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Degradation of widespread cyanotoxins with high impact in drinking water (microcystins, cylindrospermopsin, anatoxin-*a* and saxitoxin) by CWPO

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Abstract

The occurrence of harmful cyanobacterial blooms has unabated increased over the last few decades, posing a significant risk for public health. In this work, we investigate the feasibility of catalytic wet peroxide oxidation (CWPO) promoted by modified natural magnetite ($\text{Fe}_3\text{O}_4\text{-R400}/\text{H}_2\text{O}_2$), as an inexpensive, simple-operation and environmentally-friendly process for the removal of the cyanotoxins that show the major impact on drinking water: microcystins (MC-LR and MC-RR), cylindrospermopsin (CYN), anatoxin-*a* (ATX) and saxitoxin (STX). The performance of the system was evaluated under ambient conditions and circumneutral pH ($\text{pH}_0 = 5$) using relevant cyanotoxin concentrations ($100 - 500 \mu\text{g L}^{-1}$). The nature of the cyanotoxins determined their reactivity towards CWPO, which decreased in the following order: MC-RR>CYN>MC-LR>>ATX>STX. In this sense, microcystins and CYN were completely removed in short reaction times (1 – 1.5 h) with a low catalyst concentration (0.2 g L^{-1}) and the stoichiometric amount of H_2O_2 ($2 - 2.6 \text{ mg L}^{-1}$), while only 60 – 80% conversion was achieved with ATX and STX in 5 h. In these cases, an intensification of the operating conditions (1 g L^{-1} catalyst and up to $30 \text{ mg H}_2\text{O}_2 \text{ L}^{-1}$) was required to remove both toxins in 1 h. The impact of the main components of freshwaters *i.e.* natural organic matter (NOM) and several inorganic ions (HCO_3^- , HPO_4^{2-} , SO_4^{2-}) on the performance of the process was also investigated. Although the former led to a partial inhibition of the reaction due to $\text{HO}\cdot$ scavenging and catalyst coating, the latter did not show any remarkable effect, and the versatility of the process was finally confirmed in a real surface water. To further demonstrate the effectiveness of the catalytic system, the toxicity of both the initial cyanotoxins and the resulting CWPO effluents was measured with the brine shrimp *Artemia salina*. Remarkably, all CWPO effluents were non-toxic at the end of the treatment.

Nomenclature

ATX – Anatoxin-*a*
CYN – Cylindrospermopsin
CWPO – Catalytic Wet Peroxide Oxidation
DWTP – Drinking Water Treatment Plant
GAC – Granular Activated Carbon
MC-LR – Microcystin LR
MC-RR – Microcystin RR
NOM – Natural Organic Matter
PAC – Powdered Activated Carbon
STX – Saxitoxin

1. Introduction

Cyanobacteria are among the largest group of photosynthetic prokaryotes in Earth, being the main component of photoautotrophic microflora regarding total biomass and productivity (Whitton and Potts, 2012). Cyanobacteria are naturally ubiquitous in surface water, where they play relevant ecological roles such as in carbon and nitrogen cycles (Whitton and Potts, 2012). Nevertheless, under certain environmental conditions (e.g. high temperatures, calm wind, and elevated nutrient concentrations) cyanobacteria may proliferate forming dense blooms especially during summer in lentic systems (Carmichael, 1994). The risk for these episodes has increased dramatically worldwide over the past few decades due to rising nutrient levels caused by the increasing use of fertilizers and detergents (Pantelić et al., 2013), becoming an important environmental threat.

Cyanobacterial blooms lead to important changes in water, affecting its color, odor and taste. Apart from these concerns, and more importantly, such blooms can produce highly toxic compounds, called cyanotoxins (Carmichael, 1994). This collateral effect represents an important risk for human health and other ecosystem services, as it is expected that 10-95% of the recorded blooms contain cyanotoxins (Antoniou et al. 2005; Sivonen and Jones, 1999). Human exposure to these toxins may occur through ingestion of drinking-water and during recreational use of water bodies with cyanobacterial blooms (He et al., 2016; Stewart et al., 2006; Pantelić et al., 2013; Kumar et al., 2018). A number of episodes of human and animal poisoning by ingestion of cyanotoxins-bearing water have been reported since the late 1800s (Antoniou et al., 2005; Carmichael, 1994). So far, up to 40 cyanobacterial genera have been characterized as potential cyanotoxins producers (Bernard et al., 2016), with *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nodularia* and *Cylindrospermopsis* being the main genera leading to harmful algal blooms in surface water (Pantelić et al., 2013). These toxic compounds can be grouped according to their health effects in humans, viz. skin irritation (dermatotoxins), cell damage (cytotoxins), liver damage (hepatotoxins) and nervous system affection (neurotoxins) (Antoniou et al., 2005). For instance, microcystins, the best-researched cyanotoxin group, are known liver toxins; anatoxin-*a* and

saxitoxins are potent neurotoxins while cylindrospermopsins promote cytotoxicity and also hepatotoxicity and genotoxicity (Westrick et al., 2010).

Among cyanotoxins, cylindrospermopsin (CYN) has emerged as one of the most important toxins in freshwater worldwide (de la Cruz et al., 2013). It is particularly persistent, being stable in a wide range of light, heat and pH conditions (de la Cruz et al., 2013; Wormer et al., 2008). Furthermore, CYN can be produced by a large number of cyanobacteria, and compared to microcystins, it can be released into the environment at significantly higher extracellular concentrations (de la Cruz et al., 2013; Wormer et al., 2008). Therefore, it is one of the most broadly distributed cyanotoxin in water bodies, having been detected in tropical, subtropical and even in temperate areas (He et al., 2014). Although CYN is not currently regulated, a guideline safety value of $1 \mu\text{g L}^{-1}$ has been proposed for drinking water and it has been also included in the US EPA Contaminant Candidate List 3 (He et al., 2014; Westrick et al., 2010).

