NRC Publications Archive
Archives des publications du CNRC

Metagenomic study to evaluate functional capacity of a cyanobacterial bloom during oxidation

Moradinejad, Saber; Trigui, Hana; Maldonado, Juan Francisco Guerra; Shapiro, B. Jesse; Terrat, Yves; Sauvé, Sébastien; Fortin, Nathalie; Zamyadi, Arash; Dorner, Sarah; Prévost, Michèle

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.
For the publisher's version, please access the DOI link below./ Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

Publisher's version / Version de l'éditeur:
https://doi.org/10.1016/j.ceja.2021.100151
Chemical Engineering Journal Advances, 8, C, 2021-07-10

NRC Publications Archive Record / Notice des Archives des publications du CNRC :
https://nrc-publications.canada.ca/eng/view/object/?id=4f8d48ff-8906-4246-81d0-b517d26bc9aa
https://publications-cnrc.canada.ca/fra/voir/objet/?id=4f8d48ff-8906-4246-81d0-b517d26bc9aa

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at
https://nrc-publications.canada.ca/eng/copyright
READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site
https://publications-cnrc.canada.ca/fra/droits
LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at
PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

Chemical Engineering Journal Advances 8 (2021) 100151

Contents lists available at ScienceDirect

Chemical Engineering Journal Advances

journal homepage: www.sciencedirect.com/journal/chemical-engineering-journal-advances

Metagenomic study to evaluate functional capacity of a cyanobacterial bloom during oxidation

Saber Moradinejad [a,*], Hana Trigui [a], Juan Francisco Guerra Maldonado [a], B. Jesse Shapiro [b,c,d], Yves Terrat [b], Sébastien Sauvé [e], Nathalie Fortin [f], Arash Zamyadi [g,8], Sarah Dorner [a], Michèle Prévost [a]

[a] NSERC Industrial Chair on Drinking Water, Department of Civil, Geological, and Mining Engineering, Polytechnique Montréal, Montréal, Québec H3T 1J4, Canada
[b] Department of biological science, Université de Montréal, Montréal, Québec, H2V 0B3, Canada
[c] Department of Microbiology and Immunology, McGill University, Canada
[d] McGill Genome Centre, McGill University, Canada
[e] Département de Chimie, Université de Montréal, Montréal, QC, Canada
[f] National Research Council Canada, Energy, Mining and Environment, 6100 Royalmount Avenue, Montreal, QC H4P 2R2, Canada
[g] Water Research Australia (WaterRA) Melbourne based position hosted by Melbourne Water, 990 La Trobe St, Docklands VIC 3008, Australia
[8] Population Health and Immunity Division, Walter and Eliza Hall Institute of Medical Research (WEHI), 1G, Royal Parade, Parkville VIC 3052, Australia

ARTICLE INFO

Keywords:
Cyanobacteria
Oxidation
Function
Biomarker
Stress

ABSTRACT

Pre-oxidation can be used against cyanobacteria at the water treatment plant intake to improve cell removal efficiency in down flow processes and reduce cyanotoxins concentrations. In this study, shotgun metagenomic sequencing was used to describe the functional capacity of a cyanobacterial bloom (at Lake Champlain, southern Quebec, Canada) before and after pre-oxidation using Cl_2, $KMnO_4$ and H_2O_2. The bloom samples were associated with two functional profile assemblages: that of August 1st (onset of the bloom) characterized by enrichment of genes related to nutrient uptake and that of August 13th-29th (towards the end of the sampling) associated with competition for resources and repair such as Photosynthesis, Protein metabolism and DNA metabolism. Different functional profile responses to oxidation with Cl_2, $KMnO_4$ and H_2O_2 was also identified as two-time points during the bloom (at the August 1st, and August 29th). On August 1st, chlorinated samples showed a progressive shift in functional profile: starting by acquiring and sequestering nutrient sources (e.g. Iron acquisition, carbohydrates) at low chlorine exposure (CT, concentration X contact time) level, followed by showing a stronger tendency toward dormancy and sporulation genes at high CT. Our results showed that following high CT of H_2O_2, the relative abundance of the cyanobacterial biomarkers decreased, regardless of the dominant cyanobacterial genus. The toxicity of the bloom before and after oxidation samples was assessed by droplet digital PCR (ddPCR) to measure the *mcyD* gene. Our results showed significant positive correlation between the *mcyD* gene copies number and microcystin concentrations in the bloom samples (before the oxidation). However, such correlation was not observed after oxidation. These results suggest that ddPCR can only be used to evaluate bloom toxicity before oxidation.

