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REVIEW PAPER

Methods for Prevention of Mass Development of the Cyanobacterium *Microcystis aeruginosa* Kutz emend. Elenk. in Aquatic Systems

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Abstract—Methods for prevention of mass development of the cyanobacterium *Microcystis aeruginosa* Kutz emend. Elenk. in continental water bodies and industrial water supply systems are reviewed. The physicochemical, chemical, and biological methods for prevention of *M. aeruginosa* development in water bodies and water supply systems are considered; examples of successful inhibition of *M. aeruginosa* growth in laboratory experiments are demonstrated. The scientific problems are outlined that are to be solved for perfecting techniques for prevention of *M. aeruginosa* mass development in open water bodies and in closed water supply systems.

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As a result of the development of industry and power engineering over the 20th century, a number of artificial water bodies (reservoirs, ponds) and industrial water supply systems have been constructed. In the course of the operation of these objects, cyanobacteria were shown to predominate in their biocenoses and to behave as a laboratory monoculture. Mass growth of cyanobacteria leads to algal blooms in open water bodies [1] and to formation of persistent biofouling in water supply systems [2]. The species *Microcystis aeruginosa* Kutz emend. Elenk. is the most widespread among the cyanobacteria responsible for algal blooms and biofouling. Mass growth of *M. aeruginosa* significantly impairs the supply of drinking and industrial water and leads to the development of putrefaction processes, death of animals due to toxic compounds present in the water, and other consequences [3–5]. The development of *Microcystis* in water bodies is becoming a global problem [6], and the financial losses in industry and power engineering are enormous. The reasons for *Microcystis* mass development and ways of monitoring and controlling the growth of this organism are among the most urgent topics in water microbiology [7, 8]. In the present article, world experience in the development of a theoretical basis for practical measures to counter mass growth of *M. aeruginosa* in water systems is analyzed.

Mechanisms Underlying High Competitiveness of *M. aeruginosa*. Colonies of *M. aeruginosa* possess a variety of adaptive mechanisms promoting their successful growth in water. These mechanisms include effective protection against inhibition by sunlight and various mechanisms of migration in the water column. The presence of a multilayered mucous sheath, which encircles the colony and increases its resistance to unfavorable environmental conditions, is a species characteristic of *M. aeruginosa*. The forms of this species, both airborne and sedimentary, with specific features enabling them to survive unfavorable environmental conditions, form a practically inexhaustible supply of microcystis in water.

It is believed that a relatively high phosphorus content, a low nitrogen-to-phosphorus ratio (N : P < 25), and high concentrations of oxygen and trace elements promote mass growth of *M. aeruginosa* in water. However, it is still impossible to predict microcystis bloom by the presence of any of these factors, separately or in combination. The reasons for blooms or biofouling are therefore determined experimentally in each individual case; recommendations concerning microcystis elimination are then developed depending on the experimental results. The methods for countering the growth of *M. aeruginosa* described in recent publications can be conventionally subdivided into physicochemical, chemical, and biochemical.

Physicochemical methods. An experiment that involved decreasing the external phosphorus load was performed in the Villerest Reservoir (France) [9]. An almost 80% decrease in the phosphorus in its main influx did not lead to a significant decrease in the

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M. aeruginosa level. The N : P ratio in the water after the decrease in the phosphorus load was slightly above 5; this level is considered favorable for *M. aeruginosa.* The overall decrease in P concentration is therefore not sufficient to prevent microcystis growth; an N : P ratio above 25 is required for this purpose. The negative results of the work on the prevention of microcystis development in Danish water bodies confirm the conclusion that phosphorus decrease alone is insufficient [10]. Sediment withdrawal, which decreases exogenous phosphorus load should be considered a more efficient way to suppress microcystis growth. For example, in experiments that involved withdrawal of phosphorus-rich sediments from Bautsen reservoir (Germany), the growth of *M. aeruginosa* was completely prevented [11].

The results of laboratory and field experiments [12] have demonstrated that ultraviolet (UV) radiation at 75 mW/(s cm^2) stops *M. aeruginosa* growth. At the Lake Okitama (Japan), where UV lamps were placed on a specially equipped boat, UV radiation resulted in increased specific gravity of microcystis cells; the cells sank to the bottom and subsequently died. The possibility of applying UV radiation to prevent microcystis growth is still debatable. Some data of laboratory experiments indicate the existence of a unique UV-protective system in cyanobacteria. According to [13], under UV irradiation, microcystis can boost the synthesis of UV-absorbing compounds (antioxidants and extracellular polysaccharides) and resynthesize UV-sensitive proteins.

