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Review

Current and Previous Green Technologies, Their Efficiency, Associated Problems, and Success Rates to Mitigate *M. aeruginosa* in Aquatic Environments

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Abstract: Frequent *M. aeruginosa* outbreaks pose a major risk to public health and have a detrimental effect on aquatic ecosystems. Researchers are looking into ways to stop and control *M. aeruginosa* blooms, a problem that affects both the aquatic environment and human health significantly. It is important to develop proper monitoring methods to identify *M. aeruginosa* blooms. However, the existing control and monitoring techniques have some drawbacks that limit the field's applicability. Therefore, we must improve current methods for effectively monitoring and controlling *M. aeruginosa* blooms. Mitigation strategies should be customized for particular bodies of water utilizing techniques that are fast, economical, and field-applicable. This review critically identifies and evaluates green technologies, especially those focused on the presence of *M. aeruginosa* in freshwater, and compares and discusses problems with these green technologies. Furthermore, they were characterized and ranked according to their cost, effectiveness, and field applicability. A few suggestions for improvements were provided, along with ideas for future research projects that would take anticipated environmental changes into account.

Keywords: eutrophication; harmful algal blooms; physical chemical and biological cyanobacteria control; anti-cyanobacterial allelochemicals; comparative insights

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1. Introduction

Microcystis aeruginosa has been identified as one of the major bloom-forming cyanobacteria expanding globally as a result of intensifying eutrophication [1]. In May 2007, excessive nutrient concentrations and extremely warm temperatures led to a major hazardous *Microcystis* bloom in Lake Taihu, China, raising concerns across the globe [2]. Winnipeg lake has been declared "the threatened lake of the year" by the Global Nature Fund due to increased phosphorus concentrations [3]. The frequency and severity of severe summer–fall cyanobacterial harmful algal blooms (cHABs) have increased, according to satellite imagery and measurements of the lake's biomass [4]. Microcystin concentrations can reach high levels during cyanobacterial blooms in Lake Erie's western basin [5]. The city of Toledo, Ohio, received a "do not drink" warning in August 2014 after microcystin levels in the

water exceeded the recommended limit of 1 g/L set by the World Health Organization [6]. In several regions of the Laurentian Great Lakes, the poisonous cyanobacterium *Microcystis aeruginosa* has developed into a regular summertime occurrence, raising public concerns [7]. Numerous reports of cyanobacterial blooms in the Guadiana River along its downstream course through Portuguese territory have been produced. These blooms are typically dominated by the potentially toxic *Microcystis* spp. [8]. Problematic cyanobacterial blooms have also affected the Murray River in New South Wales (NSW). Their concentrations exceeded 4 mm^3 L^{-1}. The National Health and Medical Research Council (2008) established this as the red alert threshold for recreational water consumption in Australia, at which point New South Wales begins to take bloom management measures [9]. From the 1980s to 2022, *Microcystis aeruginosa* dramatically increased in frequency and abundance throughout the world, causing high levels of water contamination and affecting human health [10,11].

Reducing pollution from both point and nonpoint sources is vital to controlling eutrophication via regulation of nitrogen (N) and phosphorus (P) inputs [12]. Nitrogen (N) and phosphorus (P) are the principal nutrients of concern because their supply frequently affects aquatic life [13]. Effective treatments and preventive measures, along with their detailed mechanisms (Figure 1), must be developed to control *M. aeruginosa* blooms, which have constituted a significant threat to the security of the aquatic environment [14].

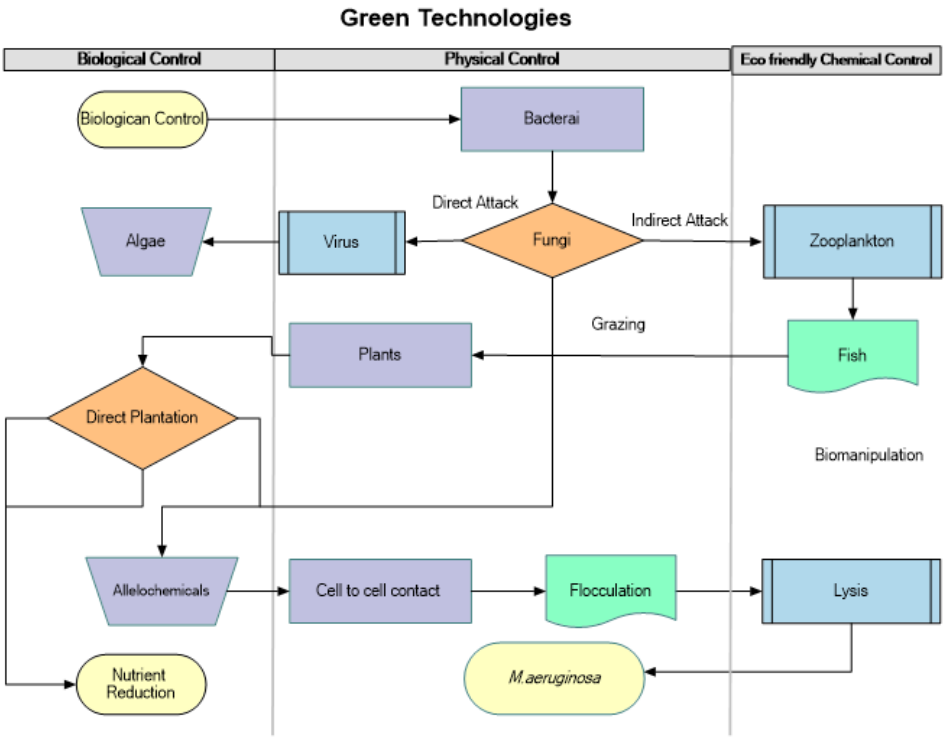

Figure 1. Green technologies and associated mechanism to control *M. aeruginosa*.

To reduce *M. aeruginosa* abundance and eliminate toxins from freshwater, the purpose of this narrative review is to focus on current green physical, chemical, and biological technologies. However, it is true that the deployment of most chemical and physical techniques is constrained due to negative ecological impacts, expensive costs, or low field operability. Since few techniques for addressing the harmful *M. aeruginosa* blooms can be applied on a wide scale in the field, it is necessary to consider a variety of strategies, including microorganisms, aquatic animals, plant allelopathy, and clay applications. We systematically compared and analyzed the development status, advantages and disadvantages, applicable conditions, and future development trends of green technologies for monitoring and controlling *M. aeruginosa* blooms.

2. Green Technologies to Control *Microcystis aeruginosa*

The three main options available to date for treating and controlling the growth of *M. aeruginosa* are:

1. Physical methods
2. Chemical methods
3. Biological methods.

2.1. Phyical Control

Freshwater treatment frequently involves physical methods and water quality standards are constantly being improved in many countries [15]. It is considered as an emergency preventive measure rather than a control method [16]. Physical preventive measures include: harvesting of *M. aeruginosa* [17], air flotation [18], magnetic flocculation [19], hydrodynamic cavitation [20], light shading [21], dredging sediments [22], ultrasound technology [23], flocculants [24], etc.

Toxic algal blooms can be removed by air extraction by using tiny bubbles to attach to the algae. Through the action of the minute oxygen bubbles, fish are protected from hypoxia and harmful algal blooms. There may be restrictions on the use of large mechanical bubblers in areas where toxic algal blooms are a problem [25].

Ultrasonic treatment has been suggested as a major control measure that can destroy algal cells on a local scale [26]. It is useful to control *M. aeruginosa* blooms due to the chemical and physical effects of sonication [27].

