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# Cyanobacteria blooms and biological control Methods

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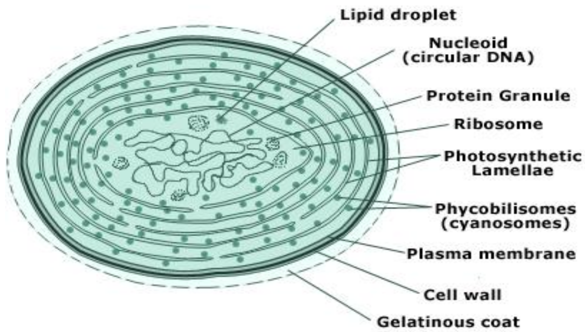
## Abstract
In last few decades, an excessive development of cyanobacteria in lakes and reservoirs occurs as a consequence of increasing freshwaters eutrophication, especially of phosphorus input. Due to production of high biomass and toxins, the cyanobacterial occurrence leads to deterioration of aquatic ecosystems and also negatively affects use of lakes for recreation and as drinking water supplies. This review focuses on cyanobacterial blooms and possible biological control methods. The review includes already well-known methods as well as currently developing biological control methods and principles. Regarding biological control methods, the use of viruses and bacteria represent a new interesting direction of research, however, they have only been based on laboratory studies up to now. Thought, there are various biological control methods, the removal of substantial amount of benthivorous fish and restoration of macrophyte domination are important or even necessary supplements for successful lake restoration, it is not sufficient to prevent cyanobacterial development if used as the only measures.

**Keywords:** cyanobacteria, biological control, fish, phytoplankton

## 1. Introduction
Cyanobacteria are often the most important group of phytoplankton in eutrophic tropical lakes [1]. Besides dominating the phytoplankton community composition, they also frequently build up dense populations. High phytoplankton abundance is normally perceived as a precursor to increases in zooplankton abundance, as is the case in several temperate lakes [2], especially for the efficient filter feeders the cladocera. Yet high cyanobacteria crops are rarely grazed down [3] and are instead associated with the disappearance or reduction in abundance of efficient grazers [4]. The occurrence of high cyanobacteria abundance in temperate lakes is often seasonal, with a few exceptions of shallow eutrophic lakes where it could last for years [5]. In some eutrophic tropical lakes, cyanobacteria blooms often last all year round and despite contributing quite significantly to the diets of herbivorous fish [6, 7] fish and zooplankton grazers in these lakes are often unable to graze down populations of cyanobacteria. This has been attributed to a number of factors, for example, formation of ‘ungrazable’ filaments and colonies [3]. Cyanobacteria tend to have a negative effect on grazer populations [8].
The objective of this review is to give some highlight on the Biological control methods of cyanobacterial blooms.

## 2. Biology of Cyanobacteria
Cyanobacteria are gram negative photosynthetic prokaryotes consisting of over 1000 species of unicellular and multicellular micro-organisms belonging to the class Cyanophyceae under the orders *Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales (Oscillatoriaceae, Nostocaceae and Rivulariaceae*) [9]. According to [9], cyanobacteria may exist in several forms that may either be unicellular (single or forming colonial aggregates) or filamentous (possessing or lacking heterocysts and akinetes). The dominance of cyanobacteria in eutrophic aquatic systems has also been attributed to a variety of factors typical to cyanobacteria including possession of phycobiliproteins [10], production of gas vesicles [14], ability of some species to fix nitrogen [13] and the ability to produce allelopathic chemicals that may inhibit growth of algae [40] and macrophytes [11].
Unlike the eukaryotic algae, cyanobacteria lack organelles, but instead have intracellular membranes (thylakoids), which incorporate the photosynthetic pigment (phycobilisomes-phycobiliproteins in a supramolecular structure) within cyanobacterial cells [13] (Figure 1).




**Source**: [43]

**Fig 1:** Cell structure of cayanobacteria

Probably the most interesting aspect about cyanobacteria is their ability to harvest light from a wide spectrum and use it to photosynthesise. All cyanobacteria possess the photosynthetic pigment chlorophyll *a* and the light harvesting phycobiliproteins; allcophyeocyanin B, allophycocyanin, and C-, or R-phycocyanin (others like phycoerythrin and C-phycoerythrin may also be present in some cyanobacteria groups - red algae) that allow them to use light energy outside the chlorophyll maxima in the fixation of carbon dioxide, converting it into organic carbon [10]. Light energy may be trapped by using both chlorophyll *a* in photosystem I (PSI) and a series of phycobilosomes in photosystem II (PSII) and is used in the production of ATP and NADPH. Cyanobacteria can thus perform better than most algae under low light conditions.