Conventional treatment processes such as chlorination and adsorption, as well as advanced systems like membrane technology, ozonation or photocatalysis have been investigated for cyanotoxins removal (Kumar et al. 2018; Rodríguez et al., 2007). In fact, most of these treatments are currently applied in drinking water treatment plants (DWTPs) but their efficiency depends greatly on the kind of cyanotoxin, the source water characteristics as well as the operating parameters (temperature, pH, cyanotoxin level), which can frequently change in a DWTP (Kumar et al., 2018). Chlorination has shown to be effective for the removal of certain cyanotoxins such as microcystin LR (MC-LR) and CYN, but relatively high chlorine doses are required ($>2 - 3 \text{ mg L}^{-1}$) (Kumar et al., 2018; Westrick et al., 2010; Senogles et al., 2000) and the generation of trihalomethanes as well as chlorinated by-products from cyanotoxins (e.g. chlorouracil and 5-chloro-cylindrospermopsin from CYN) can occur (Kumar et al., 2018; Lawton and Robertson, 1999; Banker et al., 2001). Furthermore, other relevant cyanotoxins such as anatoxin-a (ATX) and saxitoxin (STX) are resistant to chlorination (Kumar et al., 2018; Pantelić et al., 2013; Rodríguez et al., 2007). On the other hand, adsorption onto activated carbon (AC), which is another conventional technique widely used in DWTPs, allows to remove certain cyanotoxins (MCs, ATX and CYN) but high carbon dosages ($10 \mu\text{g L}^{-1}$ toxin: $20\text{-}200 \text{ mgAC L}^{-1}$) and long contact times (even above

12 h) can be required depending on the kind of AC and the operating conditions (Kumar et al., 2018; Pantelić et al., 2013; Shi et al., 2012; Kumar et al., 2018; Ho et al., 2009; Drogui et al., 2012). With regard to membrane technologies, nanofiltration and reverse osmosis appear as the most effective for cyanotoxins removal while ultrafiltration is not a reliable treatment barrier (Neumann and Weckesser, 1998; Coral et al., 2011; Westrick et al., 2010).

Advanced oxidation processes (AOPs) appear as promising alternatives for the degradation of cyanotoxins as they can destroy organic pollutants by the action of *in-situ* generated hydroxyl radicals. So far, most AOP studies have been focused on the removal of microcystins, in particular MC-LR, as they occur most frequently in fresh water; CYN and ATX have been by far less investigated and very little work can be found for STX (Kumar et al., 2018). Ozonation is widely used in DWTPs, but it is usually based on the direct action of ozone, which is much less reactive than hydroxyl radicals (Kumar et al., 2018). For instance, high degradation of MC-LR was achieved using H₂O₂/O₃ (>90% in 1 min) compared to only O₃ (60% in 30 min) under the same conditions (Kumar et al., 2018; Lu et al., 2018). In general, the application of H₂O₂/O₃ has proved to be effective for cyanotoxins removal. Nevertheless, the generation of toxic by-products is an important drawback of this treatment (Kumar et al., 2018; Lu et al., 2018). Photocatalysis has also proved to degrade cyanotoxins but the use of UV light, which is significantly more effective than solar light (e.g. CYN removal in 15 and 40 min, respectively, using Degussa P25 photocatalyst) (Fotiou et al., 2015), makes the process cost-intensive. Recently, the combination of adsorption and photocatalytic degradation by hybrid composites (CNT/TiO₂) has proved to enhance the performance of the process (Chae et al., 2019). Nevertheless, this system requires skilled supervision, strict pH control and it is also highly affected by NOM, that can absorb UV light (Kumar et al., 2018). In this context, the application of Fenton oxidation, by far less investigated than ozonation and photocatalysis for the removal of cyanotoxins, represents an interesting alternative (Bandala et al., 2004; Park et al., 2017; Gajdek et al., 2001; Zhong et al., 2009; Kim et al., 2017). This process is usually identified as the most cost-efficient AOP (Gadipelly et al., 2014) and has proved to warrant a faster cyanotoxin degradation (e.g. MC-LR and ATX) compared to ozonation and photocatalysis (Al

Momani, 2007; Al Momani et al., 2008). The use of solid catalysts in the so-called catalytic wet peroxide oxidation (CWPO) or heterogeneous Fenton allows to overcome the main limitations of the Fenton process *viz.* loss of catalyst and iron sludge generation, but scarce works can be found in the literature. Recently, Wang et al. (2016) investigated the application of zero-valent iron nanoparticles (nZVI) for MC-LR (5 mg L^{-1}) removal under ambient conditions, obtaining 59% conversion but a long reaction time (4 h) and H_2O_2 doses far above the stoichiometric were required. Nevertheless, most of the works using magnetic solids for the removal of cyanotoxins have been focused on their separation from water instead of taking advantage of the catalytic properties of iron oxides (Chen et al., 2009; Lee et al., 2011; Hena et al., 2016).

According to a recent review (Kumar et al., 2018), high energy consumption and operating costs, poorly understood kinetics and the production of harmful by-products are the main challenges that limit the widespread application of AOPs for the treatment of cyanotoxins. The aim of this work is to evaluate the feasibility of CWPO promoted by modified magnetite, as a low-cost, simple-operation and environmentally-friendly AOP, for the degradation of the most widespread classes of cyanotoxins (microcystins (MC-LR and MC-RR), CYN, ATX and STX) in raw freshwaters according to large monitoring studies (Loftin et al., 2016; Mantzouki et al., 2018) and that show a major impact on drinking water (He et al., 2016). The kinetics of the process has been investigated using relatively low doses of catalyst and H_2O_2 (mainly the stoichiometric amount for complete oxidation of toxins). The impact of the main components of freshwaters *i.e.* NOM and different inorganic ions (HCO_3^- , HPO_4^{2-} , SO_4^{2-}) on the performance of the process has been investigated. As a proof of concept, the catalytic system has been also studied using a real surface water as reaction matrix. Finally, the toxicity of the target pollutants and the resulting CWPO effluents has been evaluated with the brine shrimp *Artemia salina*.

2. Materials and methods

2.1. Chemicals

MC-LR ($\geq 99\%$), MC-RR ($\geq 97\%$), CYN ($\geq 99\%$), ATX ($\geq 99\%$) and STX ($\geq 99\%$) were provided by Laboratorio CIFGA S.A. (Spain). Their molecular weights and structures are collected in Table 1. Hydrogen peroxide solution (30% w/w), nitric acid (65%), acetic acid ($> 99\%$), methanol (HPLC grade), humic acid and formic acid ($> 98\%$) were obtained from Sigma-Aldrich. Acetonitrile (HPLC grade) was purchased from Fluka. With the exception of MC-LR and MC-RR, the chemicals were directly used, without any further purification. As MCs were provided in methanol, these samples were evaporated and reconstituted in deionized water prior use. The magnetite mineral was supplied by Marphil S.L. (Spain) (ref. 50121500). CWPO runs were performed in deionized water, unless otherwise indicated.