Chemical methods. The colonies of *M. aeruginosa* are less sensitive to tin- and chlorine-containing toxic compounds (biocides) than filamentous cyanobacteria. The complex multilayered mucous sheath, which prevents the penetration of biocides inside the microcystis colonies, is the main reason for their increased resistance to chemical toxins. However, analysis of available publications demonstrated that the search for efficient chemical means to fight microcystis continues. For example, it has been shown [14] that the addition of potassium (as KCl and $KHCO_3$) results in selective suppression of *Microcystis* growth. The authors noted that no growth of *M. aeruginosa* occurred in water containing more than 2.8 mM potassium. The most probable mechanisms for the inhibition of microcystis growth by potassium have been termed "sodium-related phenomena." Increased potassium influx into the cell is believed to result in a corresponding decrease in the uptake of sodium, which is necessary for various aspects of microcystis metabolism, including maintenance of intracellular pH.

The experiments were often successful when combined biocides were used [15]. In [16], for instance, an example was given of a successful prevention of algal bloom in Courtille Lake (France) by sequential treatment with aluminum sulfate and copper sulfate. The introduction of $Al_2(SO_4)_3$ in May resulted in a noticeable decrease in *M. aeruginosa* numbers, and the addition of $CuSO_4 \cdot 5H_2O$ in June prevented its growth completely. The mechanism of the action of copper on microcystis colonies is not completely understood [17]. The pathway by which copper-containing compounds penetrate microcystis cells is unknown. Copper is believed to bind with proteins in relatively stable complexes and to penetrate into cells practically without loss. The toxic effect of copper is possibly a result of its ability to block the SH groups of proteins.

The use of $Al_2(SO_4)_3$, $KHCO_3$ and $CuSO_4 \cdot 5H_2O$ often leads to three problems. The first one is termed "the phase reactions principle." This means that, while a low concentration of a biocide enhances the cell metabolism, a higher dose suppresses it and a still higher one kills the cell. The second problem is related to the ability of *M. aeruginosa* to rapidly adapt to lethal concentrations of most biocides by increasing the rate of random mutations, which provides for selection of metal-resistant cells [18]. Death of aquatic animals caused by nonselective action of the biocides is yet a third problem. Thus, biocides will hardly be widely used for the prevention of microcystis growth unless the above-mentioned problems are solved.

Herbicides (diuron, simazine, atrazine), which block the electron flow in photosystem II, are known to inhibit the growth of microcystis. However, I failed to find any recent publications dealing with the use of these herbicides. The negative effect of herbicides on aquatic animals seems to prevent further use of these xenobiotics to counter algal blooms and biofouling.

Biochemical methods. The introduction or spreading of aquatic organisms that excrete allelopathic compounds (exometabolites) inhibiting the growth of microcystis is a biochemical method. These compounds act as natural biocides involved in regulation of the composition of aquatic flora. For example, the introduction or recolonization of higher plants (macrophytes) in the zones of algal bloom suppresses the growth of microcystis. Exometabolites of *Myriophyllum spicatum* L. are believed to be the most promising inhibitors of microcystis growth [19]. The mixture of four polyphenols excreted by *M. spicatum* (ellagic, gallic, and pyrogallic acids and (+) catechol) causes synergetic inhibition of *M. aeruginosa* growth. Since algal blooms are initiated in the shallow-water zone, the introduction of macrophytes has an additional effect. Colonization of the shallows by macrophytes drastically decreases the probability of microcystis transition from sediments into the water column and decreases the internal phosphorus load by stabilizing bottom sediments. Macrophytes and associated bacteria also act as a trap for biogenic elements, including those arriving from tributaries and with dispersed flow from shores.

