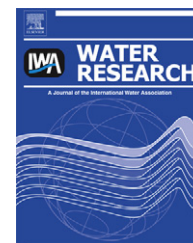


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Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide

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ABSTRACT

Although harmful cyanobacteria form a major threat to water quality, few methods exist for the rapid suppression of cyanobacterial blooms. Since laboratory studies indicated that cyanobacteria are more sensitive to hydrogen peroxide (H_2O_2) than eukaryotic phytoplankton, we tested the application of H_2O_2 in natural waters. First, we exposed water samples from a recreational lake dominated by the toxic cyanobacterium *Planktothrix agardhii* to dilute H_2O_2 . This reduced the photosynthetic vitality by more than 70% within a few hours. Next, we installed experimental enclosures in the lake, which revealed that H_2O_2 selectively killed the cyanobacteria without major impacts on eukaryotic phytoplankton, zooplankton, or macrofauna. Based on these tests, we introduced 2 mg L^{-1} ($60\text{ }\mu\text{M}$) of H_2O_2 homogeneously into the entire water volume of the lake with a special dispersal device, called the water harrow. The cyanobacterial population as well as the microcystin concentration collapsed by 99% within a few days. Eukaryotic phytoplankton (including green algae, cryptophytes, chrysophytes and diatoms), zooplankton and macrofauna remained largely unaffected. Following the treatment, cyanobacterial abundances remained low for 7 weeks. Based on these results, we propose the use of dilute H_2O_2 for the selective elimination of harmful cyanobacteria from recreational lakes and drinking water reservoirs, especially when immediate action is urgent and/or cyanobacterial control by reduction of eutrophication is currently not feasible. A key advantage of this method is that the added H_2O_2 degrades to water and oxygen within a few days, and thus leaves no long-term chemical traces in the environment.

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1. Introduction

Cyanobacterial blooms are favoured by high temperatures and nutrient load, and have increasingly become a major

nuisance in many freshwater and brackish ecosystems (Chorus and Bartram, 1999; Jöhnk et al., 2008; Paerl and Huisman, 2008). Dense cyanobacterial blooms shade away light for other phytoplankton (Mur et al., 1999; Huisman et al.,

Abbreviations: H_2O_2 , hydrogen peroxide; ROS, reactive oxygen species.

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2004), and generally offer low food quality for herbivorous zooplankton compared to most eukaryotic phytoplankton species (Ghadouani et al., 2003; Wilson et al., 2006). The high turbidity of cyanobacterial blooms may also smother the growth of aquatic macrophytes, suppressing important underwater habitat for invertebrates and fish (Scheffer et al., 1993; Gulati and Van Donk, 2002). Furthermore, some cyanobacteria produce toxins, which can cause serious and sometimes fatal liver, digestive and neurological diseases (Carmichael, 2001; Codd et al., 2005). Toxic cyanobacterial blooms pose a significant threat to birds, mammals and human health, and make the water less suitable for drinking water, agricultural irrigation, fishing and recreation (Chorus and Bartram, 1999; Huisman et al., 2005).

Nutrient reduction is arguably the best strategy to reduce the incidence of harmful cyanobacterial blooms (Dokulil and Teubner, 2000; Conley et al., 2009; Smith and Schindler, 2009). Additional measures, such as artificial mixing (Visser et al., 1996; Huisman et al., 2004) and flushing (Verspagen et al., 2006; Mitrovic et al., 2011) of lakes, may also suppress cyanobacterial populations. Some lakes have been treated with clays that bind phosphate and coagulate with cyanobacterial cells, causing their sedimentation (Robb et al., 2003; Van Oosterhout and Lüring, 2011). Chemicals such as aluminium and copper have been used as cyanobacterial algicides (Griffiths and Saker, 2003). Each of these strategies has its drawbacks. Artificial mixing of lakes is costly. Flushing of lakes is not always feasible, and may cause water deficits in upstream areas during dry summer periods. Algicides can induce massive release of cyanotoxins by lysing cyanobacterial cells, thus engraving rather than resolving water-quality problems (Kenefick et al., 1993; Griffiths and Saker, 2003). The results of nutrient reduction programs often become effectively visible only after several years due to, e.g., sustained nutrient input from diffuse sources or internal nutrient loading from the sediment (Gulati and Van Donk, 2002; Søndergaard et al., 2003). This contrasts with societal demands, as bans on recreation and the economic damage caused by the closure of recreational waters or diminished access to irrigation and drinking water often request for immediate results (Verspagen et al., 2006; Guo, 2007). Hence, there is a clear need for effective intervention techniques that can rapidly suppress the proliferation of upcoming cyanobacterial blooms without negative side-effects on overall water quality.