1. Introduction

Cyanobacterial blooms and water bodies eutrophication are happening more frequently due to increasing temperatures (climate change) and human activities (nutrient management in the watershed) [1, 2]. Over 40 toxic cyanobacterial species are known to produce five major groups of cyanotoxins (microcystin, anatoxin, saxitoxin, cylindrospermopsin and β-Methylamino-L-alanine) [3-5]. Cyanotoxins bioaccumulate in fish and shellfish and are poisonous to nearly all livestock, wildlife and humans [6, 7]. Cyanobacteria metabolites can also lead to taste and odor (T&O) problems [8-10]. The most commonly detected T&O compounds are geosmin and 2-methylisoborneol (MIB).

Cyanobacterial cells and their metabolites (cyanotoxins-T&O) pose a challenge to the drinking water treatment processes [10-12]. Cyanobacteria entering and accumulating within the plant can be dampened using pre-oxidation. Pre-oxidation has been proposed as a tool to

---

* Corresponding author.
E-mail address: saber.moradinejad@polymtl.ca (S. Moradinejad).

https://doi.org/10.1016/j.ceja.2021.100151
Received 4 June 2021; Received in revised form 22 June 2021; Accepted 8 July 2021

prevent the accumulation of cyanobacteria and cell-bound toxin in sludge using ozone [13, 14], H_2O_2 [15, 16], $KMnO_4$ [17-19] and Cl_2 [8, 18, 20]. The oxidation efficiency to degrade cyanobacterial cells and their associated harmful metabolites may vary according to the presence of different bacterial communities, growth phase, cell agglomeration and background water quality parameters such as pH and dissolved organic matter [21-24]. Considering cyanobacterial blooms' complex dynamics, the improvement of oxidation efficiency requires an understanding of the cyanobacterial structural and functional profile in response to oxidation processes. The fate of bacterial communities associated with cyanobacterial bloom have been studied using molecular tools such as high throughput sequencing [25-31] 16S rRNA [31-35] and polymerase chain reaction (PCR) [26, 31, 35-38].

The analysis of functional structure of bacterial communities using high throughput metagenomic shotgun sequencing can provide insights on how a community may respond and adapt to the imposed stress (chemical oxidation). Gomez-Smith et al. (2017) [39] highlighted the importance of microbial functions (e.g. nitrification) in drinking water treatment. For the cyanobacterial community, several studies have focused on succession (for nutrient up take), co-presence of nitrogen fixation, inorganic phosphorous scavenging, and toxin-producing strains in cyanobacterial blooms [40]. Limited studies have focused on the functional structure of the bacterial community and metabolic functions such as phosphorous and nitrogen metabolisms, cell division and cell cycle [41-44]. A high relative abundance of nitrite/nitrate reductase was identified in an early summer bloom dominated by *Aphanizomenon/Dolichospermum* in lake Utah [42]. At the same time, severe nutrient starvation was observed for phosphorus and carbohydrate metabolisms [42]. Shi et al. (2017) studied the functional structure of a cyanobacterial bloom (Lake Taihu) during a 95-day microcosm experiment. Results showed an increase in nitrogen, amino acid-related, pyruvate and methane metabolisms during lysis of the cyanobacterial community [45]. The microbial functional composition has been studied in stratified drinking water reservoir [46], ice-covered lakes [47] sewage sludge [48] and a river [49]. Shilei et al. (2020) [46] reported that dissolved oxygen, pH, temperature, nitrate, ammonia, total phosphorous, and chlorophyll-a were the critical factors in the drinking water reservoir's structural and functional composition. Shilei et al. (2020) [46] observed that metabolic functions such as cell growth and death, energy metabolism and environmental adaptation were enriched in the reservoir's mixing period compared to the stratified condition [46]. Also, results from Shilei et al. (2020) [46] suggested that total dissolved phosphorous, $NH4^+$, pH, dissolved oxygen, and temperature have an impact on the functional bacterial profile of a stratified reservoir. Besides the functional capacity, cyanobacterial bloom toxicity can be evaluated using a targeted PCR to quantify toxin-producing genes in cyanobacterial bloom. Microcystin (MC) producing cyanobacteria carries the *mcy* genes. Ten *mcy* (A-J) genes were identified as MC synthesis genes [50]. Previous studies have suggested that the monitoring of *mcyD* gene could be an appropriate approach to predict toxic cyanobacterial bloom in water resources [37, 51, 52].