2.2. Magnetite modification and characterization

The procedure followed for the preparation of Fe₃O₄-R400 has been previously reported (Álvarez-Torrellas et al., 2018). Briefly, powdered magnetite mineral was reduced at H₂ atmosphere (250 N mL min⁻¹ of 25 vol. % H₂ in N₂) for 3 h at 400 °C. A detailed characterization of the catalyst, including XRD, textural properties, and magnetic measurements, can be found elsewhere (Álvarez-Torrellas et al., 2018; Serrano et al., 2018). Summarizing, the iron content was 73% wt., the specific surface area 7 m² g⁻¹ and the particles showed a mean size of 0.2 μm. On the other hand, the solid showed a crystalline structure and strong magnetic properties (81.5 emu g⁻¹). The pH_{PZC} was determined following a method described in a previous work (Munoz et al., 2018).

2.3. Typical reaction procedure

CWPO experiments were performed at ambient conditions (25 °C, 1 atm) in a glass batch reactor (20 mL), equipped with a stirrer (750 rpm) and temperature control. Unless otherwise indicated, the initial concentration of cyanotoxins was fixed at 500 μg L⁻¹, the amount of H₂O₂ at the stoichiometric dose for the complete oxidation of the target pollutants (Table 1), the catalyst concentration at 0.2 g L⁻¹ and the initial pH was adjusted to 5.0 with nitric acid.

Another set of experiments was carried out with CYN to evaluate the effect of humic acid and several inorganic ions on the performance of the process, as well as to evaluate the effect of water matrix composition using a real surface water.

Blank experiments in the absence of catalyst were carried out with all the cyanotoxins tested in this work, and negligible conversion (<5%) of these substances was observed. On the other hand, possible adsorption of cyanotoxins onto the catalyst was discarded as observed in the tests performed in the absence of H₂O₂. All the experiments were performed in triplicate, being the standard deviation lower than 5%.

2.4. Analytical methods

The evolution of the CWPO experiments was followed by periodically withdrawing liquid samples from the reactor, which were immediately analyzed. The catalyst was previously separated using a magnet. MC-LR, MC-RR, CYN and ATX were analyzed by high performance liquid chromatography, HPLC-UV (Shimadzu, mod. Prominence-i, LC-2030C LT) using a diode array detector (SPD-M30A). An Eclipse Plus C18 5 µm column (15 cm length, 4.6 mm diameter) (Agilent) was used as stationary phase while the mobile phases were varied depending on the cyanotoxin analyzed. In all cases, the temperature of the column was fixed at 35 °C and the mobile phase flow rate at 0.8 mL min⁻¹. The analyses were carried out at 239 nm for both MC-LR and MC-RR while CYN and ATX were measured at 261 and 228 nm, respectively. A mixture of 37/63, 30/70% (v/v) of acetonitrile and acetic acid aqueous solution (75 mM) was used for the analyses of MC-LR and MC-RR, respectively. The quantification of CYN was performed using 2.5% methanol and 0.1% trifluoroacetic acid in water (v/v) as mobile phase whereas that of ATX was accomplished with a mixture 0.2/99.8% (v/v) of acetonitrile and acetic acid aqueous solution (3.75 mM). The quantification of STX was performed by HPLC-MS (HPLC-1200, 6410 TQ Agilent). In this case, the column ACE Excel 3 (C18-Amida, 15 cm length, 4.6 mm diameter, 5 µm particle size) was used. The analyses were performed using a mixture 50/50% (v/v) of water (0.1% formic acid) and acetonitrile (0.1% formic acid) as mobile phase. External mass

calibration for the positive ESI mode was conducted prior to analysis in the mass range of m/z 180 – 1000.

The concentration of H_2O_2 and dissolved iron were measured by colorimetry with a UV 2100 Shimadzu UV-VIS spectrophotometer using the titanium sulfate (Eisenberg, 1943) and the *o*-phenantroline (Sandell, 1959) methods, respectively. Short-chain organic acids were quantified by ionic chromatography (Metrohm 790 IC). A 3.2 mM Na_2CO_3 aqueous solution was used as mobile phase and a Metrosep A sup 5-250 column (25 cm length, 4 mm internal diameter, 5 μm particle size) as stationary phase. The real surface water was analyzed by a total organic carbon (TOC) analyzer (Shimadzu TOC V_{SCH}).

2.5. Toxicity study

Artemia salina has been used as model organism to assess the toxicity of the initial cyanotoxin solutions as well as the resulting CWPO effluents. This is a simple, inexpensive and reliable short-term routine test (Vanhaecke et al., 1981). The procedure followed is based on previous works (Barahona and Sánchez-Fortún, 1999; Ozkan et al., 2016; Vanhaecke et al., 1981). *Artemia salina* cysts (1.0 g) were incubated in glass bottles containing 0.5 L synthetic seawater at 30 °C under continuous aeration and illumination. Under these conditions, the cysts hatched within approximately 24 h. The artificial seawater was prepared by dissolving 35 g of synthetic sea salt (Marinium[®]) in 1 L of deionized water, stirring for 24 h with aeration and filtering through cellulose filter.

The standard conditions used to determine the acute toxicity were: 30 °C, salinity (35 g L⁻¹), pH 7 and darkness. The tests consisted of 4 concentrations of each cyanotoxin and one control which contained the seawater medium alone. Test solutions were previously adjusted to the same salinity of the stock brine shrimp media (35 g L⁻¹) by addition of concentrated salt solution. The vials containing 20 organisms in 2 mL of sample (cyanotoxin solution or CWPO effluent) were placed in an incubator for a period of 24h under the abovementioned standard conditions. There were three replicates of each

test concentration. Nauplii were considered dead if no movement was observed during 10 s of observation. The lethal concentration (LC_{50}) was used to determine the toxicity of the samples. It is defined as the lethal concentration of the compound ($\mu\text{g L}^{-1}$) that kills 50% of the *A. salina* nauplii within 24, 48 or 72 h.

3. Results and discussion

3.1. Cyanotoxins degradation by CWPO

The first set of experiments was focused on evaluating the versatility of CWPO for the treatment of the group of cyanotoxins (MC-LR, MC-RR, CYN, ATX and STX). They were performed with a low catalyst concentration (0.2 g L^{-1}) and the stoichiometric amount of H_2O_2 for the complete oxidation of the target pollutants ($2.0 - 3.0 \text{ mg L}^{-1}$), under ambient conditions and circumneutral pH ($\text{pH}_0 = 5$). The evolution of cyanotoxins upon CWPO is shown in Fig. 1. As observed, two main groups of cyanotoxins can be distinguished. The first one, including MC-LR, MC-RR and CYN, was much more reactive towards CWPO than the second one (ATX and STX). In this sense, while MC-LR, MC-RR and CYN were removed above 90% in 1 – 2 h reaction time, less than 50% was achieved with the ATX and STX. In fact, the removal yield of ATX and STX after 5 h was still around 80% and 60%, respectively. The experimental data was successfully described by a pseudo-first order kinetic equation. The resulting apparent rate constant values are collected in Table 2, confirming that cyanotoxins reactivity towards CWPO decreases in the following order: MC-RR>CYN>MC-LR>>ATX>STX.