Hydrogen peroxide (H_2O_2) is a reactive oxygen species produced in natural waters mostly by the photolysis of dissolved organic matter exposed to UV radiation (Cooper and Zika, 1983). H_2O_2 is also produced biologically, as by-product of photosynthesis, respiration and other metabolic processes (Apel and Hirt, 2004; Asada, 2006) and as signalling molecule (Veal et al., 2007). H_2O_2 decays to water and oxygen by chemical and biological oxidation–reduction reactions, with decay rates in the order of a few hours to a few days depending on biological activity and the presence of redox-sensitive metals such as iron and manganese (Cooper and Zepp, 1990; Häkkinen et al., 2004). H_2O_2 concentrations in surface waters of lakes range from 1 to 30 $\mu g L^{-1}$ (30–900 nM) (Cooper et al., 1989; Häkkinen et al., 2004). Light exposure of H_2O_2 in the presence of iron or manganese may produce trace amounts of the highly reactive hydroxyl radical (Zepp et al., 1992). These

radicals cause damage to cells by the oxidation of proteins, lipids and DNA, resulting in severe oxidative stress (Mittler, 2002; Apel and Hirt, 2004; Latif et al., 2009).

Several laboratory studies have indicated that cyanobacteria are more sensitive to hydrogen peroxide (H_2O_2) than green algae and diatoms. Barroin and Feuillade (1986) showed that as little as 1.75 ppm (corresponding to 1.75 $mg L^{-1}$) of H_2O_2 had a deleterious effect on laboratory cultures of the cyanobacterium *Planktothrix rubescens* (formerly known as *Oscillatoria rubescens*), while a 10 times higher concentration proved totally harmless to the green alga *Pandorina morum*. Subsequently, Drábková et al. (2007a,b) investigated several more species, and showed that H_2O_2 had generally a much stronger inhibitory effect on the photosynthesis of cyanobacteria than of eukaryotic phytoplankton. Barrington and Ghadouani (2008) found that cyanobacteria declined twice as fast as green algae and diatoms after H_2O_2 addition to wastewater samples, and their recent work shows that application of H_2O_2 to wastewater treatment ponds removed more than 50% of the cyanobacterial biomass within 48 h (Barrington et al., 2011).

Hence, these studies suggested the use of low concentrations of H_2O_2 for the selective removal of cyanobacteria in lakes. Although adding chemicals to natural waters appears a somewhat strange management strategy, H_2O_2 addition might not be as bad as it seems. H_2O_2 occurs naturally in small concentrations in all surface waters (Cooper and Zika, 1983), and many organisms produce H_2O_2 (Asada, 2006; Veal et al., 2007). Furthermore, since low H_2O_2 concentrations are intended to work selectively against cyanobacteria, this method, if successful, may have the advantage that other aquatic organisms will remain largely unharmed. Finally, H_2O_2 rapidly breaks down into water and oxygen, such that the added H_2O_2 is unlikely to stay in the ecosystem for long. Yet, until now, the idea of adding H_2O_2 to suppress cyanobacterial blooms has never been tested in natural waters.

In this study, we investigate whether H_2O_2 addition is able to selectively suppress cyanobacteria in an entire lake without affecting other biota. Our lake experiment was carried out in Lake Koetshuis, a small lake in the Netherlands. This lake suffered from frequent closure for recreation due to dense blooms of the nuisance cyanobacterium *Planktothrix agardhii*, which produced high concentrations of the hepatotoxin microcystin. The nearby Lake Langebosch served as control. The lake experiment was carried out in three steps. First, we ran laboratory tests with water samples taken from the lake to test the H_2O_2 sensitivity of *P. agardhii*. Next, we used enclosures placed in the lake to estimate which range of H_2O_2 concentrations would specifically hit the cyanobacteria while leaving other phytoplankton and zooplankton largely unaffected. Finally, we mixed the desired H_2O_2 concentration homogeneously into the entire lake. For this purpose, we designed a small boat with a special ‘water harrow’, injecting dilute H_2O_2 at different depths in the water column till just above the sediment. The entire operation was sized to accomplish treatment of the entire lake within a single day. After treatment of the lake, we monitored the H_2O_2 concentration, the photosynthetic vitality of the cyanobacteria, and the population abundances of the cyanobacteria and other biota during several weeks.

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