To our knowledge, no study has focused on the functional capacity of the cyanobacterial bloom following drinking water treatment processes such as chemical oxidation. Understanding the functional profile of cyanobacterial bloom before and after oxidation provide an insight into the mechanism of oxidation and a stronger basis to predict treatment efficacy. The objectives of this study were to 1) determine the functional capacity of bacterial communities associated with a cyanobacterial bloom before and after oxidation (Cl_2, $KMnO_4$, H_2O_2) using high throughput metagenomic shotgun sequencing, and 2) quantify *mcyD* gene copies number using ddPCR and investigate ddPCR capability to evaluate cyanobacterial bloom toxicity (microsystin total) before and oxidation.

2. Material and methods

2.1. Sampling site description

Cyanobacterial bloom samples were collected over the intake (surface) of the Bedford drinking water production plant (Missisquoi Bay, Lake Champlain) in southern Quebec, Canada. Samples were collected on August 1st, 13th, 15th, 21st, 29th of 2018. Cyanobacterial blooms have been previously reported from this site [11, 21, 53, 54]. The bloom started in late July and lasted until mid-September based on the real-time PC probes installed on-site (data not shown). Dominant species transitions were previously reported during cyanobacterial bloom monitoring in Lake Champlain [53]. Samples were collected in brown PETG bottles and transferred to the laboratory. The oxidation assays were conducted on the same day of sampling to decrease the impact of biodegradation.

2.2. Chemicals and reagents

The oxidation assays were performed using chlorine (0.2 and 0.6 mg/L), permanganate (5 mg/L), and hydrogen peroxide (10 mg/L). Detailed protocols to prepare the stock solutions and quenching reagents can be found in [55]. The selection of doses (concentration) and contact time was based on the common pre-oxidation doses and contact time used in the operation of the drinking water treatment plant.

Oxidant exposures (i.e. concentration(C) X contact time (T) (CT)) were calculated using Equation 1:

$$CT = \int_0^t [Oxidant]dt = \frac{C_0}{k_{decay}}\left(e^{k_{decay}t} - 1\right) \tag{1}$$

Where k_{decay} (min^{-1}) is the first-order decay rate of the oxidant, t (min) is the contact time, and C_0 (mg/L) is the initial concentration of oxidant at time zero.

The oxidation assays were conducted separately but at the same day (same cyanobacterial bloom for each day) for two different dates (August 1st and August 29th).

2.3. DNA extraction, metagenomic libraries preparation, bioinformatics and statistical analysis

Nucleic acid (DNA and RNA) was extracted from the filters using RNeasy power kits (Qiagen Group, Germantwon, MD, USA). 200 μL of nuclease-free water and 5μL of TATAA Universal DNA spike II (TATAA Biocenter AB) were added to evaluate extraction yields using real time qPCR. More details in DNA extraction and metagenomics sequencing procedure are presented in Moradinejad et al. (2020) [55]. Cyanobacterial bloom samples (before oxidation for all five days) and after oxidation samples for two dates (August 1st and August 29th) were sequenced and presented in this study. Variations in species community and diversity were evaluated with heat trees using the Metacoder (0.3.3) [56]. The function of protein fragments were identified by retrieving the best hits through SEED Subsystems [57], KEGG [58] and COG [59] databases. The Pearson correlation coefficient for binary data was calculated to measure the association within taxa and between taxa-and-functional reads, according to Pearson [60] and Janson and Vegelius [61]. Pearson correlation coefficients (P-value $<$0.01) were calculated in R to determine the correlation of microbial taxa (phylum level) with the main functional subsystems (level 1) before and after chlorination. The relative abundances of both taxonomic profile functional capacities were normalized before the correlation analysis. Multiple zeros were treated by adding small constant or pseudo-count to all the elements [62]. Detailed methodology for statistical analysis and permutation test is presented in Moradinejad et al. (2020) [55].