Disadvantages of Physical Control

Large blooms represent a challenge for the majority of physical strategies because they are generally expensive and slow [28]. Physical approaches are mainly utilized as emergency measures for algal blooms rather than as a control strategy, as they are not always practical [29]. The cost and impact of physical measures for reducing dangerous algal blooms are poorly understood due to a lack of field experience [30]. Ultrasound technology is highly unlikely to have any control impact on harmful algal blooms in natural systems, except for extremely high-intensity ultrasound used within an extremely small body of water. Such intense ultrasound has been shown to destroy zooplankton grazers and may have an impact on fish behavior and population [31–33].

2.2. Chemical Control

Recently, there has been much discussion concerning the use of clay additions to suppress hazardous algal species, a strategy that has been utilized successfully in several aquaculture operations worldwide [34]. Clays, the primary component of soil, have many advantages over other materials, including the fact that they are cheap, easy to use in the field, and non-polluting [35]. As a result, it is considered the most promising mitigation strategy against harmful algal blooms [36].

2.2.1. Natural and Modified Clays

A very viable and ecologically sustainable method is to use natural, nontoxic, and inexpensive clays to remove harmful algal blooms [37,38]. The most efficient flocculants have been observed to be clays such as montmorillonite, kaolinite, and phosphatic, with the lowest loading of 0.25 g/L and a removal efficiency of 90% [24,39–42]. The author of [42] stated that 5720 publications on harmful algal blooms (HABs) were examined throughout the course of the previous 30 years. While research publications regarding the use of natural clays to inhibit *M. aeruginosa* growth have been very limited until now. In two papers [16], natural clays were used to manage *M. aeruginosa* in Lake Taihu, China. There are presently few studies on *M. aeruginosa*'s reaction to naturally occurring, typical clay particles like kaolinite and montmorillonite.

Disadvantages of Natural Clays

The clay technique is still hampered by the following issues: Natural clays have low flocculating efficiency, which is the most serious drawback and often leads to the requirement of an exorbitant amount of clays to achieve an effective efficiency in the field [43]. For example, it was noted that the dosage of clay in aquaculture sites in Japan ranged from 110 to 400 t/km^2 [44], and 384 t/km^2 of loess was utilized in Korea to reduce *Cochlodinium polykrikoides* blooming [45]. Algae can be eliminated naturally and non-toxically by clay precipitation and flocculation; however, this process can also bring on new water blooms [46]. The negative charge of natural clays prevents them from being an ideal flocculant. Clays have not yet been given a standard definition. Since antiquity, these materials have been used and studied from a variety of angles, giving rise to various conflicting terminology [16]. Therefore, when using the clay method in the field, the barrier is caused by low removal efficiency, a high dose, and substantial deposition loads on the sediments [47,48]. Unclear mechanisms and a lack of systematic kinetic studies of clay–cell flocculation in fresh and seawaters are other reported issues of natural clays [24].

2.2.2. Modified Clays

Another control method is the application of modified clays to treat *M. aeruginosa* blooms. Currently, common flocculants (such as sediments [49], modified clay [50], iron salts [51], aluminum salts [50], etc.) are frequently employed to control *M. aeruginosa* blooms in freshwaters. Organic polymer chitosan is also combined with soil or other ballast component to treat *M. aeruginosa* blooms [52].

In several works, a number of modified clays have been assayed with *M. aeruginosa*. Two phosphate fixatives i.e., Phoslock®, Europe GmbH (Zug, Switzerland)and Aqual-PTM, (Tokoroa, New Zealand) have been revealed to have marginal impact on *M. aeruginosa* and reduce its growth rates by reducing phosphate [50]. They were used in conjunction with eight other compounds to combat *M. aeruginosa.* Montmorillonite modified lime-ceramic sand-lake sediments [49], hexadecyl trimethyl ammonium bromide (CTAB) modified clays [53], modified attapulgite [54], modified vermiculite [55], amphoteric starch-based bicomponent modified soil [56], cationic starch modified soils etc. are some recent modified clays used to inhibit *M. aeruginosa*. Table 1 shows the removal efficiency of modified clays and their applicability at lab and field scale.

Table 1. Removal efficiency of modified clays and their applicability at lab and field scale.

Name	Species	Dosage	R %	Field app.	Lab. app.	Ref.
poslock® aqual-PTM	*M. aeruginosa* PCC 7820)	0, 50, 100, 300, 600, 1000 mg /L	42.6% 28.4%	✘	✓	[50]
mmt modified lime-ceramic sand-lake sediments	*M. aeruginosa* 469	0.7 g/L	88 %	✘	✓	[57]
ctab	*M. aeruginosa*	0.3 g/L	92%	✘	✓	[53]
modified attapulgite	*M. aeruginosa* (FACHB 905)	0.37 g/L	95%	✘	✓	[54]
SnO_2-montmorillonite	*M. aeruginosa* (FACHB-942)	0.3 g/L	95%	✘	✓	[58]
montmorillonite–Cu (II)/Fe(III) oxides magnetic material	*M. aeruginosa*	1 g/L	92%	✘	✓	[59]
chitosan/montmorillonite nanocomposite	*M. aeruginosa* (FACHB-905)	100–500 mg/L	94.7%	✘	✓	[60]
chitosan modified kaolinite (CMK)	*M. aeruginosa* (NIES-843)	0, 40, 80 and 160 mg/L	NA	✘	✓	[60]

mmt = modified montmorillonite. ctab = hexadecyltrimethylammonium bromide. NA = not available. ✘ = No field application. ✓ = Only lab applicability. R = removal efficiency. Field app = field application. Lab app =lab application. Ref. = references.

Disadvantages of Modified Clays

It was noted that stirring operations might be one of the modified clay's biggest drawbacks in real-world applications [61]. Moreover, they also affect benthic flora and fauna. According to a study by [52], the effect of sediment loading on phytoplankton communities was studied and it was observed that dinoflagellates switched to heterotrophy in numerous degrees, and some dinoflagellates shaped impermanent cysts. In terms of practical applicability, flocs settling on surfaces is another of the biggest drawbacks [55]. According to [43], the majority of research on the eco-environmental effects of clay or modified clay (MC) has focused mostly on describing phenomena and findings, whereas mechanistic analysis of modified clay (MC) effects is rather weak. As a result, mechanistic research that necessitates both more in-depth examinations of mechanisms and theoretical knowledge must be reinforced.

2.2.3. Eco-Friendly Chemicals

Some copper-related compounds, chlorine, and oxidizing agents like H_2O_2 have historically been widely used to control *M. aeruginosa* blooms. They are considered to be relatively safe materials [62]. A novel environmentally safe and selective algaecide called 2-((1,3,4-thiadiazol-2-yl)thio)-N-(4-chlorophenyl) acetamide (Q2) was created to suppress *M. aeruginosa* blooms [10]. It was different from the impact on ecosystem functioning of the traditionally used harmful algaecide diuron. Their findings demonstrated that Q2 might be beneficial to the aquatic environment and offered a novel approach to the management of harmful cyanobacterial blooms (HCBs) in the future. Additionally, chitosan's anionic characteristics have been shown to make it an effective flocculant for removing cyano-HABs from water resources [63]. Ferric or aluminum salts are also extensively studied for controlling cyanobaterial blooms [64]. Some surfactants and engineered nanoparticles like titanium dioxide, silver nanoparticles, zinc oxide, and yttrium(III) oxide are also used to improve algae removal efficiency [65]. The results of recent studies on compounds that are eco friendly, their applicability at lab and field scales, and the factors examined are compiled in Table 2.

Table 2. Recent studies conducted on the application of eco-friendly chemicals to manage *M. aeruginosa* blooms.