Several cyanobacterial species, especially those possessing heterocysts, are capable of fixing nitrogen using the enzyme nitrogenase [13], thus compensating for any shortfalls in aquatic nitrogen that is essential for primary production. In filamentous cyanobacteria, nitrogenase which catalyses the reduction of dinitrogen to ammonia, may be contained in cells known as heterocysts which are formed particularly under conditions of nitrogen limitation, yet non-heterocyst forming cyanobacteria may also fix nitrogen [13].

Some cyanobacteria may also possess hollow gas-permeable and water-impermeable protein structures called gas vesicles that provide buoyancy [14]. The gas vesicles, which vary in width in different species, regulate buoyancy allowing the cyanobacteria to occupy the most optimum position within the water column. The cyanobacteria are an ancient and ubiquitous family of organisms, many with photosynthetic abilities. They are frequently found growing in marine, brackish and fresh waters, including freshwater surface water drinking sources [15]. In aquatic environment rich in nutrients cyanobacteria periodically exhibit significantly increased reproductive rates and total population biomass known as a “bloom.” Cyanobacterial blooms cause negative environmental impacts such as are shading, increase in pH, decrease in the oxygen content in the water via bloom respiration or degradation, and production of highly active cyanotoxins [16]. These effects lead to mortality of aquatic organisms, decreased growth of submerged aquatic vegetation, decreased biodiversity of aquatic organisms and decrease in ecosystem stability by interfering in food-web dynamics displacing normal phytoplankton species. Moreover, there are other negative consequences for humans due to production of odours and cyanotoxins in recreational lakes and drinking water supplies (Falconer, 1999). Wide spectrum of cyanotoxins (blue green algal toxins) predominantly causes neurotoxic, hepatotoxic, and dermatotoxic effects [16, 17]. The symptoms of excessive cyanobacterial development can bring economical losses in the form of decreased property values, high cost treatments of raw drinking water, illnesses, depressed recreational industries, and expenses for management and restoration of impaired lakes and reservoirs [18].

Cyanobacteria possess a variety of adaptive mechanisms enabling them to survive unfavorable environmental conditions and promoting their successful growth in water. The process of nitrogen fixation, occurrence of gas vesicles, possibility of partial heterotrophic metabolism, and a production of allelopathic compounds are especially important to the success of nuisance species of blue-greens [19]. Effects of many anti-cyanobacterial measures are dependent on sensitivity or autecology of particular cyanobacterial species. One of the worldwide most common bloom-forming toxins-producing cyanobacterial species is *Microcystis* genus, especially *Microcystis aeruginosa*. Therefore, many experiments and methods are aimed at the control of these species [20].

Although many methods for cyanobacterial blooms control are available, the question how to control growth of such successful and well adapted organisms by far has not been solved. The effectiveness of methods is controlled by a variety of circumstances (the type and the size of the lake, retention time, the tropic degree and size of nutrient load, water chemistry, the quality and amount of sediments, a season, fish stock, etc.), the methods are not universally usable, and the use of many of them is limited to special cases. Combination of several measures have a better chance to succeed have than a single treatment. The logical and most important step in preventing cyanobacterial blooms is to decrease high nutrient loads, especially phosphorus, from watershed, which is the primary cause of cyanobacterial massive occurrence. This includes rehabilitation of point and non-point sources of nutrients (wastewater discharges; agriculture runoff and erosion from urban and deforested areas). Broad range of in-lake methods may further decrease availability of phosphorus in lakes. However, sufficient reduction of nutrient inputs and accumulation requires long-term and expensive restoration of landscape and reservoirs [18]. Therefore, to reach effect in shorter time, there is a high pressure to directly treat cyanobacteria in lakes, mainly by some chemicals toxic to cyanobacteria. However, use of most of formerly used algicides/cyanocides such as copper salts is not acceptable due to nonselective effects, toxicity to non-target aquatic organisms, and negative consequences in relation to human health [21, 22, 23]. Thus, there is still a need to select or develop new *cyanocides* with lower negative impact on aquatic environments, as well as other types of methods.