2.4. ddPCR

Detection primers of the *mcyD* gene involved in microcystin production were as follows: *mcyD*(KS)F1: 5′-TGGGGATGGACTCTCTCACTTC-3′ and *mcyD*(KS)R1: 5′ GGCTTCAACATTCGGAAAACG-3′ [37]. Equal concentration of DNA 1.0 ng/μL were used for ddPCR analysis. The PCR mixture consisted of 12. 5 μL Bio-Rad 2x QX200 ddPCR EvaGreen^R Supermix (SM) (BioRad Laboratories Ltd, Mississauga, ON), *mcyD* (KS) primers at a final concentration of 0.1 μM, 0.2 mg/mL BSA and genomic DNA input concentrations ranging from 1 to 5 ng DNA per 25μL ddPCR reaction. Sample mixtures were vortexed gently avoiding the formation of bubbles, centrifuged for 20 s, then kept on ice until droplet generation. Samples were packaged into droplets by adding 20 μL of the PCR mixture in each sample well of the single-use DG8 cartridge followed by addition of 70 μL of droplet generation oil for EvaGreen^R to each of the corresponding oil wells. The cartridge was then placed into the QX200 droplet generator for droplet production. Forty microliters of generated droplets were transferred from the cartridge to a semi-skirted ddPCR 96-well plate (Bio-Rad). Samples were successively prepared in cartridges by groups of eight, transferred to the PCR plate and subsequently heat sealed with a pierceable foil seal. The plate was transferred to a thermal cycler and reactions were run under the following standard cycling conditions: 95 °C for 10 min followed by 40 cycles of 94 °C for 30 s; 55 °C for 60 s, 98 °C for 10 min, and 4 °C hold; ramp rate = 50% (2 °C/*sec*). Upon completion of the PCR phase, plates were loaded onto the QX200 Droplet Digital reader, which automatically reads the droplets from each well of the plate (17 000 droplets/well). Data analysis was performed using QuantasoftTM software (Bio-Rad). Negative droplets, lacking target and/or reference gene DNA, and positive droplets, containing either or both DNAs, were counted to give the fraction of positive droplets. Using Poisson statistics, the concentrations of both DNA were determined, and copies/ng calculated.

2.5. Microcystin analysis

Microcystin concentrations (intracellular and extracellular) were measured using an on-line solid-phase extraction ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (On-line SPE-UHPLC-MS/MS, Thermo TSQ Quantiva). Microcystin measurements were performed on a Thermo Hypersil Gold C18 column (100 mm x 2.1 mm, 1.9 μm particle size). Further details on the cyanotoxin measurement methods can be found in Munoz, et al. [63] and Roy-Lachapelle, et al. [64].

2.6. Analysis of RNA integrity following exposure to oxidants

The impact of oxidants on the integrity of nucleic acids was analyzed by measuring total RNA (cultured cyanobacteria) after exposure to oxidants. *Microcystis Aeruginosa* strain CPCC 300 and *Dolichospermum* strain CPCC 544 were cultured in BG-11 medium at 21°C under 12-hr rotating light-darkness at an intensity of 70 μmol $S^{-1} m^{-2}$. Cultures were harvested at stationary phase and spiked into intake water from the Saint Jean Sur Richelieu (Quebec, Canada) water treatment plant (6–7 mg/L of dissolved organic carbon (DOC) and pH of 7 – 7.5). A mix of 200,000 cell/mL of *Microcystis* (50%) and *Dolichospermum* (50%) was used. Cl_2 was added at 3 mg/L for contact times of 2 min, 5 min, 30 min, 1 h, 2 h) and KMnO4 at 10 mg/L for contact times of 30 min, 1 h, 2 h, 4 h. The RNA integrity was a separate test conducted to evaluate the susceptibility of cell components such RNA to oxidation. Therefore, higher concentration of oxidant (compare to natural bloom oxidation) was used, and different sampling times covered the broader exposure to see the complete view of cell component degradation.

RNA was extracted by the RNeasy PowerWater Kit (Qiagen). DNA was removed using the Turbo DNA free Kit from Ambion (ThermoFisher, Saint-Laurent, QC). 1% Formaldehyde-agarose gel was used to evaluate total RNA profiles. Thirty μl of total RNA were run in each lane. RiboRuler High Range RNA Ladder (ThermoFisher, Saint-Laurent, QC) was run in parallel with the samples to determine the size of the RNA bands. RNA profiles showed the 23S, 16S and 5S ribosomal RNA corresponding respectively, to 2900, 1500 and 120 base pairs (bp). Detailed oxidation experimental design is presented in supplementary information (Table S1).