Name	Field app.	Lab. app.	Parameters Studied	Ref.
copper ethanolamine complex, $CuSO_4 \cdot 5H_2O$, $CuSO_4$	✘	✓	chlorophyll-*a*, photosystem II efficiency (PSII), soluble reactive phosphorus (SRP) and intracellular and extracellular microcystin (MC) concentrations, total organic carbon content (TOC), membrane integrity	[50,66]
$CoCl_2 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$ and $Na_2EDTA \cdot 2H_2O$ $MnCl_2 \cdot 4H_2O$ $Na_2MoO_4 \cdot 2H_2O$	✘	✓	growth, toxin production, cell morphology, iron accumulation	[67]
benzalkonium chloride (BAC-14)	✘	✓	growth inhibition, photosynthesis endpoints, microcystin, multi-platform metabolomics	[68]
copper sulfate pentahydrate, thanolamine-chelated copper compound	✘	✓	ell density, total microcystins, cell membrane integrity	[69]
copper sulfate	✘	✓	cell counting, Fv: Fm, TTC, SOD, and MDA, microcystin-LR, superoxide dismutase, catalase, and peroxidase, F_v/F_m	[70,71]
copper sulfate	✘	✓	chlorophyll fluorescence value and chlorophyll *a* content, transcriptome analysis,	[60,72]

Table 2. *Cont.*

Name	Field app.	Lab. app.	Parameters Studied	Ref.
H_2O_2	✘	✓	cell density, chlorophyll, phycocyanin, organic matter, true color, intracellular microcystin, geosmin, total pheophytin, ROS, CAT and SOD, chlorophyll a, carotenoid, TDN, TDP, dissolved organic matter, phytoplankton community analysis, cell lysis, caspase-3 activity, terminal deoxynucleotidyl transferase labeling (TUNEL) assay, RNA analysis	[60,73,74]
novel H_2O_2 pre-oxidation	✘	✓	chlorophyll a, turbidity, algal removal efficiency, TOC, TN, TP, cell membrane assay, SOD, CAT, microcystin,	[75,76]
combined process of nanoscale zero-valent iron (NZVI) and H_2O_2	✘	✓	chl *a*, phycocyanobilin (PC), allophycocyanin, phycoerythrin, zeta potential, MDA, SOD, CAT, POD, total organic carbon,	[77]
N-acetyl-5-methoxytryptamine	✘	✓	cell density, chl a, SOD, CAT, MDA, MC-LR, mcyB and mcyD genes	[78]
H_2O_2 and copper sulfate	✘	✓	cell density, MC-LR, inhibition of *Bacillus* sp.	[79]
H_2O_2	✘	✓	effects of EPS on the killing activity of H_2O_2	[59]
$H_2O_2H_2O_2$ under light	✘	✓	cell count, cell integrity, microcystins, chlorophyll	[80–82]
H_2O_2 and ultrasound				
Ozone	✘	✓	algae removal, microcystins, cell morphology, DOC	[63,83–86]
chitosan-modified nanobubbles	✘	✓	cell intact rate, cell lysis rate, cell inactivation rate, OH radical production, ROS, MC-LR, cell density, phosphorus, chlorophyll a, carotenoids, phycocyanin, allophycocyanin, phycoerythrin, total protein content	[56,63,87–90]
Chitosan fiber				
Chitosan				
chitosan quaternary ammonium salt				
chitosan fiber				
chitosan-zinc oxide hydrogel film				
chitosan-aluminum chloride combined coagulants				

✘ = No field application. ✓ = Only lab applicability.

Disadvantages of Eco-Friendly Chemicals

Copper addition to lakes and reservoirs raises concerns about heavy metal accumulation and toxicity [85]. H_2O_2 is widely used in water treatment and in the aquaculture industry [91]. However, different types of water bodies react differently and different amounts of H_2O_2 are required to control cyanoHABs, ranging from 2 to 20 mg L^{-1} [83–86]. Varying background stressors in freshwater ecosystems may interact with H_2O_2, altering its efficacy in controlling cyanoHABs. Co-occurring stressors can have complex impacts on organisms and communities as stressors in combination can either amplify (synergistic) or attenuate (antagonistic) effects [92–94]. The variation of the dose of H_2O_2 required might therefore be linked to the growing number of background stressors faced by aquatic ecosystems. These background stressors include a temperature increase, an elevated level of CO_2, anthropogenic inputs such as pharmaceuticals, personal care products, pesticides, and, relevant to this study, tiny plastic fragments [58]. Chitosan is a non-toxic and biodegradable material, but the acidic condition of a chitosan solution sprayed over a water body for the control of *M. aeruginosa* can negatively influence water quality. Tiny chitosan particles (i.e., chitosan nanoparticles) can also cause physiological stress in aquatic biota [63].

2.3. Biological Control

One alternative approach to the control of algal blooms involves the use of biological control (biocontrol) agents [95]. Biological control includes the use of microorganisms [96], plants [97] and biomanipulation approach [98] to control *M. aeruginosa* blooms. Compounds such as biochar [99] are also in use to control *M. aeruginosa*.

2.3.1. Microorganisms Control

Microorganisms such as viruses, bacteria, actinomycetes, fungi, amoebae, and cyanophages have been shown to kill cyanobacteria.

Bacteria

Among these, antagonistic bacteria have the potential to become useful agents for algal control, as they are simple to culture and manipulate [100]. Bacteria, being one of the most common and varied species in the aquatic environment, form complex ecological interactions with cyanobacteria, including predation, competition, mutualism, commensalism, and amensalism [101]. Those bacteria that display obviously adverse effects on cyanobacterial growth are recognized as cyanobactericidal bacteria [102].

Against pathogenic *M. aeruginosa*, recently discovered algicidal bacteria *A. bestiarum* HYD0802-MK36 and *P. syringae* KACC10292T have been found to be effective [103]. The growth of *M. aeruginosa* can be hampered by a number of bacteria from the genera *Aeromonas* [104] and *Pseudomonas* [105]. These bacteria affect the growth of *M. aeruginosa* by two modes (Figure 2): direct attack and indirect attack [106].

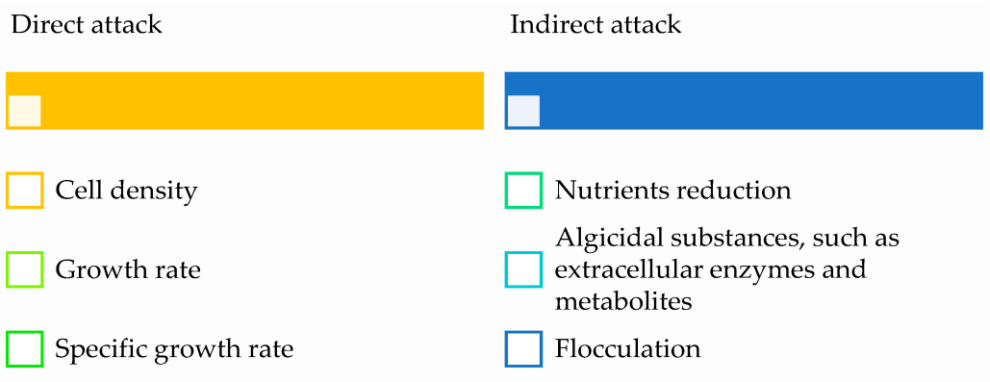

Figure 2. Attacking modes of cyanobactericidal bacteria against *M. aeruginosa*.