## 3. Harmful Algal Blooms

Intense proliferation of cyanobacteria or the so-called cyanobacterial blooms are a major symptom of eutrophication. Cyanobacteria will tend to dominate in aquatic ecosystems that have high concentrations of total phosphorus (TP) [24, 25] or as earlier suggested in environments with low TN (total nitrogen): TP ratios [26].

In deep eutrophic temperate and subtropical lakes, the summer rise in water temperature, followed by depletion of dissolved inorganic nitrogen and carbon are characteristic of a progression from a clear water phase to cyanobacteria bloom formation which eventually disappears during the cold autumn and winter. In shallow lakes, however, cyanobacteria blooms may persist for years [27]. In temperate regions cyanobacteria blooms often occur in late summer [27], whereas the warmer temperatures, intense solar radiation, and year-round 12-hour days in the tropical regions, often promote all year round cyanobacteria blooms in eutrophic lakes [28, 29].

**Source** [30]

**Fig 2:** Algae is seen near the City of Toledo water intake crib, Sunday, Aug. 3, 2014, in Lake Erie, about 2.5 miles off the shore of Curtice, Ohio

Cyanobacterial blooms may have far-reaching ecological effects on aquatic ecosystems [8]. Formation of cyanobacteria blooms typically leads to a reduction of light penetrating through the water column, causing a shading effect. This lowered transparency causes poor growing conditions for epiphytes, phytoplankton, and benthic algae [31]. Increases in pH due to carbon dioxide depletion by cyanobacteria blooms and/or anoxia resulting from a collapsed bloom could lead to massive fish kills. Additionally, when these blooms die off they sink to the bottom, where they decompose causing a depletion of bottom water oxygen or hypoxic conditions [32].

Nile tilapia (*Oreochromis niloticus*) [33] in eutrophic lakes has been attributed to their ability to utilize cyanobacteria. This, however, is not always the case [34], since blooms are often dominated by colonial and filamentous forms, which, among the already mentioned undesirable traits, may cause mechanical interference for zooplankton grazers and clogging of gills in fish [8]. A few studies have also found some species of cyanobacteria to be nutritionally deficient to a number of zooplankton species [35]. For a number of zooplankton species and for juveniles of most fish species, under certain conditions, some cyanobacteria species can be a poor source of nutrition [36, 37].

## 4. Cyanotoxins

Cyanobacteria produce secondary metabolites the so called cyanotoxins; these may be cytotoxic or biotoxic (hepatotoxins and neurotoxins). Cyanotoxins may have allelopathic effects on dinoflagellates [38] green algae, and other cyanobacteria [39, 40], affecting photosynthesis and growth. Cyanotoxins may also account for the disappearance of submerged macrophytes [41].

The toxicity of cyanobacteria to zooplankton can be attributed not to one but to several toxic compounds including the non-ribosomal oligopeptides that may lower ingestion rate, disrupt moulting, and lower survival. The most commonly assayed of these are the hepatotoxic microcystins and the neurotoxic anatoxins. Studies examining the effects of these toxic compounds in cyanobacteria on the growth and survival of zooplankton indicate that though they may have no effect on population growth they greatly affect survival [37].

Whereas fish have been considered quite tolerant to the toxicity of cyanobacteria, there are some reports linking toxic cyanobacteria to massive fish kills. However, not enough evidence is available to implicate cyanobacteria toxins in occurrences of fish kills and related cyanobacteria blooms either in tropical or temperate regions [42]. Fish kills may be a result of other cyanobacteria bloom related circumstances such as oxygen depletion [32] (figure 3). Nonetheless, several laboratory and field experiments have demonstrated that the presence of aqueous and cell-bound cyanotoxins in the diet of the fish is not good for their physiology, morphology, and behavior [44].