3. Results and discussion

3.1. Bacterial functional profile of the cyanobacterial bloom metagenome

Cyanobacterial bloom samples were collected at the intake of water treatment plant from the Lake Champlain. Cyanobacterial taxonomic cell count results showed maximum 3.3 × 10^3 cells/mL on August 1st and minimum 5.4 × 10^4 cells/mL on August 29th. Taxonomic cell count and biovolume results are presented in the Table S2. Taxonomic cell count results were higher than the cell number alert level for drinking water treatment plants 6.5 × 10^4 cells/mL except the last day of sampling. The bacterial and cyanobacterial community composition has been described recently in Lake Champlain during the bloom sampling period (from August 1st to 29th) using metagenomic shotgun sequencing (Fig. S1) [55]. Proteobacteria was the most abundant phylum within the whole bacterial community, with more than 70% relative abundance on August 1st. From August 13th to 29th, the major bacterial phyla were Proteobacteria, Bacteroidetes, and Actinobacteria, followed by Cyanobacteria, which accounted between 5 and 10% of the total relative abundance in all samples (Fig. S1a). The first bloom period was on August 1st to August 15th and corresponded to the dominance of *Dolichospermum* with the contribution of *Synechococcus* (Fig. S1c). The second period was on August 21st and it corresponded to the dominance of *Synechococcus* and the higher abundance of *Microcystis.* The last cyanobacteria bloom sample was taken on August 29th and coincided with *Microcystis* dominance (Fig. S1c).

In the current study, the functional profile of these samples was explored to correlate it with the taxonomic pattern described above. Fig. 1 illustrates the 29 most dominant functional categories (Level 1) based on the relative abundance of assigned reads within bloom samples retrieved from Lake Champlain from August 1st to August 29th. On August 1st, “Carbohydrates” was the most abundant functional category, followed by “Protein metabolism” and “Amino acids and derivatives”, suggesting that the microbial populations on August 1st were well adapted to degrade accessible carbon substrates such as soluble carbohydrates or polysaccharides and amino acid derivatives. On August 13th, the most relative abundant reads shifted to the “Protein metabolism” category, and it remained abundant until the end of the sampling date, on August 29th (Fig. 1). Genes related to “DNA metabolism”, “RNA metabolism”, “Phage, Prophage Transposable elements, Plasmids”, “Photosynthesis” and “Secondary metabolism” also showed slightly higher abundance during the period of August 13th-29th in comparison with August 1st. Interestingly, an opposite trend has been observed for “Membrane transport”, “Metabolism of aromatic compounds” and “Motility and chemotaxis”, “Iron acquisition and metabolism”, “Sulfur metabolism”, “Cofactors, Vitamins”, “Prosthetic groups, Pigments”, “Potassium metabolism” “Stress response” categories. Their relative abundance were lower on August 13th-29th compared to those on August 1st. Song et al. (2017) used functional metagenome analysis of natural soils in laboratory microcosms to characterize different ecological strategies adopted by the microbial community [65]. Gong et al., (2017) showed that nutrient acquisition systems were highly upregulated during cyanobacterial bloom conditions [66]. Building on their findings, we suggest that the conditions of the August 1st bloom were associated with lower nutrient availability and early successional stage (bacteria developing early and ready to use available resources) due to the higher abundance of genes involved in the regulation of amino acids, carbohydrates, sulfur, iron acquisition, potassium and aromatic compounds metabolisms. The period of August