Direct attack mode requires physical contact between predatory bacteria and cyanobacteria [107]. The *Bdellovibrio*-like bacteria lysed *M. aeruginosa* by breaking down cell structures after penetrating the host cell [108]. Indirect attack mode occurs when bacteria suppress or kill cyanobacteria without physically contacting them. Indirect assault methods include:

1. Releasing cyanobactericidal substances, such as extracellular enzymes [105]
2. Release of metabolites (e.g., indole, 3-oxo-α-ionone) [109]
3. Deteriorating algal survival environment, e.g., by competing nutrients [110]
4. Flocculating algae cells [111,112]

Cyanobactericidal bacteria affecting *M. aeruginosa* are listed in Table 3, according to the published literature.

Table 3. Recent studies conducted on application of bacteria to manage *M. aeruginosa* blooms, Removal efficiency: RE, References: Ref.

Strain Name	Species	Mode of Action	RE %	Ref.
Bacillus mycoides	*M. aerugi-nosa*PCC7806	shadowing and photo-inhibition	NA	[113]
Brevibacillus laterosporus	*M. aeruginosa* FACHB 905	efflux pump transporters, hydrolytic enzymes, antibiotics, proteases, and other secondary metabolites	92.30%	[114]

Table 3. *Cont.*

Strain Name	Species	Mode of Action	RE %	Ref.
Ochrobactrum sp. FDT5	*M. aeruginosa*	active cellular Components	34–58.6%	[115,116]
Alcaligenes aquatilis F8	*M. aeruginosa* FACHB-905	cell membrane damage, disappearance of photosynthetic lamellae, cyanelles disorder	88.45%	[117]
Bacillus sp. AF-1	*M. aeruginosa* NIES-843, NIES-90, NIES-44	increased intracellular ROS buildup, cell death, and intracellular component efflux	93%	[118]
Arthrobacter sp.443 and UN 383	*M. aeruginosa* BCPUSP232	antimicrobial agents Production	24.87 and 23.85%	[119]
Shewanella maltophilia	*M. aeruginosa* FACHB-905	hexahydropyrrolo [1,2-a] pyrazine-1,4-dione, 2,3-indolinedione Secretion	NA	[120]
Pseudomonas putida	*M. aeruginosa* FACHB 905	extracellular antialgal chemicals are secreted, characterized as anti-heat shock.	98.8%	[121]
Streptomyces globisporus	*M. aeruginosa* NIES-843, NIES-44, NIES-90	cell-to-cell contact	96.7%	[122]
Rhizobium AQ_MP	*M. aeruginosa*		100%	[123]
Alcaligenes Denitrificans	*M. aeruginosa* NIES 298	cell lysis	96.4%	[124]
Streptomyces neyagawaensis	*M. aeruginosa* NIES-298	secretion of extracellular antialgal substances	84.5%	[100]
Xanthobacter autotrophicus HYS0201-SM02 (SM02)	*M. aeruginosa* NIER-100001	algicidal substance secretion	95.6%	[96]
Stenotrophomonas F6	*M. aeruginosa* 9110	excretion of extracellular algicidal compounds (Cyclo-(Gly-Pro)	50%	[17]
Serratia marcescens	*M. aeruginosa* TH1, TH2, and FACHB 905	secretion of a red pigment identified as prodigiosin ($C_{20}H_{25}N_3O$)	87.7%	[125]
Salvia miltiorrhiza	*M. aeruginosa* FACHB-905	neo-przewaquinone *A* oxidative stress, inhibition of three genes involved in photosynthesis (psaB, psbD, and rbcL).	74.08%	[121,126]
Pedobacter sp.	*M. aeruginosa* NIES-843	algicidal activity	50–80%	[125]
Acinetobacter sp. J25	*M. aeruginosa*	lysing and denitrification	100% 87.7%	[127]
Paucibacter aquatile DH15	*M. aeruginosa* KW	oxidative stress, alteration of fatty acid profile, damage to photosynthetic system, carbohydrate, and protein metabolism	94.9%	[128]

Table 3. *Cont.*

Strain Name	Species	Mode of Action	RE %	Ref.
Pseudomonas aeruginosa UCBPP-PA14	*M. aeruginosa* NIES 298,44	lysis and toxin Degradation	92%	[50,129]
Acinetobacter guillouiae A2	*M. aeruginosa* FACHB-905	algicidal compound 4-hydroxyphenethyl-amine secretion	91.6%	[130]
Paucibacter toxinivorans 2C20	*M. aeruginosa*	toxin degradation	90%	[131]
Achromobacter spp.	*M. aeruginosa* CAAT 2005-3	lysis activity	79.5%	[132]
Pseudomonas grimontii	*M. aeruginosa* FACHB-905	oxidative stress	91.81%	[32]
Bdellovibrio species	*M. aeruginosa* Kützing	lysis activity	NA	[108]
Exiguobacterium A27	*M. aeruginosa* PCC7806	production of extracellular algicidal compounds	64.4%	[133]
Bacillus sp. B50	*M. aeruginosa* FACHB905, FACHB1023 PCC 7806, M. NIES-843, CHAB440, CHAB109, CHAB456, CHAB587, CHAB439, CHAB2162, CHAB2170, CHAB724, CHAB4370	algicidal activity	15–71.8%	[134]
Aeromonas bestiarum HYD0802-MK36 and *Pseudomonas syringae* KACC10292T	*M. aeruginosa*	direct attack and cell-to-cell contact	100%	[103]
Raoultella ornithinolytica	*M. aeruginosa* FACHB-905	low-molecular-weight organic acids	96.2%	[75]
Raoultella sp. R11	*M. aeruginosa* FACHB 905	oxidative stress	94.28%.	[127]
Raoultella planticola and *Aeromonas* sp.	*M. aeruginosa* FACHB-905	algae lysis	90%	[135,136]
Halobacillus sp. H9	*M. aeruginosa* PCC7806 and TAIHU98	secretion of active flocculating substance	95%.	[111,112]
Shewanella sp. Lzh-2	*M. aeruginosa* 9110	hexahydropyrrolo [1,2-a] pyrazine-1,4-dione and 2, 3-indolinedione (isatin) secretion	92.3%	[20]
Hahella sp. KA22	*M. aeruginosa* TAIHU98	prodigiosin secretion	71–88%	[118]

Table 3. *Cont.*

Strain Name	Species	Mode of Action	RE %	Ref.
Citrobacter sp. R1	*M. aeruginosa* FACHB-905	glycogen synthase gene glgA	81.6%	[137]
Stenotrophomonas sp. KT48	*M. aeruginosa* PCC7820	oxidative stress	88.47%	[138]
Enterobacter hormaechei F2	*M. aeruginosa* FACHB-315	prodigiosin and PQS Secretion	84.2%	[114,139]
Enterobacter sp. NP23	*M. aeruginosa*	algicidal activity	70 %	[140]
Shigella sp. H3, Alcaligenes sp. H5	*M. aeruginosa*	cells-to-cells direct contact and secretion of algicidal metabolites	96% and 74%	[141]
Aquimarina salinaria sp. Nov	*M. aeruginosa* MTY01	phosphatidylethanol-amine, diphosphatidylglycerol	100%	[142]
Chryseobacterium species	*M. aeruginosa* FACHB 905	algicidal activity	80%	[111]
Chryseobacterium sp. GLY-1106	*M. aeruginosa* 9110.	1106-A (cyclo(4-OH-Pro-Leu)), 1106-B (cyclo(Pro-Leu))	90%	[143]
Aureispira sp. CCB-QB1	*M. aeruginosa* NISE 102 strain	floculation	75.39%	[144]
Streptomyces rameus	*M. aeruginosa* KKU-13	cell lysis	82% to 95%	[145]
Streptomyces aurantiogriseus	*M. aeruginosa* KKU-13	production of metabolites	83.3%	[146]
Streptomyces amritsarensis strain HG-16	*M. aeruginosa* FACHB-905	secretion of active Substances	91.2%.	[147]
Streptomyces jiujiangensis JXJ 0074T	*M. aeruginosa* FACHB-905	antialgal amino acid: L-Valine 2′-deoxyadenosine	80%	[126,148]
Rhodococcus sp. p52	*M. aeruginosa* FACHB927, FACHB 975	trans-3-indoleacrylic acid, DL-pipecolic acid, and L-pyroglutamic acid secretion	93.5%	[149]
Aeromonas veronii	*M. aeruginosa* PCC7806 and MGK	lumichrome production	NA	[150]
Bacillus fusiformis	*M. aeruginosa*	secretion of metabolites	90%	[151]
Bacillus licheniformis Sp34	*M. aeruginosa* DCM3, DCM4	oxidative stress, lipid Peroxidation, DNA damage, and a malfunction in the DNA-repair system	75.6%	[152]
Bacillus methylotrophicus ZJU	*M. aeruginosa*	algicidal effect	89%	[153]
Deinococcus metallilatus MA1002	*M. aeruginosa* PCC7806	deinoxanthin Production	100%	[154]