Introduction of barley straw into aquatic ecosystems is considered one of the most efficient biochemical methods for countering microcystis growth. Decomposing straw is known to inhibit microcystis growth [20]. It was previously believed that inhibitory com-

pounds are not excreted from straw but rather produced by the associated microflora. Subsequent research demonstrated that short-term suppression of microcystis growth is caused by extraction of phenolic compounds from straw, and long-term suppression, by oxidative decomposition of lignin. Straw has been successfully used to prevent mass growth of microcystis in reservoirs and in return water supply systems in England, Australia, the United States, and South Africa. The recommended amount of straw is no less than 2.5 g/m^3 water. The long duration of the inhibitory action (6–8 months) and the absence of hazardous ecological effects are the advantages of this method.

Microcystis growth was reported to have been suppressed by exometabolites excreted from decaying wood, forest litter, fallen oak leaves, banana peel, and tangerine skin (see, e.g., [21]). Fallen oak leaves are often used to counter microcystis in the European countries. The oak leaf exometabolites have a prolonged inhibitory effect (up to 2.5 years). A short-term effect of the oak leaves (4–90 days) is caused by excretion of tannins; a long-term effect (90–900 days), by the oxidative decomposition of lignin [22]. Moreover, bacteria associated with fallen leaves can potentially excrete compounds inhibiting microcystis growth. Combined introduction of barley straw and fallen oak leaves is also possible.

Some researchers [23, 24] consider typical aquatic bacteria and actinomycetes with inhibitory action to be the most promising biological agents countering algal blooms. For instance, the combined action of *Alcaligenes denitrificans* exometabolites inhibits growth of *M. aeruginosa* [23], and the combined action of *Peridinium bipes* exometabolites causes dissolution of cell membranes of *M. aeruginosa* [25]. Cyanobacterin and fischerellin are the best-studied natural inhibitors of microcystis growth. The first compound is produced by cyanobacteria of the genera *Nostoc* and *Oscillatoria*; the second one, by cyanobacteria of the genus *Fischerella.* Both these compounds cause rupture of the thylakoid membranes in microcystis. According to [26], approximately a half of the 83 actinomycetal genera collected in the bottom sediments of Japanese lakes were capable to cause lysis of the microcystis cells. *Streptomyces phaeofaciens* was used to investigate the nature of the lysing effect of actinomycetes on microcystis. It was found to excrete L-lysine, which caused irreversible damage to microcystis cell walls in 48 h. The mixtures of lysine with malonate or with copper in a concentration of 0.5 to 20 mg/l were later also shown to inhibit the growth of *M. aeruginosa* [27].

Lysine has a selective effect on microcystis growth. Selective suppression of microcystis growth is also characteristic of certain surface-active substances. In [28], for example, the broth culture of *Bacillus subtilis* C1 containing 10 mg/l of a surface-active substance was demonstrated to completely inhibit the growth of natural *M. aeruginosa.* In general, recent publications permit the conclusion that biochemical methods for preventing microcystis growth are the most promising due to their efficiency and cost-effectiveness. Wide-scale field tests and the development of scientific methods for the introduction of biological preparations into aquatic systems are, however, necessary.

Microcystis has recently been much considered as a valuable industrial resource, e.g., for the production of essential fatty acids or of compounds inhibiting the growth of other harmful microorganisms [29]. The progress in this field calls for future application of technologies for mechanical collection of microcystis from the surface of water bodies and water supply systems.

The common shortcoming in most experiments on preventing microcystis growth is the lack of preliminary calculations and an analysis of the results of long-term monitoring of hydrobiological, hydrochemical, and hydrophysical characteristics of water. Mathematical modeling is seldom used, although it is important for predicting the results of action on microcystis growth. Complex ecological technologies that imply a combined use of inexpensive physicochemical and biochemical techniques that do not affect ecological norms have almost completely been ignored.

In summing up the published data, two scientific problems should be mentioned which must be solved for successfully developing methods to prevent microcystis growth in aquatic systems. First, technical procedures for the introduction of microorganisms producing exometabolites toxic to microcystis should be worked out. Second, experiments are required in order to develop genetically modified organisms with enhanced synthesis of exometabolites that inhibit microcystis growth.

Most of the methods for controlling *M. aeruginosa* growth in water are not selective. Although some chemical and biochemical methods exhibit selectivity, the prospects of their application on the scale of a water body as a whole remain unclear. The joint use of physicochemical and biochemical methods to counter mass growth of *M. aeruginosa* is the most promising field.

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