In-lake treatment with hydrogen peroxide for cyano-HABs control at Delft, NL (Lake Delftse hout) during the summer of 2015

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Abstract The presence of harmful cyanobacterial blooms (cyano-HABs) in surface waters negatively impacts the surrounding ecosystem while at the same time they limit its usages (for drinking water, irrigation and recreational purposes). Current research activities are focusing on treating Cyano-HABs at source as part of prevention ormitigation strategy. Application of hydrogen peroxide (2.5 mg/L H_2O_2), for the selective and rapid termination of Cyano-HABs has been successfully applied in fresh water lakes. It was therefore decided to treat the undesirable symptoms of cyano-HABs in the lake Delftse hout (NL) with H_2O_2. The lake was treated with 5 mg/L H_2O_2. A higher concentration was used because of the floating layers of cyanobacterial scum. H_2O_2 rapidly reacted with cyanobacteria and left no residual concentration within minutes of the application. The cyanobacteria population, mainly consisted of *Aphanizomenon* and *Dolichospermum* (formerly known as *Anabeana)*, was reduced to less than 1% and 3%of their initial concentration, respectively, 24 hours after the treatment. No adverse effects were observed on other flora and fauna. Following treatment microcystin concentration was below warning levels, and entrance to the public was allowed two days after treatment.

Keywords: *Dolichospermum* sp., *Aphanizomenon* sp., cyanotoxins, hydrogen peroxide, in-lake treatment.

1. Introduction

Harmful cyanobacterial blooms (cyano-HABs) and the formation of surface scum are among the most noticeable and infectious consequences of eutrophication besides oxygen depletion and the addition of taste and odor in water. Most importantly, cyanobacteria can produce potent toxic metabolites, cyanotoxins, leading to the prohibitive use of the lakes. Cyanotoxins are categorized based on their chemical structure (cyclic peptides, alkaloids, and lipopolysaccharides-LPSs) and on the toxicological effects they cause (cytotoxins, dermatotoxins, hepatotoxins, and neurotoxins).

In-lake treatment of cyano-HABs can potentially reduce and inhibit further blooming of cyanobacteria and the concentration of cyanotoxins in the affected water body (Douglas *et al*. 2016, Ibelings *et al*. 2016, Visser *et al*. 2016). So far, an array of physical and chemical in-lake treatments have been tested for Cyano-HABs control.

To prevent cyanobacterial blooms, internal and external nutrient loading should be reduced in the lake (see e.g. Ibelings *et al*. 2016). Artificial aeration can be an effective technique since it can provide better oxygenation in the entire water column, leading to reduced phosphorous release from sediments, increase light scattering, and alter phytoplankton biomass and composition (Drabkova and Marsalek, 2007). as it creates conditions more beneficial for the growth of other green algae rather than cyanobacteria (Visser *et al*. 2016). Chemical treatment by adding phoshorus binding chemicals can be effective in isolated lakes (Douglas *et al*. 2016). Many treatments are not always effective on a short term and therfore the use of algaecides are also in use.Chemical treatment with copper algecides is the most widely used in-lake treatment for Cyano-HABs for more than a century.

Copper-based algaecides, such as copper sulfate ($CuSO_4$), interfere with a variety of biological functions and chemical transformations by uptaking and substituting copper for magnesium within chlorophyll in the living cells. One of major concerns about the copper-based algaecides is the ecotoxicological effects that this treatment can have on the remaining ecosystem since a variety of non-target aquatic species are also affected. Another issue is the release of the intracellular concentration of cyanotoxins from ruptured cells leading to unusually high levels of the cyanotoxins in the surrounding water which exceed orders of magnitude the World's Health Organization (WHO) guideline of 1.0 μg L^{-1} for minimum

allowable concentration levels in drinking water (Antoniou *et al*. 2013).

