preparation of research material

ANTX-a was obtained from an axenic culture of *Dolichospermum flos-aquae* (Lyngb.) de Bréb. strain SAG 30.87 purchased from the University of Göttingen (Germany). The cyanobacterium was cultivated in BG11 medium (Stanier et al., 1979) in a phytotron (Bolarus S-711S/P, Poland) at 20 ± 1 °C with 80% humidity and 25 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) under a 12 h photoperiod. The Erlenmeyer flasks containing cyanobacteria cultures were regularly shaken.

2.1.1. ANTX-a extraction and purification

The toxin was extracted and purified from the cyanobacterial cells in the following steps. 21 day old culture was centrifuged 10 min at 5000 ×*g*, cell pellet was sonicated in water (Omni sonic ruptor 400, USA) for 30 min on ice. The degree of cell lysis was determined under a microscope (sample volume 20 μL). ANTX-a was concentrated and initially purified by using solid phase extraction (SPE) in 6 mL Baker Bond glass columns (USA) filled with 2 g of C18 octadecyl phase, protected from the bottom and top sides with PTFE frits (20 μm porosity). The columns were placed in a vacuum manifold (Supelco, USA). The general scheme of the SPE procedure was as follows: the flow rate of all reagents was set as 1 mL$\cdot min^{-1}$, sorbent activation with 20 mL of methanol followed by 20 mL of MilliQ water (both acidified with 0.1% trifluoroacetic acid, TFA, Sigma-Aldrich), loading of 250 mL of cyanobacterial extract on each column, washing with 20 mL of distilled water, drying the columns, elution of the toxin with 20 mL of methanol +0.1% TFA, concentration of the collected ANTX-a by evaporating at room temperature under nitrogen stream. The flow rate of solvents and sample through the columns was set at 1 mL$\cdot min^{-1}$. The dry residues were dissolved in MilliQ water. The toxin was purified by using high-performance liquid chromatography (HPLC) system using the method described by Meriluoto and Codd (Meriluoto and Codd Editors, 2005). Final toxin concentration was determined as described by Kaminski et al. (Kaminski et al., 2014).

2.2. Experimental procedure

All experiments were performed in 20 mL transparent glass bottles, placed in a photoreactor with three independent replicates. The volume of the analyzed sample was 5 mL, the concentration of purified ANTX-a was 10 μg$\cdot mL^{-1}$, and the concentration of TiO_2 was 10 mg$\cdot mL^{-1}$ (*w*/*v*). The initial amount of living *Dolichospermum flos-aquae* cells was equal to 0.2 mg dry weight$\cdot mL^{-1}$ ± 7%. To obtain an extract from cyanobacteria, the culture (20 mL) was lysed by repeated (5×) alternating freezing and thawing.

2.2.1. UV-A irradiation source

Photoreactor was constructed as described in Skillen et al. (2016) with modifications. Briefly, photoreactor consisted of four fluorescent lamps which emitted irradiance in the UV-A (max emission peak at λ = 365 nm, irradiance 255.84 W$\cdot m^{-2}$), nine air flow control fans and a magnetic stirrer, with a centrally located glass bottle holder (3 mm height plastic ring). The speed (240 rpm) of the stirrer was constant. The temperature in photoreaction was constant and similar to room temperature (21 ± 1 °C).

2.2.2. Test conditions

2.2.2.1. Dark adsorption experiment. To MilliQ water (resistivity equal to 18.2 MΩ$\cdot$cm), ANTX-a (10 μg$\cdot mL^{-1}$) and TiO_2 (10 mg$\cdot mL^{-1}$ (*w*/*v*)) were added. The bottle was covered with aluminium foil, placed in a photoreactor, in which the magnetic stirrer and fans were turned on. Samples (100 μL) were collected at t_0 (turning on the UV-A lamps), and after 2, 4, 6, 8, 10, 15, 20, 25, 30, 60, 90 and 120 min. Three independent replicates were performed.

2.2.2.2. Effect of UV-A radiation on ANTX-a stability. An aqueous solution of ANTX-a was placed in the photoreactor, and the magnetic stirrer and fans were turned on. Samples (100 μL) were collected 5, 15 and 30 min before UV-A lamp was switched on, at t_0, and after 2, 4, 6, 8, 10, 15, 20, 25, 30 and 60 min of irradiation.

2.2.2.3. TiO_2 photocatalytic experiments. In the first part of experiments to an aqueous solution of purified ANTX-a, TiO_2 was added (10 mg·mL^{-1} (*w*/*v*)). The sample was placed in the photoreactor, and the magnetic stirrer and fans were turned on. In the second part the two-week-old culture of *D. flos-aquae* cultivated in BG11 medium in 250 mL Erlenmeyer flask was gently shaken for 8 h. Culture (5 mL) was transferred to glass bottles, and TiO_2 was added (10 mg·mL^{-1} (w/v)). The mixture was placed in the photoreactor and the fans and stirrer were turned on. Samples (100 μL) were collected 5, 15, and 30 min before UV-A source was turned on, at t_0 (turning on the UV-A lamps), and after 2, 4, 6, 8, 10, 15, 20, 25, 30 and 60 min of irradiation. Control was performed in the same conditions without the addition of TiO_2.