**Source**: [43]

**Fig 3:** Lake Erie algae bloom

## 5. Biological Control Methods of Algal Bloom

### 5.1. Bio-manipulation

The term bio manipulation was coined by [45] and later used by [46] as a term for lake water quality management methods based on biological interventions. Shapiro included effects of algal biomass from top-down control of zoo planktivores by piscivores and bottom-up effects on algae such as nutrient cycling supported by benthivorous fish. In the contemporary limnology this method is usually referred to as a top-down control. The principle is based on manipulations of a trophic cascade. As a result of the reduction of feeding pressure of fish on zooplankton, large species of zooplankton predominate, which are capable of keeping the phytoplankton levels down. The desired composition of fish populations can be achieved by harvesting non-predatory fish and by introducing predatory fish. The effectiveness of the top-down bio manipulation method is limited. The number of reviews has written on this topic shows that if the method is to be successful, specific condition needs to be fulfilled [47]. Bio manipulation is usually not very effective in the case of highly eutrophic lakes and reservoirs where the total phosphorus concentration exceeds 100 µg $L^{-1}$. Most effective examples of bio manipulation apply to relatively small water bodies because of a great difficulty to continuously manipulate fish populations in large ones. Also, the bio manipulation procedure cannot be considered as a routine method, since it depends on a number of circumstances and can be performed only with the participation of a skilled limnologist. Often, the continuous maintenance is required [47].

In the case of *Microcystis* dominance the zooplankton grazing is limited, mainly because of bigger colonies, which are poorly edible for most of zooplankton species [48, 49]. In the case of increased numbers of smaller zooplankton species, the green algae are preferred for grazing, which may even support the cyanobacterial dominance. The toxic effects of cyanobacteria on daphnia species are reported, which may also play a role in poor effectiveness if the bio manipulation is focused on cyanobacterial blooms control. However, daphnids in lakes with commonly present cyanobacterial blooms might be already resistant to cyanotoxins [50], thus the effective grazing cannot be excluded.

More probably, the bio manipulation may reduce cyanobacterial growth via the bottom-up effect in the case of fish removal. Due to fisheries and economical interests, lakes or ponds are commonly overstocked by benthivorous cyprinids. Benthivorous fishes re suspend large quantities of sediments, which enhance transfer of phosphorus and cyanobacterial cells in to the water column. Besides, they uproot aquatic plants and their excretion contributes to phosphorus loads. Therefore the removal of substantial amount of benthivorous fishes is strongly recommended if control of cyanobacterial blooms is the aim of lake-restoration project [51].

### 5.2. Herbivorous fishes
Potential method how to reduce cyanobacterial blooms development is direct grazing by herbivorous fishes. Phytoplankton is the main food especially for the silver carp (*Hypophthalmichthys molitrix*) and partially also bighead carp (*Aristichthys nobilis*). However, number of studies reported that metabolic activity of phytoplankton after gut passage remains unaffected or even increases. There are some indications promoted by ecosystem studies describing the effective use of Nile tilapia (*Oreochromis niloticus*) in cyanobacterial bloom control. Nile tilapia is known to have an extremely low pH value in its stomach, [52] therefore enhanced damage of ingested cyanobacteria is probable.

### 5.3. Macrophytes and periphyton
The presence of macrophytes in lakes and reservoirs is beneficial for many reasons. Macrophyte-dominated lakes are resistant to development of algal or cyanobacterial dominance because rooted plants reduce wind and boat-generated re suspension of sediments, provide a daytime refuge to algae-grazing *Daphnia*, and shade and therefore cool water in the littoral zones. Macrophytes also remove part of nutrients and act as careers for periphyton, which further removes quantum of dissolved phosphorus. Some macrophytes also release allelopathic compounds inhibitory to cyanobacteria. Especially, the inhibiting effects of *Miriophyllum* sp., *Chara* and *Elodea* have been reported [53].

However, there is a nutrient-based stability threshold for a macrophyte-dominated state of the lake of about 50-100 μg P $L^{-1}$. If the nutrient loads remains higher, the phytoplankton or cyanobacterial growth will still overwhelm. Reduction of P loads increases the probability that the cyanobacterial or algal dominated state of the lake can switch to the macrophyte-dominated, clear-water state. However, there is a resistance of the lake to both, the increasing and decreasing nutrient loading. The macrophyte-dominated lake can maintain clear water even in the case of high nutrient load, while water quality in the phytoplankton-dominated lake may not improve even if the nutrients concentrations are substantially reduced [18].

### 5.4. Other organisms
Many other aquatic organisms have been considered and studied to limit potentially the cyanobacterial growth. Their use is based on principles of predation, parasitism or release of metabolites suppressing cyanobacterial growth. Studies with viruses, bacteria, algae, fungi, and protozoa have been reported in the literature. Especially the parasitism of bacteria and viruses seems to be interesting due to the high specificity only to particular cyanobacterial species and, therefore, no effects to other aquatic organisms. However, the large-scale cultivation of many of these organisms is problematic. It is also common that cyanobacteria develop resistance in the case of the effect based on production of antibiotics (bacteria, fungi). Knowledge in this area is mostly only based on laboratory studies and no successful direct applications in the whole lake scale have been reported [53].