NA = not available.

(1) Disadvantages of Bacteria

Several cyanobactericidal bacteria or their released chemicals have proven useful in reducing cyanobacterial blooms in the environment. There are many uncertainties when it comes to using cyanobactericidal bacteria or compounds to effectively control or eliminate cyanobacterial blooms in natural waters. Cyanobacteria in natural water tend to have stronger resistance than laboratory culture due to the colonial form of algal cells [155,156] and that the single- or two-celled Microcystis used in cyanobactericidal research is less resilient than the colon [157]. The financial expenses of using cyanobactericidal bacteria or allelopathic chemicals for bloom-control on a wide scale should be a concern. Simply put, the size of their contribution must be sufficient to overcome the relevant cutoff. If the channels for agent generation (i.e., bacterial culture) are not cost-effective, the application will be constrained. Before using cyanobactericidal bacterial agents, it is important to weigh the potential consequences for the environment [152].

The use of the cyanobactericidal bacterium *Lysobacter* enzymogenes subsp. enzymogenes AL-1 to eradicate *M. aeruginosa* in a microcosm was determined to be of high ecological concern [158]. A considerable decrease in ciliates, flagellates, and fungi was seen as a result of the use of cyanobactericidal bacteria. For example, using L-lysine to reduce Microcystis blooms led to blooms of *Euglena* sp. and *Phormidium* sp. in ponds, [159], suggesting that the removal of certain cyanobacterial blooms with these agents can generate other unforeseen algal bloom problems. Therefore, in order to achieve an algicidal effect on target cyanobacteria, the use of cyanobactericidal bacteria and substances in the control of cyanobacterial blooms in natural waters must overcome biological and abiotic uncertainties. The widespread use of cyanobactericidal microorganisms or chemicals in aquatic ecosystems requires first conducting biosafety studies [135,136].

Fungi

Degradation by fungal strains and the elimination of cyanobacterial cells are mainly unexplored [160]. There are just 15 known fungus species that may inhibit and lyse cyanobacterial cells. There are several structural similarities between the two fungus groups to which these species belong: Ascomycetes (nine of the species) and Basidiomycetes (six of the species). In addition, it has been claimed that fungi play a significant role in water treatment, with strong data showing that microcystins (MC) breakdown by fungal stains is faster [155] than that occurring by bacterial strains [156,157]. Some fungi (such as *Aurobasidium* pullulans and *Trichoderma citrinoviride*) have been shown to restrict the development of cyanobacteria while leaving the growth of more beneficial algae alone [155,161]. As an interesting side note, certain fungal strains (*Trichaptum abietinum, Trichoderma citrinoviride*) showed a dual-functional feature, efficiently lysing cyanobacteria and decomposing MCs produced by the decaying cells [162]. In Table 4, we have a brief overview of several recently discovered algicidal fungi that are effective against *Microcystis aeruginosa*.

Table 4. Summary of some recent algicidal fungi active against *Microcystis aeruginosa*.

Strain Name	Species	Mode of Action	RE %eff.	Ref.
Aureobasidium pullulans KKUY070	*M. aeruginosa* DRCK1	N-β-acetylglucos-aminidase.	100%	[155]
Bjerkandera adusta T1	*M. aeruginosa* PCC7806	Protease, polysaccharide lyases8 (PL8)	98.27%	[163]
Irpex lacteus T2b	*M. aeruginosa* PCC7806	Cell-to-cell contact	99.1%	[164]
Lopharia spadicea	*M. aeruginosa* FACH-918	Oxidative stress	100%	[165]

Table 4. *Cont.*

Strain Name	Species	Mode of Action	RE %eff.	Ref.
Phanerochaete chrysosporium	*M. aeruginosa*	Release of fungal metabolites	88.6%	[160]
Trametes versicolor F21a	*M. aeruginosa* PCC7806	Cellulase, β-glucanase, trypsase, and pepsin	85%	[166]
Trichaptum abietinum 1302BG	*M. aeruginosa* FACH-918	Cell-to-cell contact and lytic enzymes release	100%	[167]
Trichoderma citrinoviride kkuf-0955	*M. aeruginosa*	Excretion of algicidal compounds	100% removal	[161]
Aspergillus niger 7806F3	*M. aeruginosa* 7820, 7806, 1752	Indirect attack	80%	[75]
Penicillium chrysogenum	*Microcystis aeruginosa*	Secreting extracellular substances	69.56%	[168]
Aureobasidium pullulans strain KKUY0701	*M. aeruginosa* DRCK1	Cell lysis	84%	[155]

(1) Disadvantages of Fungi

Although fungi have been infrequently reported as potential biological controllers, there are parasitic associations between freshwater microalgae and Chytridiomycetes fungi [169,170] and with biflagellate fungi belonging to Oomycetes. However, this latter association occurs to a lesser extent [171]. This fungal infection in planktonic diatoms has been associated with mortality of host organisms, suppression or retardation of phytoplankton blooms, and changes in the size, distribution, and composition of planktonic populations and communities [172–175]. Moreover, there is scarce information concerning the relationship between freshwater toxic microalgae and pathogenic fungi [16].

Virus

Viral treatment may be one of the important factors that can control HABs [176]. The virus typically uses species-specific interaction [177], the bursting of cells, and the viral lytic cycle. Viral degradation has the advantage of a species-specific attack [178]. Cyanophage (Ma-LMM01) specifically infects a toxic strain of the bloom-forming cyanobacterium *Microcystis aeruginosa* [179,180]. Cyanophage infection may have a significant impact on the succession of cyanobacteria in the pond. Cyanophage from *Myoviridae* family isolated from Chinese freshwater; GenBank, accession number KF356199.1., named MaMV-DC, was thought to have a half-life of between 24 and 48 h and 80 infectious units per cell (*Microcystic aeruginosa* FACHB-524). Cyanophages are found to be effective biocontrol agents of *M. aeruginosa*. [181–183].

A novel, wide-ranging freshwater cyanophage called MinS1has the ability to infect multiple different cyanobacterial orders and could be used as a biological control measure against cyanobacterial blooms [184]. In host–range experiments, a novel freshwater cyanophage called Mae-Yong1326-1 was effective in lysing *M. aeruginosa* FACHB-1326 [181].