2.2.2.4. Sample preparation. Samples were centrifuged for 10 min at 13,000 ×*g* to separate cyanobacterial cells from the medium, after which supernatants were transferred to fresh microcentifuge tubes (1.5 mL). Both the supernatant (95 μL) and cells was evaporated in the Genevac EZ-2 centrifugal evaporator (Great Britain). To each sample 100 μL of 10% aqueous methanol was added. All samples were vortexed by 15 min at 2300 rpm and after that, centrifuged for 10 min at 13,000*g*. The supernatant (50 μL) from each sample was transferred to HPLC vials. ANTX-a in the cells was expressed as μg·mL^{-1}.

2.2.2.5. Determination of the dry weight of cyanobacterial cells. One mL of culture was transferred to six independent previously weighted 1.5 mL vials. Samples were centrifuged by 10 min at 13,000 rpm, supernatants were removed and water from the cyanobacterial cells was evaporated in Genevac EZ-2 centrifugal evaporator. After that, vials were weighed again. The difference in weight between the mass of vials with cyanobacteria material and the same empty vials was calculated to give the dry weight of cyanobacteria.

2.2.3. Influence of TiO_2 on Dolichospermum flos-aquae cells homogenate under UV-A radiation

D. flos-aquae culture (20 mL) was lysed by repeated (5×) alternating freezing (−20 °C) and thawing (room temperature 21 °C). After that, the sample was transferred to an ultrasonic bath for 10 min. Then, 5 mL of homogenate was poured into glass bottles, and TiO_2 was added. Sampling and experiment were performed as before (Subsection 2.2.4).

2.2.4. Analysis of samples by UPLC-qTOF

In order to determine the changes in ANTX-a concentration over time and confirm the formation of potential decomposition products, all of the samples were analyzed on a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) connected with Waters Xevo quadrupole Time-of Flight (qTOF). The gradient mobile phase consisted of water/acetonitrile (both acidified with 0.1% formic acid) where the organic phase increased from 2% to 35% over 5 min at a flow rate of 0.35 mL·min^{-1}. Samples were separated on an ACQUITY UPLC BEH C18 Column (130 Å, 1.7 μm, 2.1 mm × 100 mm) maintained at 40 °C. ANTX-a was identified by comparing the retention time and UV-spectra determined for an ANTX-a standard and quantified by absorbance at 227 nm. A multilevel calibration curve was obtained using commercial ANTX-a (from 0.01 to 100.00 μg·mL^{-1}). The post-reaction samples were analyzed by MS using positive ion electrospray (ESI-MS) as follows: a source temperature of 80 °C, a desolvation temperature of 350 °C, a desolvation gas flow rate of 500 L h^{-1}, a capillary