The obtained results can be explained by the different nature of cyanotoxins. As can be seen in Table 1, their physicochemical properties vary significantly in terms of molecular size and structure. Among microcystins, MC-RR was more reactive than MC-LR under the operating conditions tested in this work. This could be attributed to the different amino acids present in these MC variants. MC-LR shows the amino acids leucine (L) and arginine (R) while MC-RR has two R amino acids. The reported second-order rate constants of these amino acids with hydroxyl radicals (generated by UV/ H_2O_2) were 1.7×10^9 and $3.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (He et al., 2015) for L and R, respectively, which explains that MC-RR

can be removed faster than MC-LR. This order of reactivity is also consistent with a previous work where the removal of four variants of microcystins by UV/H₂O₂ was investigated (He et al., 2015). In fact, it is well-known that amino substituents are quite reactive towards HO· attack (Anbar et al., 1966), and while leucine only presents one amino group, arginine contains four. Apart from microcystins, CYN also showed to be quite reactive towards CWPO. This cyanotoxin contains a number of functional groups such as uracil ring, sulfate and guanidine. The uracil group is the most susceptible to oxidation (de la Cruz et al., 2013), as it includes two quinone groups, species readily attacked by HO· (Pignatello et al., 2006). On the other hand, the presence of a sulfate functional group in the CYN molecule must be also taken into account as it is also highly reactive towards hydroxyl radicals (Anbar et al., 1966). ATX, the smallest cyanotoxin among those tested, is a bicyclic secondary amine with two functional groups slightly susceptible to oxidation, the amine and α,β -unsaturated ketone. In fairly good agreement with the results obtained in the current work, Onstad et al. (2007) also found that MC-LR and CYN showed similar reactivity towards hydroxyl radical oxidation while the removal rate for ATX was one order of magnitude slower. Finally, STX only presents two guanidine functional groups susceptible to oxidation (de la Cruz et al., 2013), being the most refractory cyanotoxin under the operating conditions tested in this work.

The obtained results can be favorably compared with those previously reported in the literature. The catalytic system H₂O₂/Fe₃O₄-R400 has proved to be effective for the removal of a wide range of cyanotoxins even operating under relatively gentle conditions. For instance, Wang et al. (2016) reached only 59% removal of MC-LR (5 mg L⁻¹) after almost 4 h reaction time using zero-valent iron (0.6 g L⁻¹) and a dose of H₂O₂ far above the stoichiometric amount (224 mg L⁻¹). The pseudo-first order kinetic constant value obtained was 0.024 L min⁻¹ g_{cat}⁻¹ while the one obtained in this work (considering that a catalyst concentration of 0.2 g L⁻¹ was used) is almost five times higher (0.11 L min⁻¹ g_{cat}⁻¹). Up to 30 mg L⁻¹ H₂O₂ and a UV dose of 1285 mJ cm⁻² were required to achieve an ATX (600 μ g L⁻¹) conversion above 85% (Afzal et al., 2010). Apart from the relatively fast removal of the cyanotoxins, it must be noted that only traces of oxalic, acetic and formic acids were identified in the final CWPO effluents.

As our results indicated a significantly less efficiency of the CWPO process with ATX and STX, a new set of experiments was carried out under more severe conditions *viz.* increasing the concentration of catalyst (1 g L^{-1}) and H_2O_2 (up to 10 times the stoichiometric amount: 30 and 24 mg L^{-1} , for ATX and STX, respectively). The results obtained are shown in Fig. 2. As observed, the increase of catalyst and H_2O_2 concentrations allowed to completely degrade the cyanotoxins in significantly shorter reaction times. In particular, the use of 10 times the stoichiometric amount of H_2O_2 and 1 g L^{-1} of catalyst allowed to reach similar rate constant values to those previously obtained with microcystins and CYN (8.90×10^{-2} and $5.93 \times 10^{-2} \text{ min}^{-1}$ for ATX and STX, respectively), being the cyanotoxins eliminated in 1 – 1.5 h reaction time.

With regard to the catalyst stability, it should be noted that dissolved iron concentration was below 0.5 mg L^{-1} in all cases, which represents 0.3% wt. of the initial iron concentration in the solid.

3.2. Effect of organic matter on CWPO performance

Natural organic matter is ubiquitous in the aquatic environment and thus, it is usually present in the source waters of DWTPs, affecting significantly the efficiency of their treatments (Kumar et al., 2018). NOM can consume chlorine leading to the formation of trihalomethanes upon chlorination treatment, saturate AC in adsorption systems or cause fouling of the membranes in separation processes (Kumar et al., 2018). It has been also demonstrated that NOM has a negative impact on AOPs performance, mainly through $\text{HO}\cdot$ scavenging, preference adsorption onto the active sites of the catalyst or light absorption (Kumar et al., 2018; Karci et al., 2018; Wang et al., 2016; Park et al., 2017; Pignatello et al., 2006). Nevertheless, it must be also noted that the occurrence of NOM can also present a positive effect, mostly in homogenous Fenton process, as it can provide a better availability of $\text{Fe}^{2+}/\text{Fe}^{3+}$ due to the formation of iron complexes, facilitating electron transfer (Pignatello et al., 2006). In this work, the impact of NOM on the degradation of CYN was investigated using deionized water fortified with humic acid, a major component of NOM, within the typical concentration values of organic matter in natural waters ($1 - 10 \text{ mg L}^{-1}$) (Park et al., 2017; He et al., 2010). As can be seen in Fig. 3a, the presence of

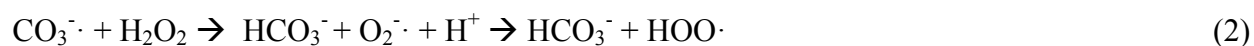
NOM in the reaction matrix partially hindered the degradation of CYN, leading to a decrease on the apparent rate constant values (Table 3). Up to 2.5-fold decrease was observed with the highest concentration of NOM tested (10 mg L^{-1}). Nevertheless, almost complete conversion of CYN was achieved regardless of the concentration of NOM after 2 h reaction time. On the other hand, the stability of the catalyst was not affected by the presence of NOM, reaching dissolved iron concentration values below 0.5 mg L^{-1} .