(1) Disadvantages of Virus

Many biological phenomena related to viruses are poorly understood because of host specificity and seasonal issues. For example, several reports suggested that algal viruses often existed in stable numbers, even when their hosts were absent [185]. Reports claimed that the summer and spring seasons are showing the highest decay rates of cultivated viruses after four seasons of analysis [186]. The seasonal study found that the low decay rates of the algal virus during the winter allowed for the survival of about 126 days under

the ice cover in a frozen freshwater pond [182]. Another thing is that these agents show high specificity and efficiency, but they have the limitation of high cost and require upscaled level experiment confirmation [183].

Phytoplankton and Zooplankton

Recent efforts to control toxic algal blooms have concentrated on isolating anti-algal active compounds from micro- and macro-algae [187]. Marine macro-algae (seaweed) extraction has yielded many compounds with the potential to inhibit many other micro-algae, including red tide dinoflagellates [188]. Micro- and macroalgae are reported as effective biocontrol agents, but these studies are mainly focused on the removal of red tides [65]. Research studies regarding the use of algae against *M. aeruginosa* removal are limited.

Zooplankton are able to limit the dominance of cyanobacteria in aquatic ecosystems up to a certain density [189]. The majority of the data on the function of MC comes from research with generalist grazers (*Daphnia*), which only occasionally coexist with cyanobacterial blooms [190,191]. *M. aeruginosa* physiological and metabolic changes are affected by *Daphnia magna* exudates [192]. However, another investigation using copepods suggested that MC may serve as a warning to avoid *Microcystis* [193].

(1) Disadvantages of Phytoplankton and Zooplankton

Mechanisms of cyanobacterial metabolites and their impacts in the presence of grazers are scarce [194]. While some studies demonstrate that the production of MC is induced by zooplankton [195], other studies contend that the zooplankton may even impede the formation of MC [196]. Although it has been proposed that zooplankton can affect the dynamics of cyanobacterial metabolites, their chemical structures are unknown since detection and isolation of these organisms are difficult [197]. Limitations on the predator's potential usage outside the lab result from logistical challenges in applying the predator and growing the culture to produce enough zooplankton predators [65].

2.3.2. Fish

Due to some fish's ability to consume and digest the poison directly, fish species have always employed this technique for bloom clearance. To regulate HABs for the lake ecology, bio-manipulation is a viable method [198]. There have been numerous attempts to reduce cyanobacterial blooms in China and other regions by utilizing filter-feeding fish such as bighead carp and silver carp, which have occasionally proved successful [57,199,200].

Field trials were conducted to eliminate *Microcystis* blooms by stocking tilapia in Lake Yuehu and other eutrophic lakes in Ningbo, China, between 2000 and 2003, as well as feeding studies to evaluate tilapia's consumption and digestion of *M. aeruginosa* in a lab setting [201]. *Microcystis* could be consumed and digested in significant amounts by tilapia. At a water temperature of 25 °C, the digestion efficiency ranged from 58.6% to 78.1%. Salazar Torres [202] study provides evidence of reducing cyanobaterial biomass almost 60% in the presence of *Oreochromis niloticus* in eutrophic reservoirs. Hybrids of silver carp *Hypophthalmichthys molitrix* and *bighead carp H. nobilis* have been reported to alter phytoplankton species composition [203].

Disadvantages of Fish

Animal growth may be slowed down or impeded because of the potential for poor digestion of *Microcystis* species and potential low/imbalanced nutritional values. The digestive proteases trypsin and/or chymotrypsin can be inhibited by the protease inhibitors produced by *Microcystis* spp., such as aeruginosins, cyanopeptolins, micropeptins, microviridins, and microcins [204]. The reallocation of energy to the detoxification of MCs and other cyanotoxin also resulted in reduced animal growth. This strategy for managing algal blooms is not highly suggested due to the difficulties (Figure 3) linked with health hazards for animals from the digestion of *Microcystis aeruginosa* and the reported enormous mortality of fish species [32].

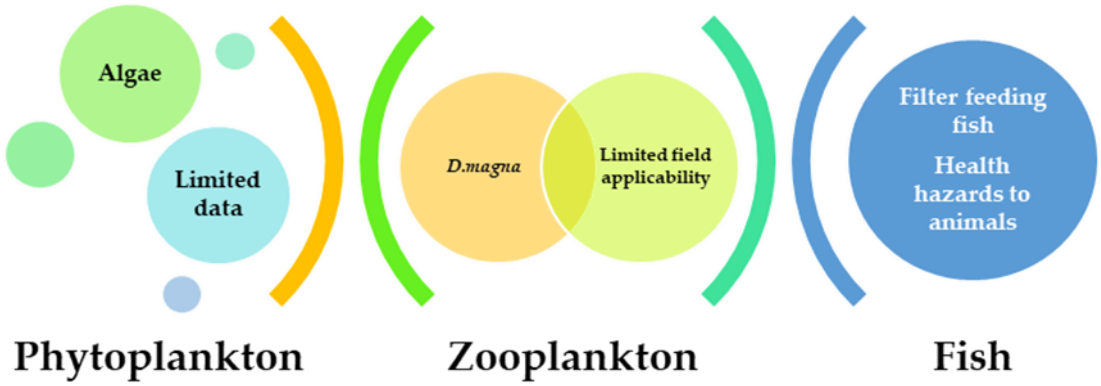

Figure 3. Major issues limiting the application of phytoplankton, zooplankton, and fish species to control *M. aeruginosa*.

2.3.3. Plants

Allelopathic application is a promising strategy to control HABs. As a method inspired by natural phenomena, the effectiveness of allelochemicals in inhibiting microalgae cells has been discovered and confirmed for many years [205]. Both planting macrophytes [142] and adding extracted allelochemicals [97] were effective for introducing inhibition effects on microalgae cells. Four main categories of allelochemicals, including polyphenolics [206], N-containing compounds [207], fatty acids/eaters [208], terpenoids [209], and their derivatives, were proved to be efficient in *M. aeruginosa*-inhibiting capacities, respectively. Figure 4 shows an overview of allelochemicals and their effects on algae.