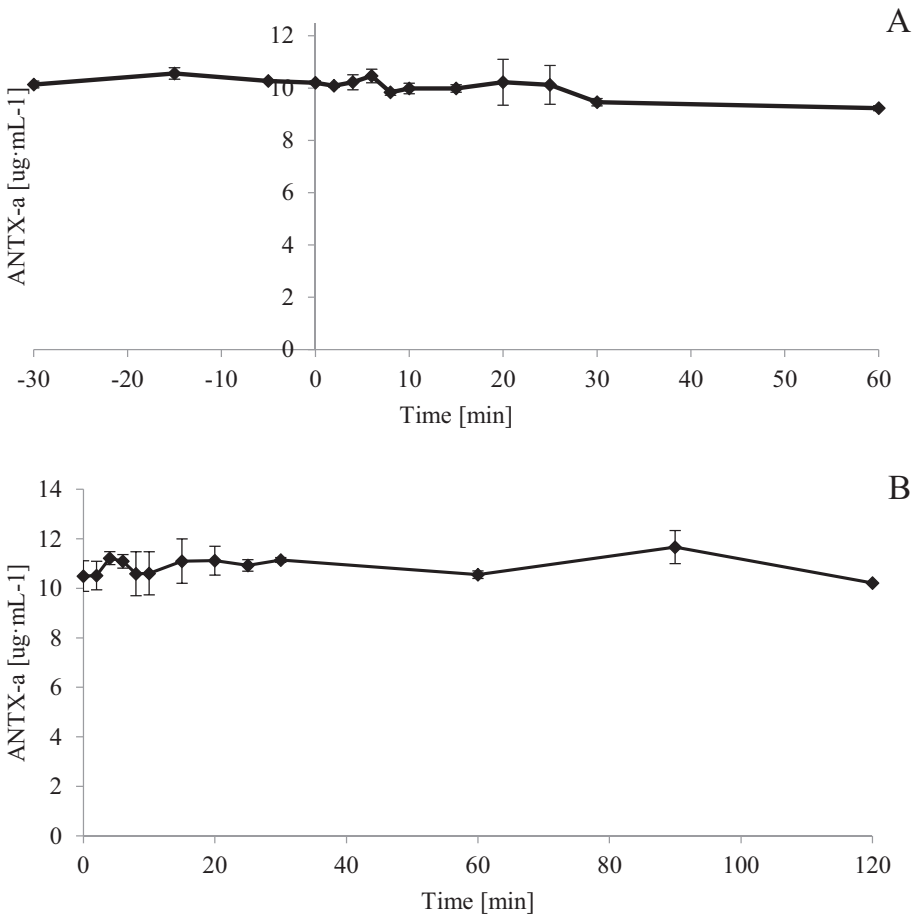

Fig. 1. Changes of ANTX-a concentration: within 60 min of UV-A irradiation (A), or under TiO2 impact in constant darkness (120 min, B), $n = 3 \pm$ s.d.

potential of 3.3 kV, and a cone potential of 25 V. The positive-ion mode was applied and the scan range was 50–400 *m/z* in the MS and MS/MS modes. Data acquisition and processing was achieved using MassLynx 4.1.

2.2.5. Toxicity test

Evaluation and comparison of the toxicity of ANTX-a or its decomposition products were performed using a commercially available Thamnotoxkit F™ toxicity test according to the respective standard operational procedures. The Thamnotoxkit F™ assay is based on the determination of the percentage mortality of *Thamnocephalus platyurus* larvae (10 larvae in each test well, 3 replicates) after 24 h of exposure to the analyzed solutions. The assay was carried out for the sample of ANTX-a completely degraded by TiO_2 photocatalysis after 60 min of UV-A irradiation (starting concentration of 10.0 μg ANTX-a·mL^{-1}), and the control, ANTX-a solution (10.0 μg·mL^{-1}). In order to remove TiO_2, both samples were subjected to the SPE procedure described above. The toxicity test was performed in four independent replicates.

2.2.6. Chemicals

ANTX-a standard was purchased from National Research Council Canada (Canada), TiO_2 P25 nanopowder from Merck (Germany). All other solvents and reagents were high purity LC-MS quality.

3. Results

The data obtained from the analyses of samples subjected to the impact of UV-A irradiation confirmed the high stability of ANTX-a molecule, while the dark adsorption experiment shows that toxin is not attached to TiO_2 molecules (Fig. 1A, B) within 60 or 120 min, respectively. UV-A irradiation of an aqueous solution of ANTX-a with the addition of TiO_2 resulted in accelerated degradation of neurotoxin (Fig. 2). The toxin was completely degraded after 30 min. Additionally, the data obtained in the dark phase of the experiment indicates that toxin is not adsorbed to TiO_2 particles as is often the case with aquatic pollutants.

During the irradiation of ANTX-a several degradation products were determined, (*m/z* ratio presented in Table 1). Most of the detected products, were created in 2 min of exposure to UV-A radiation. As the ANTX-a decreased in concentration, the decomposition products appeared to increase reaching a maximum around 15 to 20 min, after that their concentrations also decreased (Fig. 3) as a result of photocatalysis.

Some of the observed decomposition products have been previously characterized (epoxyANTX-a and hemiacetalANTX-a), while some of them seem to be newly described here. On the basis of MS/MS spectra, and other publications (James et al., 1998; Tak et al., 2018) in which ANTX-a degradation pathways were presented, a photocatalytic toxin decomposition pathway has been determined (MS/MS spectra were attached to supplementary materials) (Fig. 4). The MS/MS spectra of the products with m/z equal to 156.11, 200.14 and 216.13 show some similarity to hemiacetalANTX-a ($[M + H]^+ = 198.12$).

In the second stage of the experiment, where the impact of UV-A/TiO_2 reaction on *D. flos-aquae* cell culture was examined, it was microscopically determined that some cells, before turning on UV-A lamps ($T = -5$ min), were damaged by mixing of culture (cyanobacteria trichomes cracking was observed), but total amount of toxin in samples was relatively stable (Fig. 5). After switching on UV-A lamps (t_0) it was determined that the accelerated degradation of toxin in the solution and constant damage of cyanobacterial cells, was manifested by the extracellular release of ANTX-a. After 30 min of irradiation, the presence of neurotoxin was not determined in the samples. In the control samples without addition of TiO_2, UV-A irradiation did not affect the toxin concentration significantly.