Based on the above, current research efforts are focusing on establishing appropriate in-lake treatment strategies that can selectively oxidize cyanobacteria without affecting the remaining ecosystem. These substances must primarily act as cyanocides. Addition of H_2O_2 appears to be a promising in-lake chemical oxidation treatment since it does not generate hazardous waste and has minimum effect on the remaining aquatic life (Matthijs *et al*, 2012; Matthijs *et al*, 2016). The selective suppression of harmful cyanobacteria in an entire lake with homogeneously injected H_2O_2 has been found to be promising since the presence of flavoproteins flv 1 and flv2 in cyanobacteria excludes formation of the superoxide anion radical ($O_2^{-\bullet}$) and formation of H_2O_2 does not take place in the chloroplast when there is an excess of electron flow. Therefore cyanobacteria cells do not have the proper mechanisms for eliminating H_2O_2, like algea do.

This study focused on the actual treatment of Lake Delftse hout (NL). The H_2O_2 treatment took place on July 14, 2015. A specially designed boat that evenly disperses H_2O_2 was used (Matthijs *et al*, 2012). Chemical addition occurred once and then samples were taken from 3 different points every 4 hours for 24 hours to evaluate the photosynthetic vitality of the treated lake (Figure 1). It was also important that the cyanotoxin concentration was below the guideline levelfollowing treatment to allow access to the public.

2. Materials and Methods

Lake water samples were analyzed to determine temperature, pH, dissolved O_2, chlorophyll-a levels and nutrient concentrations. Also water samples were analyzed for phyto- and zooplankton communities. The specific inhibitory effects of hydrogen peroxide on the phytoplankton's photosynthetic activity were measured by using a mini-PAM fluorometer (Walz, Germany). Samples from the lake, before and after the H_2O_2 addition (1, 3, and 5 hours) were collected in triplicates from different locations (Fig. 1). A volume of 100 mL of each location was filtered through GF/C filters (25 mm diameter) and after the measurements filters and filtrate were stored at -20^{o}C for toxin analyses. The residual concentration of H_2O_2 was monitored with indicator sticks (QUANTOFIX). The concentration of total microcystins was initially monitored with an ELISA kit to determine the intracellular (extraction of filter with methanol) and the extracellular (filtrate water) toxin concentration. Accurate measurements of microcystin concentration were performed using Acquity UPLC system with photodiode array (ACQUITY UPLC PDA) equipped with Tandem Quadruple Time of Flight (Xevo QToF) in series (Waters, Elstree, UK). Samples were separated on Acquity UPLC ® BEH C18 column (2.1 i.d. x 100 mm; 1.7 µm particle size; Waters, UK) maintained at 40°C. Milli-Q water (A) and acetonitrile (B) both containing 0.1% Formic acid (FA) constituted the mobile phase and used in a gradient.

3. Results and Discussion

Lake Delftse Hout has five sampling points. In this study, water samples were taken only from the three following locations OW203-112, OW203-113 and OW203-117 (Figure 1).

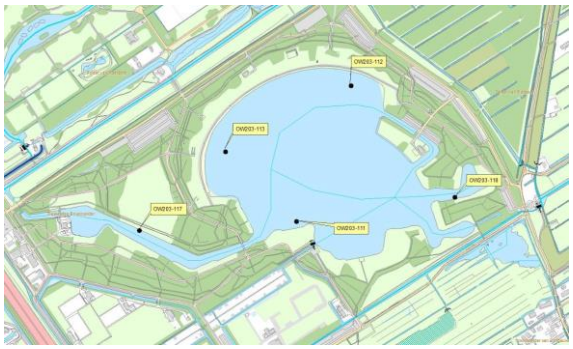

Figure 1 Sampling locations in Delftse Hout

In order to determine the degradation rate of hydrogen peroxide in the water for accurate assessment of the concentration HP in the lake treatment, samples were taken one day in advance, Since the dose can be affected by the type and population of cyanobacteria, an empirical optimum H_2O_2 dose needed to be found. For the entire lake and the type of cyanobacteria species it was found that treatment would be successful if for 5 hours, at least 2 mg/L is present in the water (Matthijs *et al*. 2106). For dense populations (floating layers) hydrogen peroxide is broken down quickly to the target, thus the concentration for treating those areas was set to 5.0 mg/L.