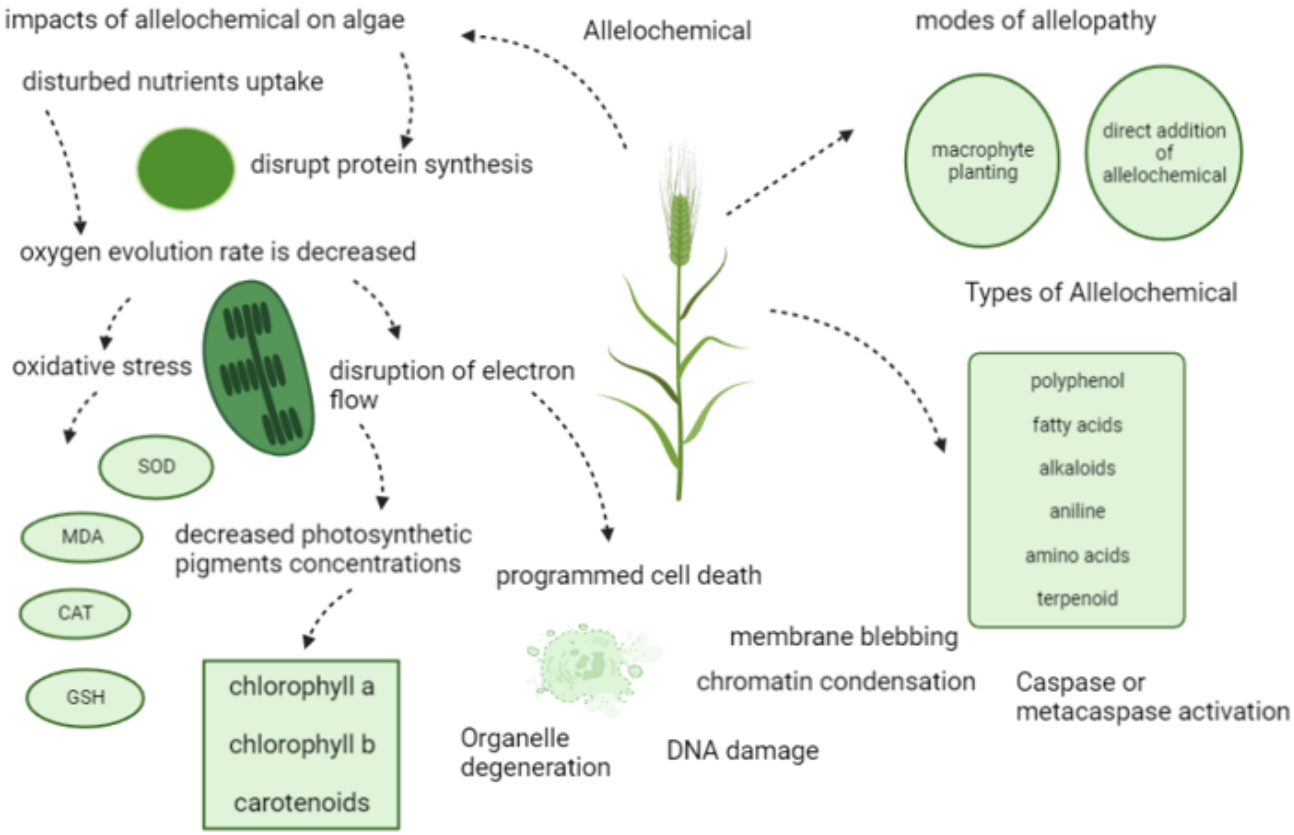

Figure 4. An illustration of allelochemicals and their mode of action.

The sensitivities of microalgal species upon allelochemicals were significantly different and *M. aeruginosa* was widely confirmed as the most sensitive microalgal species to allelochemicals [210].

Allelochemicals induced damages on multiple levels of microalgal cells, including interfering the photosynthesis, generating oxidative stress, triggering programmed cell death (PCD), and disturbing other physiological and biochemical processes (Figure 5) [211].

Figure 5. Plants and their released allelochemicals effective against *M. aeruginosa*.

Disadvantages of Plants

Though many plant derived allelochemicals have been screened, few are feasible candidates for application in field environments. Few reasons discourage the application of plant-derived allelochemicals in field environments (Figure 6).

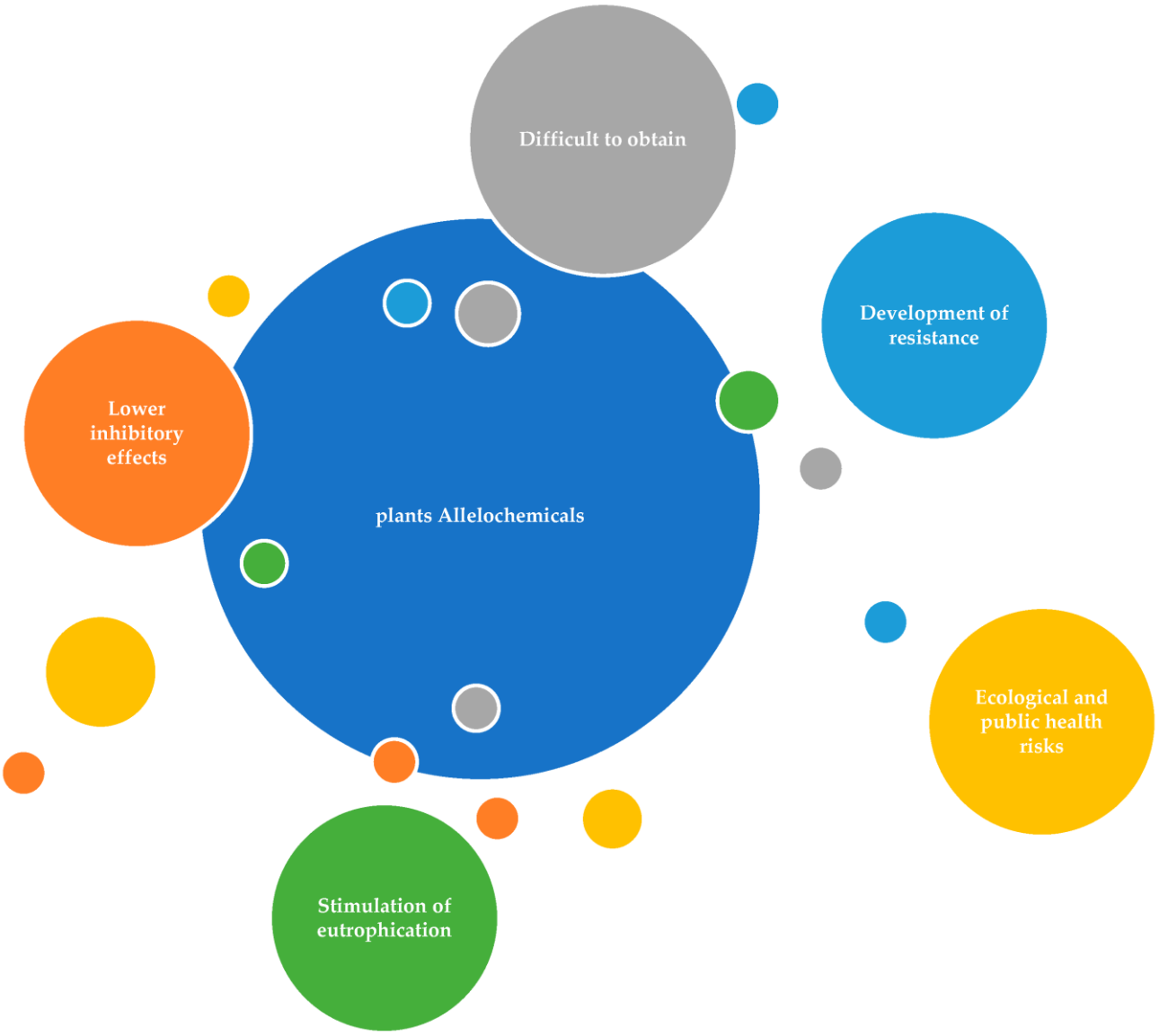

Figure 6. Problems associated with the application of allelochemicals.

Some allelochemicals show only weak inhibitory effects on cyanobacteria. For example, bionone is an antialgal compound that inhibits *Microcystis*, but the EC50 is 22 mg/L [212]. Cyanobacteria can adapt to the inhibitory effect of some biologically derived substances (BDS) and become resistant to them. Nonanoic acid was reported as an allelochemical showing a strong inhibitory effect on *M. aeruginosa*, with a median effective concentration (EC50) as low as 0.5 mg/L [208]; however, a following study indicated that, under the stress of nonanoic acid, cells of *M. aeruginosa* soon adapt to this environment [213]. Some allelochemicals are difficult to obtain. Even though some natural antialgal chemicals strongly inhibit cyanobacteria, the supply of these biologically derived chemicals is limited, and the structures of those antialgal chemicals are very complex, so their chemical synthesis is difficult or prohibitively expensive. For example, Tellimagrandin II originating from *M. spicatum* shows a strong inhibitory effect on Anabaena [171], but the content of Tellimagrandin

II in *M. spicatum* is very low, and the structure of this chemical is too complex for facile synthesis by chemical engineers. In this way, the extract of plants, such as barley straw, may be more applicable in algae control since it is cheaper and easier to obtain. Beside the potential damage of allelochemicals to nontarget aquatic organisms, the health risks of allelochemicals to humans are also not known. Allelochemicals such as lysine, rice hull, and wheat bran leachate include N and/or P, which may increase bioavailable N and/or P in waters where they are applied, thereby exacerbating eutrophication [214].