A lysed extract of *D. flos-aquae* cells resulted in cell disruption and release of the entire toxin pool into the medium. In the control samples

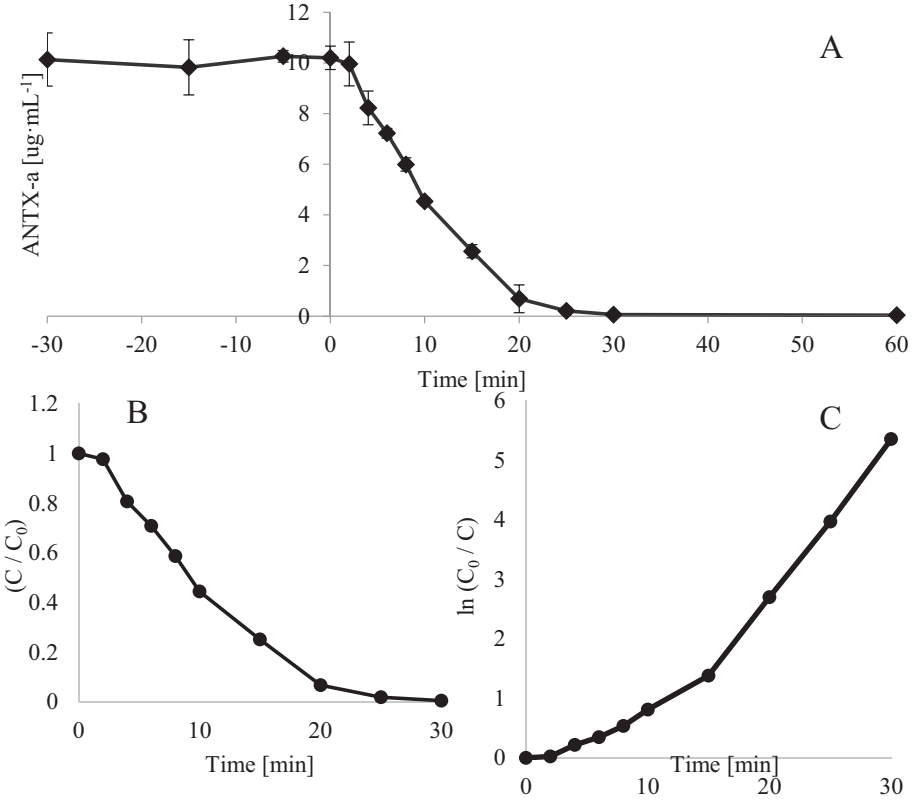

Fig. 2. Kinetic of ANTX-a concentration changes under the simultaneous impact of TiO2 and UV-A irradiation, n = 3 ± s.d.. Bars indicate standard deviation (A), the photocatalytic disappearance of 10 μg·mL^{-1} ANTX-a (B), transformation of ln(C0/C) versus time (C).

Table 1
Decomposition products of ANTX-a in the presence of TiO_2. "+" indicates the presence of product, RT is retention time shown.

				Hemiacetal-ANTX-a			ANTX-a	epoxy-ANTX-a	
	m/z	156.11	216.13	198.12	180.11	200.14	166.13	182.13	140.08
Time (min)		RT = 0.83	RT = 1.11	RT = 1.27	RT = 2.14	RT = 2.32	RT = 2.38	RT = 2.45	RT = 2.56
UV-A irradiation	60								
	30	+	+	+	+	+		+	+
	25	+	+	+	+	+	+	+	+
	20	+	+	+	+	+	+	+	+
	15	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+
	4	+	+	+	+		+	+	
	2	+	+	+	+		+	+	
UV lamp on darkness	0						+		
	-5						+		
	−15						+		
	−30						+		

(darkness, UV-A irradiation without the addition of TiO_2) ANTX-a was very stable, and changes in its concentration were not greater than 2% of the initial value. With the addition of TiO_2 to the samples subsequently exposed to UV-A irradiation resulted in much faster toxin degradation in comparison to the previously described experiment. After 8 min only 2% of its initial concentration was remaining, while after 10 min it was completely absent (Fig. 6). Between 2nd and 20th min of the treatment of the sample three chemical compounds were determined with *m/z* equal to 136.07, 160.08, and 158.96, but because of the very large number of substances in the homogenate, we cannot assume that they are derivatives of toxin degradation.

The rate constants (min^{-1}) of photocatalytic degradation of ANTX-a, confidence coefficients and half-times (min) are listed in Table 2. Separate results were calculated for purified toxin with initial concentration equal to 10.0 and to compare to other results with concentration equal to 1.05 $\mu g \cdot mL^{-1}$. The confidence coefficient is the highest for purified toxin, and cyanobacterial cells homogenate. In case of ANTX-a in a medium with live cyanobacteria, its concentration and removal depended on the rate of toxin degradation by TiO_2 photocatalysis and its replenishment by cell lysis. The rate constant increased from 0.12 min^{-1} for living cells to 0.48 min^{-1} for *D. flos-aquae* homogenate. The shortest half-time (2.85 min) was observed for previously degraded cells, and the longest, 6.28 min was for living cells, and for the 10 times higher initial purified toxin concentration (10.72 min).