These results are consistent with those recently reported by Park et al. (2017) and Wang et al. (2016) in homogeneous and heterogeneous Fenton oxidation of MC-LR, respectively, and can be mainly attributed to $\text{HO}\cdot$ scavenging as well as the coating of magnetite particles by humic acid. At pH 5, the surface of the catalyst is positively charged ($\text{pH}_{\text{PZC}} \sim 7.5$) while humic acids are negatively charged (average $\text{pK}_a \sim 4.0$ (Fukushima et al., 1996) and thus, they are electrostatically attracted to the surface of the catalyst. All in all, it must be noted that the efficiency of the catalytic system was not strongly reduced when compared with other AOPs (Zhang et al., 2014; Al Momani et al., 2008). Al Momani et al. (2008) observed a strong decrease in the ozonation of CYN, from 90% removal to 50% and 30% in the presence of 2 and 10 mg L^{-1} NOM, respectively. In the same line, up to 95% decrease on the reaction rate was found upon CYN degradation by photocatalysis with polymorphic titanium dioxide (Zhang et al., 2014). In the latter, apart from the scavenging effect, the inhibition of the reaction was also attributed to the role of NOM as UV-Vis blocker.

3.3. Effect of inorganic ions on CWPO performance

Apart from considering the effect of organic matter on CWPO performance, the influence of several inorganic anions commonly occurring in natural waters (HCO_3^- , HPO_4^{2-} and SO_4^{2-}) was also evaluated. The effect of these anions on the degradation of CYN upon CWPO at a concentration of 3 mg L^{-1} (World Health Organization, 2004; Wang et al., 2016) can be seen in Fig. 3b (see Fig. S1 of the Supplementary Material for the experimental data obtained within the concentration range of 3 – 50 mg L^{-1} with sulfate and bicarbonate). As observed, the presence of the anions had no significant influence

on CYN removal rate, being the cyanotoxin eliminated in 90 min regardless of the water matrix composition tested. The resulting rate constants showed a slight influence of the anions on the kinetics of the process (Table 3). Remarkably, only bicarbonate led to some decline in the oxidation rate of CYN while phosphate and mainly sulfate promoted it. On the other hand, it should be highlighted that none of the anions tested led to a higher leaching of iron from the solid compared with the results showed in previous sections. It is well-known that bicarbonate and carbonate anions are potent HO· scavengers and that they can also lead to an inefficient decomposition of H₂O₂, yielding HOO· which shows a lower oxidation potential and less reactivity than HO·, according to the following reactions (Beltrán et al., 1998):



In fact, the experiment carried out with a higher concentration of HCO₃⁻ (50 mg L⁻¹) led to an inefficient consumption of H₂O₂ and thus, the complete conversion of CYN was not achieved (see Fig. S1 of the Supplementary Material for experimental data). Karci et al. (2018) also observed a partial inhibition in the degradation of CYN upon photo-Fenton reaction in the presence of carbonate anions. Nevertheless, the effect of the anion on the performance of the process was significantly higher than that obtained in the current work. The rate constant was reduced around 77% when the concentration of NaHCO₃ was adjusted to 3 mg L⁻¹ (total alkalinity = 1.8 mg CaCO₃ L⁻¹) while only a 9% decrease was observed in the presence of 3 mg L⁻¹ HCO₃⁻ with the H₂O₂/Fe₃O₄-R400 catalytic system.

According to previous works (Ratanatamskul et al., 2010; Pignatello et al., 2006), sulfate and phosphate ions can inhibit the homogeneous Fenton reaction due to their interaction with ferrous and ferric ions (Ratanatamskul et al., 2010; Pignatello et al., 2006). This fact allows to explain why the effect of these anions at low concentrations was not negative on the removal of CYN although it must be noted that a slight inhibition was observed at the highest concentration of sulfate tested (50 mg L⁻¹) (see Fig. S1 of the Supplementary Material for the experimental data obtained within the concentration range of 3 – 50 mg L⁻¹ with sulfate). These results are in agreement with those achieved in the Fenton

oxidation of 4-chlorophenol in the presence of different inorganic anions, where the kinetic constants were found to decrease in the following order: $\text{ClO}_4^- \sim \text{NO}_3^- > \text{SO}_4^{2-} > \text{Cl}^- \gg \text{HPO}_4^{2-} > \text{HCO}_3^-$ (Lipczynska-Kochany et al., 1995). It must be also noted that, the effect of sulfate and mainly phosphate has proved to decrease the efficiency of the oxidation in different works, they were carried out using significantly higher anion concentrations than the typical found in natural waters (Lipczynska-Kochany et al., 1995; Pignatello et al., 2006).

3.4. Operation in real surface water

As a proof of concept, the potential application of the catalytic system $\text{H}_2\text{O}_2/\text{Fe}_3\text{O}_4\text{-R400}$ was finally evaluated using a real surface water ($[\text{TOC}] = 2.7 \text{ mg L}^{-1}$, $[\text{IC (inorganic carbon content)}] = 14.9 \text{ mg L}^{-1}$; $[\text{SO}_4^{2-}] = 11.2 \text{ mg L}^{-1}$; $[\text{PO}_4^{3-}] = 0.1 \text{ mg L}^{-1}$; $[\text{Cl}^-] = 14.1 \text{ mg L}^{-1}$; conductivity = $200 \mu\text{S cm}^{-1}$) spiked with CYN. In this case, the effect of the initial concentration of the cyanotoxin was examined within the representative range of 100 to $500 \mu\text{g L}^{-1}$. The obtained results are depicted in Fig. 4 and the resulting rate constants are summarized in Table 3. A marked decay on the oxidation rate of CYN was observed compared with the results obtained in deionized water (Fig. 1). CYN conversion was around 90% after 6 h of treatment while only 1.5 h were enough to achieve its complete degradation in deionized water, which means a 6.6-fold decrease of the rate constant. According to the results showed in the previous sections, this decrease could be related to the presence of bicarbonate and also NOM in the real surface water while sulfate, phosphate and chloride would be of minor importance given their low concentration and poor $\text{HO}\cdot$ scavenging effects. Nevertheless, it must be noted that complete conversion of the cyanotoxin was achieved in 3 h reaction time with a more representative concentration of the target pollutant ($100 \mu\text{g L}^{-1}$). Furthermore, it must be noted the low concentration of catalyst used in this case (0.2 g L^{-1}). In fact, less than 60 min reaction time were required to achieve the complete conversion of CYN at a concentration around one order of magnitude higher than the commonly found in real water reservoirs ($100 \mu\text{g L}^{-1}$) with 1 g L^{-1} catalyst load.