A special boat, as it was described in Matthijs *et al*. (2012) for a homogeneous dispersal of a low concentration of H_2O_2 throughout the lake, was designed by ARCADIS and the application was done by professionals experienced in handling the oxidant. Stocks of concentrated H_2O_2 were stored in big containers located in a restricted area and every time the boat was reloading from there. Prior to treatment, the lake was close for public use and warning signs were placed around it indicating that swimming was prohibited due to treatment of the lake to mitigate cyanobacteria in the water.

On the day of the treatment, the concentration of H_2O_2 was carefully monitored. By using the dosing boat (Matthijs *et al*., 2012) it appeared to be possible to accurately dose the quantity of H_2O_2. Following H_2O_2 application the entire lake was maintained at 2-3 mg/L for more than 5 hours.

The decrease in the phytoplankton vitality on the day of the treatment is shown in Figure 2. The presented values give the mean of samples taken at three sampling sites. The initial value of the photosynthetic yield was 600 corresponding to a vitality of 100 %. The small but steady increase in the vitality measurements with time at point OW203-112 let to the decision to apply an additional treatment in zone t =4. Based on the results of Figure 2 and the drastic decrease in the photosynthetic vitality of the sample at t=4 hour, the additional treatment was essential to inhibit further growth of cyanobacteria. Although H_2O_2 decomposed faster at OW203-111 compared with other locations, cyanobacteria did not survive (decrease in photosynthetic vitality). At location OW203-112 a more gradual degradation was observed, with a lower decrease in photosynthetic vitality compared to site OW203-111.

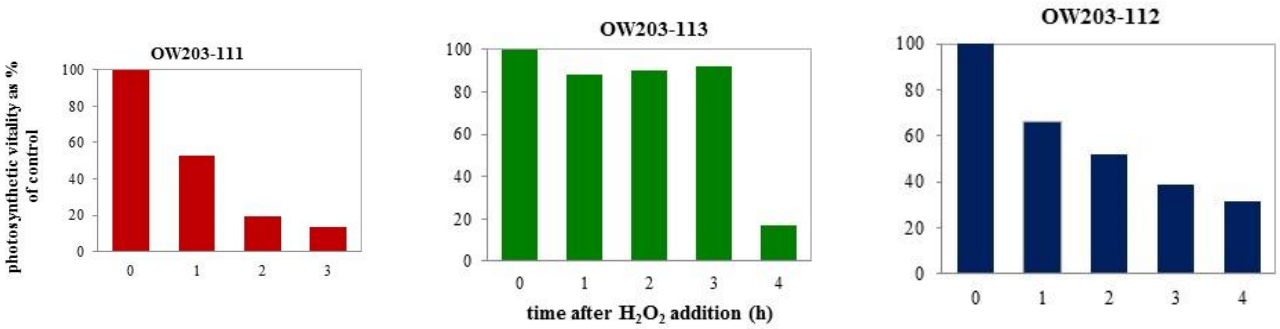

Figure 2: Reduction of photosynthetic vitality in the lake as a function of time after the hydrogen peroxide addition. The photosynthetic vitality is expressed as percentage of the control.

Overall, the two dominant cyanobacteria species found in the lake were *Dolichospermum* sp and *Aphanizomenon* sp. The concentration of both species rapidly declined following H_2O_2 treatment, with an average decrease of 99.7% and 97.3% for *Dolichospermum* sp. and *Aphanizomenon* sp., respectively. Intracellular and extracellular toxins of the samples collected were tested using the ELISA kit. Since these assays have a lot of false positive/negative and cannot quantify for the actual amount of specific derivatives, a more accurate and advanced analytical technique (LC-MS/MS) was used. The analysis indicated that the major cyanotoxins (MC-LR, MC-RR, and MC-YR and nodularin) were below the detection limit of the method. **Acknowledgments**

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