T. platyurus test confirmed 100% toxicity of ANTX-a solution (10 $\mu g \cdot mL^{-1}$) while the similar toxin sample irradiated 60 min by UV-A with the addition of TiO_2, had no effect on tested organism within 24 h

4. Discussion

Due to the documented harmful impact of cyanotoxins on health and lives of various organisms (microorganisms, plants, and animals including humans), researchers have focused on developing effective and cheap methods and/or technologies for their removal or ideally destruction of the cells and/or toxins. It is thought that some of the most effective methods are based on AOPs. Usually, for this purpose, O_3, H_2O_2, O_3/H_2O_2, O_3/Fe(II) and Fenton treatment have been investigated at laboratory and industrial scale. Nowadays, processes based on photocatalysis are starting to play a key role in the detoxification of hazardous organic substances and proved to be very effective and efficient method for detoxification of water (Tahir et al., 2019).From the 1970s, TiO_2 photocatalysis has been studied as a practical technology for removing various pollutants from water, including cyanotoxins. TiO_2 exists in three different crystalline forms: anatase, rutile, and brookite. Some researchers reported that these three forms of TiO_2 vary in photoactivity, and TiO_2-P25 with the structure of anatase and rutile (80:20), used in our experiments, showed higher photoactivity than other materials containing, for example, 100% anatase (Behnajady et al., 2008; Hoffmann et al., 1995).

Experiments performed under controlled laboratory conditions in constant darkness with the addition of TiO_2 (Fig. 1) showed, not only the previously described high stability of ANTX-a, but also lack of toxin adsorption to photocatalyst which has often been thought to be an important aspect of TiO_2 photocatalysis. The lack of so called dark adsorption might be responsible for the relatively slow ANTX-

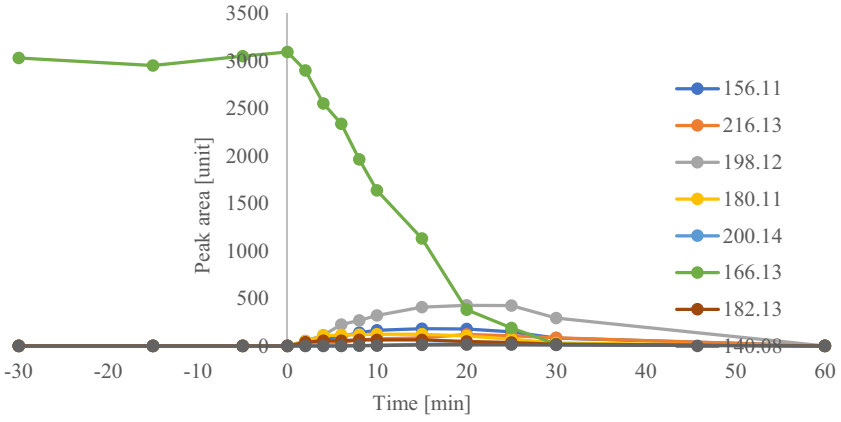

Fig. 3. Peak area changes of ANTX-a and decomposition products during UV-a/TiO2 reaction. Numbers in legend correspond to masses $[M + H]^+$ of analyzed chemicals, $n = 3$.