3.5. Toxicity study

The toxicity of the cyanotoxins as well as the CWPO effluents was analyzed using the brine shrimp *Artemia salina*. This organism filters a large amount of water per hour and thus, it faces a higher risk of exposure to pollutants compared to other aquatic species, being recognized as an outstanding model organism for toxicity testing (Zhu et al., 2017; Ozkan et al., 2016; Rajabi et al., 2015). Table 4 collects the toxicity values obtained for each of the cyanotoxins studied (parent compounds) in terms of lethal concentration (LC_{50} , $mg L^{-1}$) using 24-old nauplii at different exposure times (24, 48 and 72 h). Clearly, CYN was the most toxic cyanotoxin followed by MC-LR and MC-RR. In the case of ATX and STX, the toxicity was significantly lower and LC_{50} values could only be calculated at 72 h exposure time. The obtained results obtained are consistent with those previously reported for MC-LR and CYN (Metcalf et al., 2002; Cornish et al., 2000). Nevertheless, it must be noted that these results cannot be directly extrapolated to the toxicological effects produced by the tested cyanotoxins in humans. For instance, pure CYN has an LD_{50} in mice (intraperitoneal) of $200 \mu g kg^{-1}$ (Ohtani et al., 1992), though LD_{50} values in mice vary depending on the exposure time. The LD_{50} of CYN in mice (intraperitoneal) is lower than that reported for ATX ($250 \mu g kg^{-1}$) and MC-RR ($50 \mu g kg^{-1}$) but higher than that of MC-LR ($50 \mu g kg^{-1}$) or STX ($10 \mu g kg^{-1}$) (Sivonen and Jones, 1999; Pearson et al., 2016). All in all, CYN poisoning can cause liver, kidney, thymus and heart damage in mammals (Terao et al., 1994; Wiegand et al., 2005). Particularly in humans, CYN has shown hepatotoxic, general cytotoxic, and neurotoxic effects and is considered a potential carcinogen (Pearson et al., 2016).

The mortality promoted by the initial target solutions ($1000 \mu g L^{-1}$) and the final CWPO effluents ($[Cyanotoxin]_0 = 1000 \mu g L^{-1}$; $[H_2O_2]_0 = 4 mg L^{-1}$; $[Fe_3O_4-R400] = 0.5 g L^{-1}$; $T = 25 \text{ }^\circ C$; $pH_0 = 5$) after 72 h exposure was also evaluated. In line with the calculated LC_{50} values, the mortality values promoted by the cyanotoxins were quite low (10% – 15%) with the exception of CYN (82%). Remarkably, all CWPO effluents were non-toxic at the end of the experiments, which is consistent with their complete elimination as well as with the fact that short-chain organic acids were the last end-products identified

(traces of oxalic, acetic and formic). The evolution of toxicity along the treatment was investigated using CYN as target pollutant given its high toxicity. In this case, the mortality caused by the samples extracted from the reactor after 72 h exposure was followed. As can be seen in Fig. 5, the toxicity decreased progressively along reaction, consistent with the removal of CYN, obtaining a non-toxic effluent (mortality < 2%) in 1 h reaction time. These results can be favorably compared with those previously reported in the literature for AOPs, where the formation of higher toxicity products along reaction and, in some cases, even still present in the final effluents has been observed (Kumar et al., 2018).

4. Conclusions

The inexpensive and environmentally-friendly catalytic system $\text{Fe}_3\text{O}_4\text{-R400}/\text{H}_2\text{O}_2$ has proved to be effective for the removal of a representative group of cyanotoxins from water. Notably, they were completely eliminated along the treatment under ambient conditions and circumneutral pH although their nature clearly determined the oxidation rate, which was found to decrease in the following order: MC-RR>CYN>MC-LR>>ATX>STX. In this sense, while only 0.2 g L^{-1} catalyst and the stoichiometric amount of H_2O_2 ($2 - 2.6 \text{ mg L}^{-1}$) allowed to remove completely the most reactive cyanotoxins (MC-RR, MC-LR and CYN) in 1 – 1.5 h, up to 1 g L^{-1} catalyst and $30 \text{ mg L}^{-1} \text{ H}_2\text{O}_2$ were necessary to totally oxidize the most refractory cyanotoxins (ATX and STX) within the same reaction time. Remarkably, the versatility of the process was demonstrated using relevant reaction matrix compositions and a real surface water. The effect of NOM and several inorganic ions (HCO_3^- , HPO_4^{2-} , SO_4^{2-}) was investigated, proving that only the former can lead to a partial inhibition of the reaction. Finally, the capability of CWPO to completely eliminate not only the cyanotoxins but also their associated toxicity risk was proved with the brine shrimp *Artemia salina*. These results demonstrate the potential of the $\text{Fe}_3\text{O}_4\text{-R400}/\text{H}_2\text{O}_2$ system for the effective and fast removal of cyanotoxins in drinking water treatment, and open the door for further research in this field, especially with the least investigated cyanotoxins (ATX and STX) and also with real bloom samples containing not only cyanotoxins but also cyanobacterial

cells. Further research on the scale-up of the process in continuous flow operation should be also explored.

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Table and Figure captions

Table 1. Cyanotoxins investigated and theoretical stoichiometry for their oxidation.

Table 2. Values of the pseudo-first order rate constants of disappearance of the cyanotoxins tested.

Table 3. Values of the pseudo-first order rate constants of CYN disappearance under different conditions.

Table 4. Toxicity values (LC_{50}) of the cyanotoxins.

Fig. 1. Time-course of the cyanotoxins upon CWPO ($[\text{cyanotoxin}]_0 = 500 \mu\text{g L}^{-1}$; $[\text{H}_2\text{O}_2]_0 =$ stoichiometric amount ($2 - 3 \text{ mg L}^{-1}$); $[\text{Fe}_3\text{O}_4\text{-R400}] = 0.2 \text{ g L}^{-1}$; $T = 25 \text{ }^\circ\text{C}$; $\text{pH}_0 = 5$). Experimental (symbols) and model fit (solid lines).

Fig. 2. Time-course of ATX and STX upon CWPO at different H_2O_2 doses (solid symbols: 5 times the stoichiometric amount ($12 - 15 \text{ mg L}^{-1}$) and open symbols: 10 times the stoichiometric amount ($24 - 30 \text{ mg L}^{-1}$)) ($[\text{cyanotoxin}]_0 = 500 \mu\text{g L}^{-1}$; $[\text{Fe}_3\text{O}_4\text{-R400}] = 1 \text{ g L}^{-1}$; $T = 25 \text{ }^\circ\text{C}$; $\text{pH}_0 = 5$). Experimental (symbols) and model fit (solid lines).