3. Summary of Limitations of Green Technologies

This review thoroughly explained current and previous green technologies, their efficiency and problems associated with the success rate of all of these applications which have been applied over the years to mitigate *M. aeruginosa*. We noticed the following crucial aspects (Figure 7).

1. Physical methods are preferred to chemical methods, but they are expensive and are not easy to adapt in field conditions.
2. Chemical methods are efficient in *M. aeruginosa* removal, but they are a source of secondary pollution.
3. For the mitigation of *M. aeruginosa*, many biological control agents existed that includes bacteria, fungi, phages, zooplankton, plants, fish, etc. Many reports of laboratory success have been reported, but when it comes to field management, the success rate appears quite low.

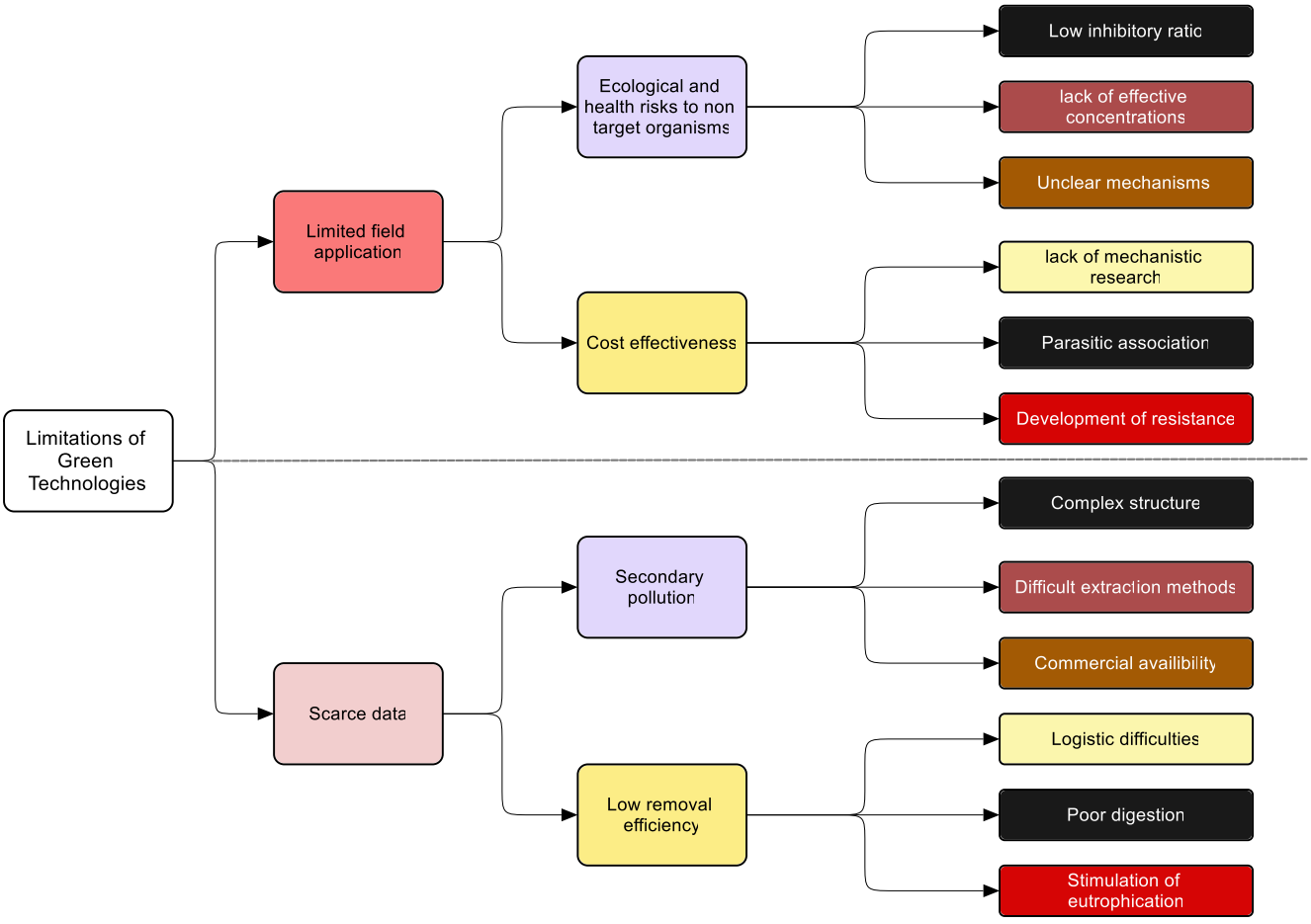

Figure 7. Summary of limitations of green technologies.

4. Conclusions and Future Solutions

Regular *M. aeruginosa* outbreaks are harmful to aquatic ecosystems and constitute a serious risk to the public's health. Research on *M. aeruginosa* removal is mainly focused on physical, chemical, or biological techniques. Each of these techniques has the potential to be effective in removing algae, there are certain drawbacks, and several techniques differ significantly from one another. It is important to do in-depth study on the integration of various approaches when assessing the social, economic, and environmental benefits as well as other comprehensive variables. Physical methods, i.e., harvesting of *M. aeruginosa*, air flotation, magnetic flocculation, hydrodynamic cavitation, light shading, dredging sediments, ultrasound technology, and flocculants, have promising applications. Physical method application has two main problems: they are expensive and difficult to implement at larger scale. Compared to physical control, chemical control—which includes natural, modified clays, and eco-friendly chemicals—is heavily debated.

However, there are conflicting results regarding the application of clay and the induction of physiological stress in aquatic biota by eco-friendly chemicals, which restricts the use of this approach. As an alternative to physical and chemical control, biological control focuses on the utilization of biological agents, such as bacteria, fungi, viruses, plants, etc., their released products (plants + allelochemicals), and biomanipulation (fish) techniques. Among biological agents, bacterial species have been frequently reported as potential biological control agents. Parasitic association of fungi, poorly understood mechanisms of viruses, zooplankton, phytoplankton, and poor digestion of *M. aeruginosa* still raise many questions about biological control techniques. In conclusion, we think that some effective, affordable, and environmentally friendly new algae removal methods and their combination processes are the future development direction.

Based on this narrative review, the following are some of the proposed solutions to get rid of *M. aeruginosa* blooms and combat currently existing problems with green technologies.

1. There is a need to further explore the use of natural clays because of their abundance, cost effectiveness, and easy application. The only problem which has been mentioned in literature is their lower removal efficiency, which has been dealt by using modified clay but still data regarding the application of natural clays on *M. aeruginosa* is scarce.
2. Combined application of ecofriendly chemicals and biological agents should be studied to evaluate their efficiency in *M. aeruginosa* blooms removal.
3. Effect of physical, ecofriendly chemicals and biological agents on nutrient concentrations is also required to understand control mechanism deeply
4. Further research is required regarding the effects of all these green technologies, i.e., physical, ecofriendly chemical, and biological methods on non-target organisms.

Keeping in mind all above proposed solutions, more research is needed to fully implement any of these methods in the field for the achievement of a sustainable environment.

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