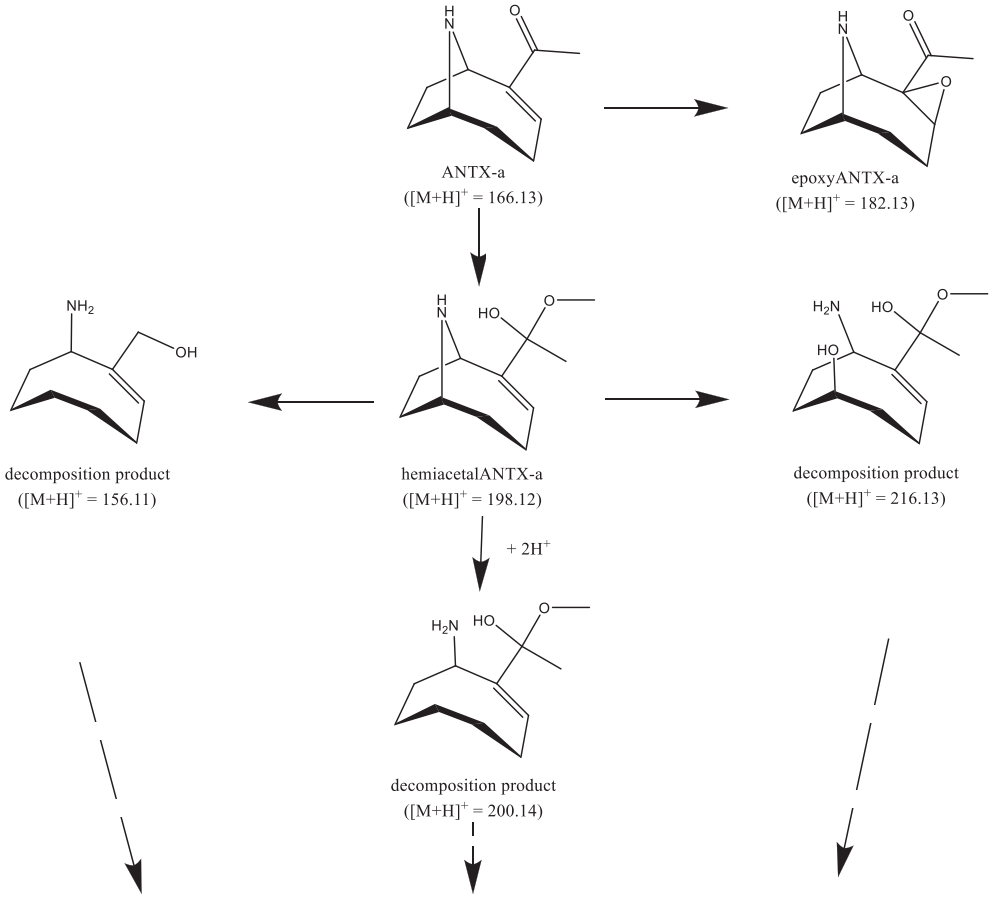

Fig. 4. Proposed degradation pathways of ANTX-a, under UV-A/TiO_2 reaction.

a degradation. Total ANTX-a degradation was observed after 30 min of treatment (Fig. 2), while MC-LR removal was achieved in 5 min (similar concentrations, and experimental procedure) In our experiments, we used a much higher concentration of ANTX-a than typically described in the natural environment (<1.75 $\mu g \cdot mL^{-1}$ (Hedman et al., 2008)). This was dictated not only by the desire to check the degradation efficiency of the toxin but also to identify the products of its decomposition.

Compared to other common freshwater cyanotoxins, MC-LR or CYN, which are larger and complex molecules than ANTX-a, with molecular weights equal to 995.19 g/mol, 415.42 g/mol, and 165.24 g/mol, respectively, photodegradation of analyzed neurotoxin resulted in the formation of a significantly smaller number of decomposition products. In comparison to toxin degradation under UV-C/H_2O_2 reaction (Tak et al., 2018), where the creation of decomposition products with lower masses than ANTX-a was observed, under UV-A/TiO_2 treatment almost all of characterized products had increased molecular weight compared to ANTX-a (Table 1). Formation of these products is related to the toxin molecule structure. In the ANTX-a molecule, there are two double bonds. One between C2 and C3 in the ring structure and one between carbon-oxygen atoms in the acetyl group. The radical addition reaction to the double bond is well-recognized in organic chemistry (Lalevée et al., 2005). Analysis of MS/MS spectra and other publications describing toxin degradation (supplementary materials available online, (James et al., 2005; Kaminski et al., 2013; Tak et al., 2018)), showed that three decomposition products with *m/z* equal to 156.11, 200.14 and 216.13 were generated by modification of hemiacetal ANTX-a ($[M + H]^+ = 198.12$).

Previously published papers have also demonstrated the ability of TiO_2 to eliminate microbes and remove odour compounds as well as improving the taste of the drinking water (Holmström et al., 1977; Sharma et al., 2018). In the second stage of planned experiments, we examined the possibility of destroying cyanobacteria and degrading toxin simultaneously by using TiO_2. This is a very important advantage that this treatment provides because some of the currently used drinking water treatment technologies cause lysis of cyanobacterial cells and release of intracellular cyanobacterial toxins, which are not degraded or removed during coagulation, flocculation, filtration, and chlorination disinfection processes. The initial total amount of toxin in our culture (in medium and intracellular) was 1.06 $\mu g \cdot mL^{-1}$. The larger portion (58%) of the toxin was dissolved in the medium. During the first 4 min of culture irradiation in the presence of TiO_2, a 10-fold decrease in the extracellular toxin was observed (Fig. 5). The extracellular ANTX-a pool was supplemented with its intracellular toxin being released as the cyanobacterial cells degraded. It was determined that cyanobacterial cell walls and membrane were degraded throughout the first 25 min. After 30 and 60 min of irradiation, no toxin was detected (Fig. 5).

Assessment of photocatalysis of a cell lysate indicated very rapid decomposition of the toxin. It was completely degraded after 10 min (Fig. 6). Probably several factors influenced this, such as earlier degradation of cell walls and membranes, as well as creation of increased ROS enhanced by phycocyanin soluble in medium (Robertson et al., 1999). In comparison to the samples with living cells, in case of homogenate the medium had a blue coloration.