Fig. 3. a) Effect of the concentration of humic acids (a) and inorganic ions (3 mg L^{-1}) (b) on the degradation of CYN upon CWPO ($[\text{CYN}]_0 = 500 \text{ } \mu\text{g L}^{-1}$; $[\text{H}_2\text{O}_2]_0 = 2 \text{ mg L}^{-1}$; $[\text{Fe}_3\text{O}_4\text{-R400}] = 0.2 \text{ g L}^{-1}$; $T = 25 \text{ }^\circ\text{C}$; $\text{pH}_0 = 5$). Experimental (symbols) and model fit (solid lines).

Fig. 4. Time-course of CYN upon CWPO in real surface water at different catalyst concentrations ($[\text{H}_2\text{O}_2]_0 = 2 \text{ mg L}^{-1}$; $T = 25 \text{ }^\circ\text{C}$; $\text{pH}_0 = 5$). Experimental (symbols) and model fit (solid lines).

Fig. 5. Evolution of *Artemia salina* mortality upon CWPO of CYN ($[\text{CYN}]_0 = 1000 \text{ } \mu\text{g L}^{-1}$; $[\text{H}_2\text{O}_2]_0 = 4 \text{ mg L}^{-1}$; $[\text{Fe}_3\text{O}_4\text{-R400}] = 0.5 \text{ g L}^{-1}$; $T = 25 \text{ }^\circ\text{C}$; $\text{pH}_0 = 5$).

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Table 1
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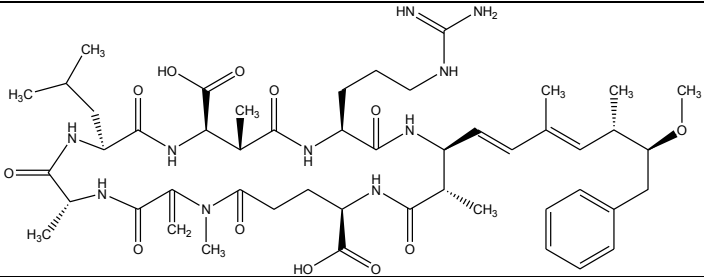
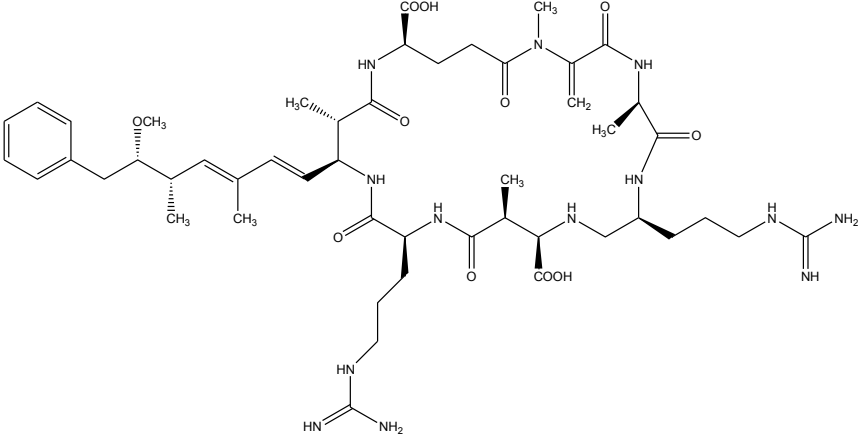
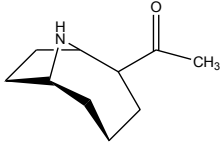
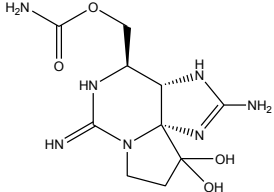
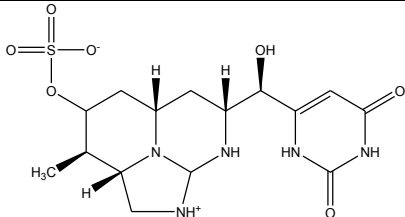
Toxic effect in humans	Cyanotoxin	Structural formula	Reaction	H ₂ O ₂ (mg L ⁻¹)*
Hepatotoxins	Microcystin-LR (MC-LR) (995.2 g mol ⁻¹)		$C_{49}H_{74}N_{10}O_{12} + 148 H_2O_2 \rightarrow 49 CO_2 + 180 H_2O + 10 HNO_3$	2.5
	Microcystin-RR (MC-RR) (1038.2 g mol ⁻¹)		$C_{49}H_{75}N_{13}O_{12} + 156 H_2O_2 \rightarrow 49 CO_2 + 187 H_2O + 13 HNO_3$	2.6
Neurotoxins	Anatoxin-a (ATX) (165.2 g mol ⁻¹)		$C_{10}H_{15}NO + 29 H_2O_2 \rightarrow 10 CO_2 + 36 H_2O + HNO_3$	3.0
	Saxitoxin (STX) (299.3 g mol ⁻¹)		$C_{10}H_{17}N_7O_4 + 42 H_2O_2 \rightarrow 10 CO_2 + 47 H_2O + 7 HNO_3$	2.4
Cytotoxin	Cylindrospermopsin (CYN) (415.4 g mol ⁻¹)		$C_{15}H_{21}N_5O_7S + 49 H_2O_2 \rightarrow 15 CO_2 + 56 H_2O + 5 HNO_3 + H_2SO_4$	2.0

Table 1

* Theoretical stoichiometric dose of H₂O₂ for the complete oxidation of cyanotoxins (initial concentration = 500 µg L⁻¹).

Table 2[Click here to download Table: Table 2.docx](#)

Table 2

Cyanotoxin	$k \times 10^2$ (min⁻¹)	r^2
MC-LR	2.29	0.99
MC-RR	6.01	0.99
ATX	0.64	0.99
STX	0.31	0.99
CYN	4.45	0.99

Table 3

[Click here to download Table: Table 3.docx](#)

Table 3

CYN initial concentration ($\mu\text{g L}^{-1}$)	Catalyst (g L^{-1})	H_2O_2 (mg L^{-1}) ¹⁾	Reaction matrix	Humic acid (mg L^{-1})	Inorganic ions (mg L^{-1})	$k \times 10^2$ (min^{-1})	r^2
500	0.2	2	Deionized water	0	--	4.45	0.99
				1		2.86	0.99
				10		1.82	0.98
				--	HCO_3^- (3 mg L^{-1}) ¹⁾	4.08	0.99
				--	HPO_4^{2-} (3 mg L^{-1}) ¹⁾	4.72	0.99
				--	SO_4^{2-} (3 mg L^{-1})	6.50	0.99
				--			
500	0.2	2	Real surface water	(not additionally added)	(not additionally added)	1.01	0.99
100						1.21	0.99
						6.45	0.99
	1.0						

Table 4

Cyanotoxin	LC ₅₀ (mg L ⁻¹)		
	24 h	48 h	72 h
MC-LR	3.3	1.8	0.9
MC-RR	9.2	5.7	1.4
ATX	ND*	ND*	5.2
STX	ND*	ND*	4.3
CYN	1.1	0.5	0.3

ND*: non-detected, LC₅₀ > 10000 µg L⁻¹ (mortality < 15% at all the concentrations tested).