#### Viruses
Viruses of cyanobacteria (cyanophages) commonly occur in marine and freshwater aquatic environment [54, 55], where they play an important role in determining the cyanobacteria during the season. The first evidence of potential use of viruses to control cyanobacterial blooms were reported by [56]. The sudden decline of the cyanobacterial biomass accompanied by the cyanophage occurrence has been reported [57]. There are few recent studies dealing with the possible control of cyanobacterial development by viruses [58]. However, there are many problems that make the use of viruses nearly impossible in praxis. The isolation and the cultivation of cyanophage are problematic or nearly impossible. Cyanobacteria became resistant to cyanophage so the effect will be temporary [59]. Moreover the virus is specific to a particular strain and often does not affect cyanobacteria from another locality even if the species is the same [60]. Even after the decline of particular cyanobacteria by viruses, the cyanobacteria can be easily replaced by other cyanobacterial species [61].

#### Bacteria
Bacteria can lyses cyanobacteria by the production of extracellular lytic enzymes or by contact lyses. The lytic effects selective to bloom forming cyanobacteria are reported in the case of bacteria *Alcaligenes denitrificans* [62], *Bacillus* sp. [63], *Bdellovibrio*-like bacteria [64], *Myxococcus* sp. [65], *Flexibacterium* [66], and *Pseudomonas* sp. or *Spingomonas* sp. producing lytic agent Argimicin A [67]. Actinomycete *Streptomyces exfoliatus* caused 50% mortality of *Anabaena*, *Microcystis Oscillatoria* [68]. Some cyanobacterial extracellular metabolites also cause inhibition of cyanobacterial growth, photosynthesis or metabolism. Metabolite from *Flexibacterium* similar to lysozymes inhibited photosynthesis and enzymatic activity of cyanobacterium *Oscillatoria williamsi*. More recently [69] describes strong selective growth inhibition of cyanobacterium *Microcystis aeruginosa* and *Anabaena affinis* by surfactin produced by *Bacillus subtilis* C1. Similarly, cyanobacteriolytic substance from *Bacillus cereus* has been described and its lytic activity toward *Aphanizomenon flos-aquae* has been found as well. Lyses of *Microcystis aeruginosa* by *Streptomyces neyagawaensis* has been reported [70].

#### Algae
Besides macrophytes, some planktonic algae may also produce allelopathic compounds inhibiting cyanobacterial growth. [71] demonstrated that extract from dinophyte *Peridinium bipes* causes changes in membrane permeability in cyanobacterium *Microcystis aeruginosa*.

#### Fungi
Parasitism of a cyanobacterium by the chytridiaceous fungus *Rhizophidium planktonicum* was shown [73] however, chytridiaceous fungi were later considered to be of limited use due to difficulties in their large-scale culture. Redhead and [72] demonstrated specific antagonistic effects of 62 non chytrid fungi on *Anabaena flos-aquae* and also other filamentous or unicellular cyanobacteria.

**Protozoa**

Within aquatic ecosystem, protozoa play an important role in the reduction of phytoplankton populations by grazing and phagocytosis [73]. The predation on cyanobacteria was described in the case of ciliates *Furgasonia* [73], *Nassula* and *Pseudomicrothorax Stos*, [74], *Amoeba* [75] and flagellate *Monas guttula* [76]. However, most of bloom-forming cyanobacterial species form colonies, which prevent them from protozoan grazing. Therefore, the use of protozoa as bio-control agents is very limited and questionable

## 6. Conclusion

Cyanobacteria are often the most important group of phytoplankton in eutrophic tropical lakes. Besides dominating the phytoplankton community composition, they also frequently build up dense populations. There are various control options were implemented in the control of blooms but the one which is environmentally affable are biological control methods. Biological control is a group of methods involving living organisms. These methods are mainly based on principles of food web manipulation, grazing, predation, parasitism or release of metabolites suppressing cyanobacterial growth. Use of viruses and bacteria represent a new interesting direction of research, however, they have only been based on laboratory studies up to now. Thought, there are various biological control methods, the removal of substantial amount of benthivorous fish and restoration of macrophyte domination are important or even necessary supplements for successful lake restoration, it is not sufficient to prevent cyanobacterial development if used as the only measures.

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