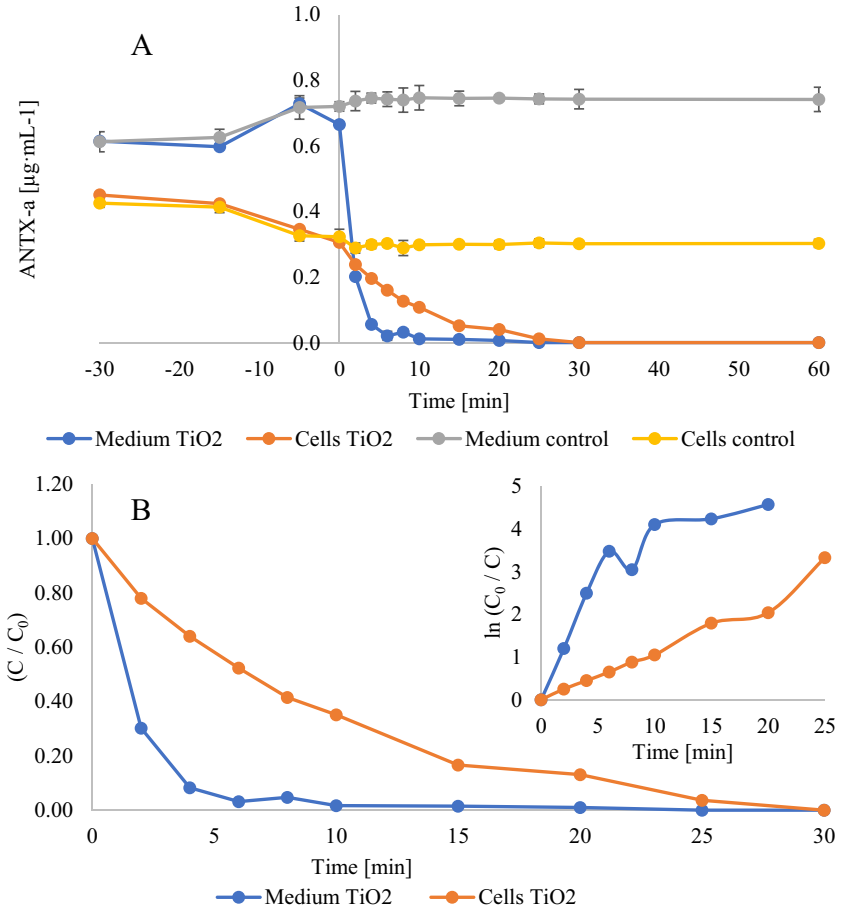

Fig. 5. Changes of ANTX-a concentration in media and cyanobacterial cells under TiO_2 photocatalysis, and in the control sample without TiO_2, n = 3 (A), the photocatalytic disappearance of ANTX-a in cyanobacterial cells and medium (B). Inset: transformation of ln(C0/C) versus time.

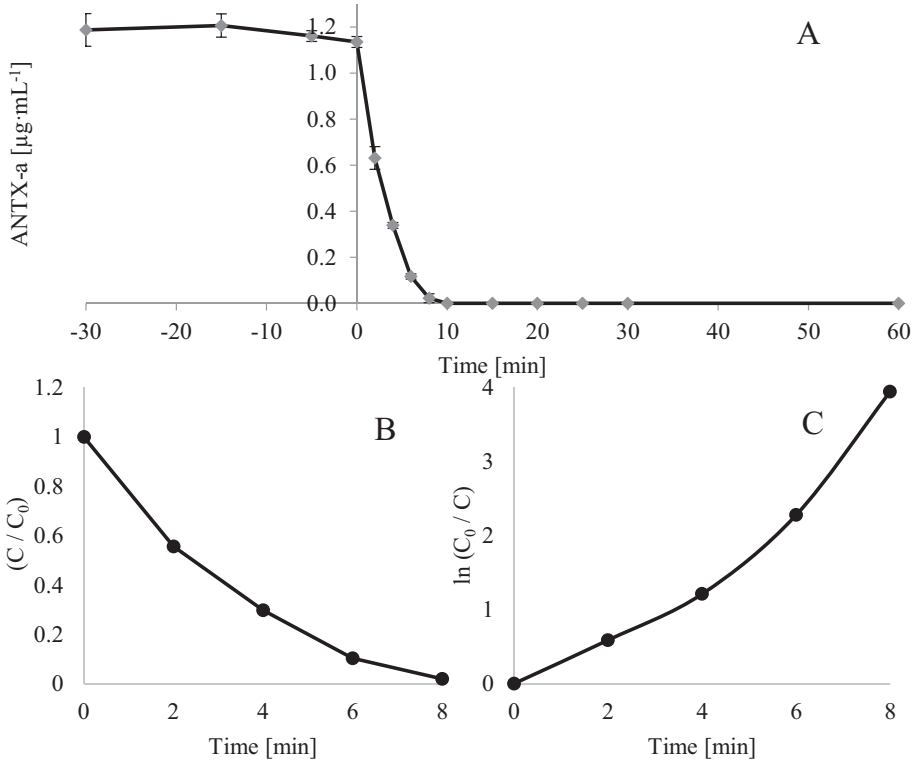

Fig. 6. Kinetic of ANTX-a degradation in *D. flos-aquae* homogenate under the impact of TiO2 and UV-A irradiation, n = 3 ± s.d. (A), the photocatalytic disappearance of ANTX-a in cyanobacterial cells and medium (B), transformation of ln(C0/C) versus time (C).