Figure 1 revised

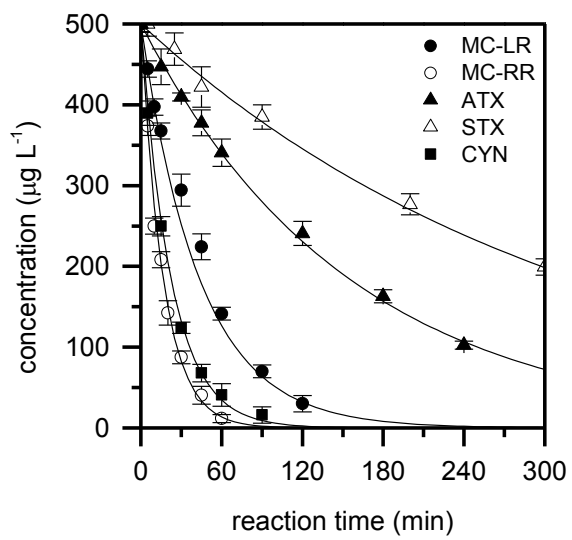


Figure 2

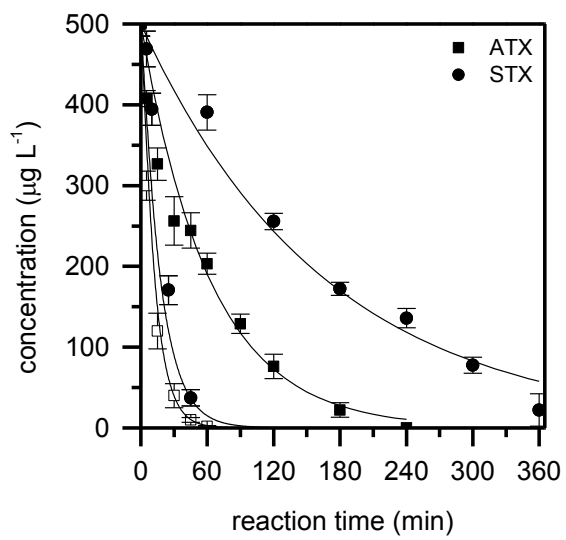


Figure 3 Revised

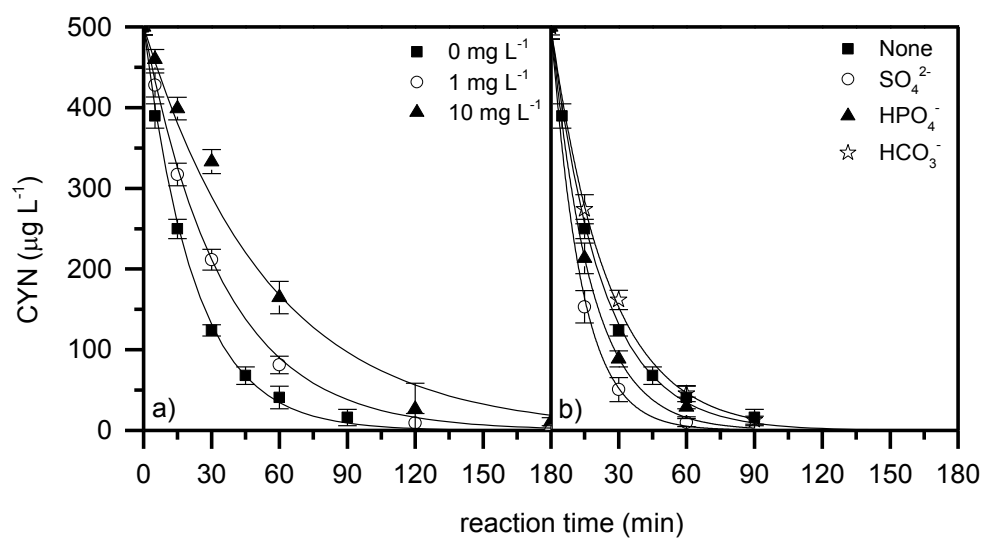


Figure 4

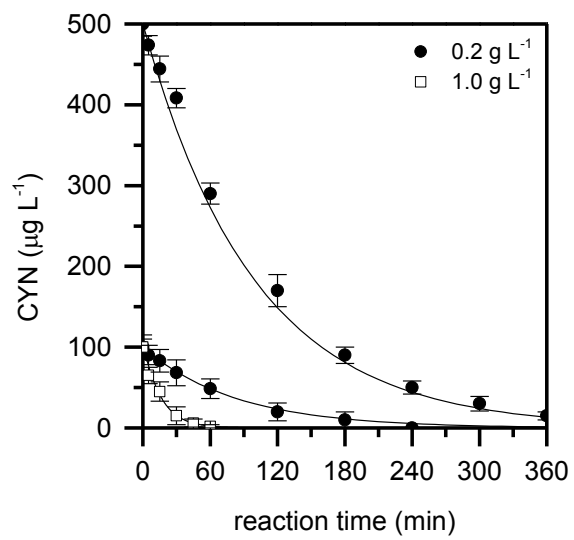
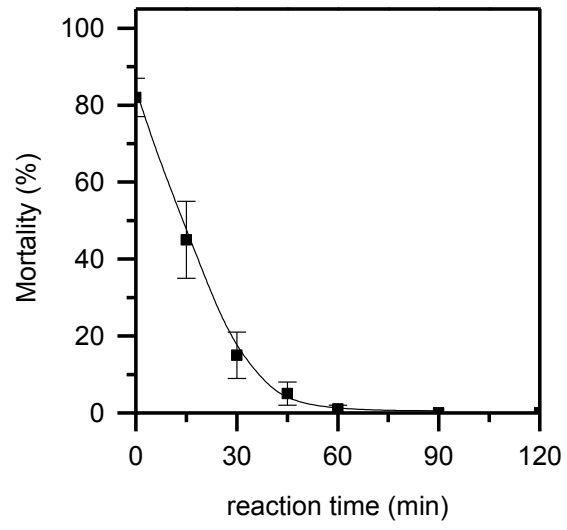


Figure 5



Electronic Supplementary Material (for online publication only)

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