Table 2
Photocatalytic degradation rate constants (from 2nd min to total toxin degradation and half-lives of previously purified ANTX-a from cyanobacterial cells or in living cells/medium or in a homogenate in the presence of TiO_2/UV.

Toxin source	Initial concentration [$\mu g \cdot mL^{-1}$]	Confidence coefficient	Rate constant [min^{-1}]	Half-time [min]
Purified toxin	10.0	0.989	0.11 ± 0.01	10.72
Living cells	0.34	0.971	0.11 ± 0.01	6.28
Medium from living cells	0.72	0.764	0.16 ± 0.04	3.09
D. flos-aquae homogenate	1.1	0.947	0.55 ± 0.08	2.85

AOPs based on the TiO_2 photocatalysis are testing and applying for removing for water various organic contaminations. Previous studies confirmed its effectiveness and application potential for removing natural cyanotoxins such as microcystins, nodularin or cylindrospermopsin (Chen et al., 2015; Liu et al., 2005; Pestana et al., 2015). Pseudo-first-order rate constants (k) in case of CYN solution depended mainly on the initial toxin concentration $[CYN]_0$ and varying from 0.37 min^{-1} for 16 ppm to 1.18 min^{-1} for 2 ppm (Chen et al., 2015). In our study we used much higher toxin concentrations and obtained for purified toxin of *D. flos-aquae* homogenate similar reactions rate constant. Cyanotoxins are natural toxic compounds, but human use has led to increasing ubiquitous presence of pharmaceuticals and their metabolites in aquatic environments and even in drinking water. The studies on TiO_2/UV degradation of sulfa pharmaceuticals (sulfapyridine, sulfisoxazole) or β-blockers such as atenolol, metoprolol or propranolol confirmed the possibility of using this method also for these pollutants (Yang et al., 2010b, 2010a). In case of sulfa pharmaceuticals 60 min irradiation removed up to 92.2% of initial concentration of chemical compounds.

TiO_2 photocatalysis is also being tested to removing pollutants on soil surface. It was tested under laboratory studies on degradation of polycyclic aromatic hydrocarbons (PAHs) on soil surface (Zhang et al., 2008), were photocatalytic degradation rates in this case depends mainly on analyzed wavelength, with dependence greater energy - faster decomposition, environmental pH, and analyzed PAHs.

From the economical and practical points of view, it is important to verify the potential toxicity of created decomposition products. In our study, we used a simple, commonly available and cheap toxicity test based on the mortality of *T. platyurus* larvae which is used to determine the toxicity threshold of many cyanotoxins (Sierosławska et al., 2014). Decomposition products obtained by 60 min irradiation of purified ANTX-a with addition of TiO_2 did not show any effect on *T. platyurus* larvae within 24 h. The lack of toxicity of degradation products is very important information because is some cases the generated intermediates products are more toxic than initial compounds. Antiviral pharmaceutical acyclovir can be fully degraded within 90 min using g-C_3N_4/TiO_2 photocatalysis generating three persistent intermediates. One of them, guanine, was more toxic to daphnia than acyclovir itself (Li et al., 2016).

In all experiments, we confirmed the complete anatoxin-a degradation, the creation of decomposition products and the lack of their toxicity in *T. platyurus* test. However, to improve the effectiveness of this process by obtaining higher decomposition ration, and shorter reactions times, this technique in a water treatment plant or environmental usage should be adjusted. Higher energy input, photocatalyst doses, or addition of other compounds should positively affect on degradation process (Aliabadi et al., 2020; Antoniou et al., 2018; Robertson et al., 1999). New technological processes using TiO_2 as among others: nanoparticles in nanotubes or water resistant cellulose – TiO_2 composite, are currently under development (Chang et al., 2019; Ciambelli et al., 2019; Garusinghe et al., 2018). For each application the optimal operating parameters, such as the source and range of radiation or temperature, differ from each other and are selected individually. Therefore, in any case, the degradation efficiency time for various organic water pollutants is difficult to predict and should be measured experimentally.

5. Conclusion

These studies confirm the hypothesis that TiO_2 photocatalysis efficiently degrades ANTX-a, no matter whether the toxin was previously purified from cyanobacterial cells or is in living cells or in a homogenate. Additionally, UV-A/TiO_2 is able to simultaneously damage cyanobacterial cells and degrade the released toxin. Decomposition products were not toxic to *T. platyurus*. The fastest toxin decomposition was observed in the cell lysate, half-time decomposition of 1.1 $\mu g \cdot mL^{-1}$ took 2.85 min, and the total degradation 10 min.

CRediT authorship contribution statement

Ariel Kaminski: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Christine Edwards:** Methodology, Investigation, Resources, Writing - review & editing. **Ewelina Chrapusta-Srebrny:** Investigation, Writing - review & editing. **Linda A. Lawton:** Conceptualization, Methodology, Resources, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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