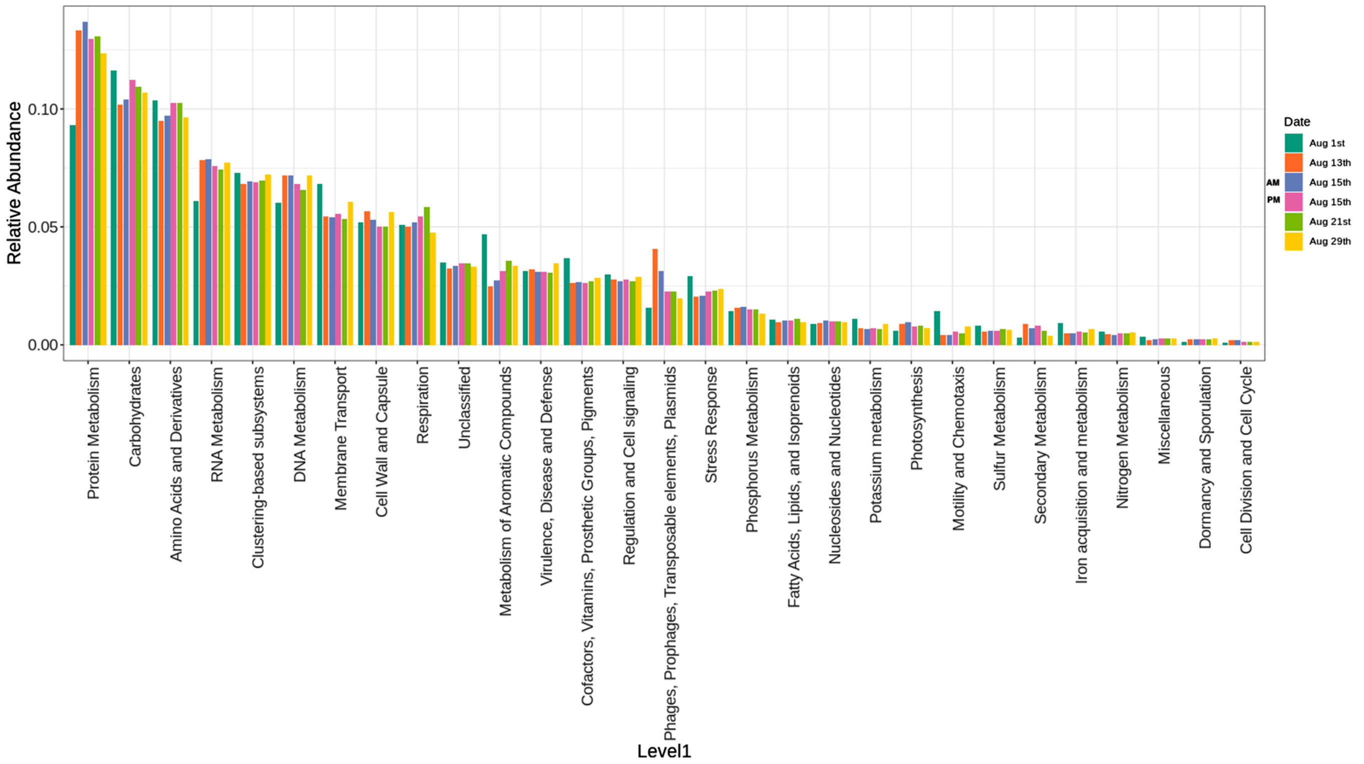

Fig. 1. Functional profiles of the cyanobacterial bloom samples collected at the intake of the drinking water production plant in Missisquoi Bay, Lake Champlain during the month of August 2018.

13th-29th was more associated with a late successional stage (sustained crowding, intense competition for resources and replication/repair) since "Photosynthesis, "Protein metabolism" and "DNA metabolism" categories were relatively more abundant.

Interestingly, genes belonging to the "Phage, Prophage Transposable elements, Plasmids" subsystem were much more abundant (on August 13th), indicating that the bloom community structure on August 13th −29th acquired more genome plasticity (Fig. 1). It has been reported that the activity of transposable elements in bacteria generates genome plasticity, leading to genetic variability and subsequently, strong fitness benefit to environmental changes [67]. For cyanobacteria, genome plasticity is an important adaptive driver of evolution, unveiling the means of their persistence [29, 68]. The taxonomic profile at the end of the bloom period exhibited higher diversity, where *Proteobacteria, Actinobacteria* and *Bacteroidetes* shared the bacterial dominance (Fig. S1-a). Song et al. (2017) [65] suggested that the high taxonomic diversity was associated with more functional diversity, which may be relevant to the overall bacterial adaptation strategies.

3.2. *Oxidation impact on the bacterial functional response*

The functional profile dynamics observed in bloom samples (before oxidation) led us to hypothesize that these bacterial communities may have different functional responses to chemical oxidation. This

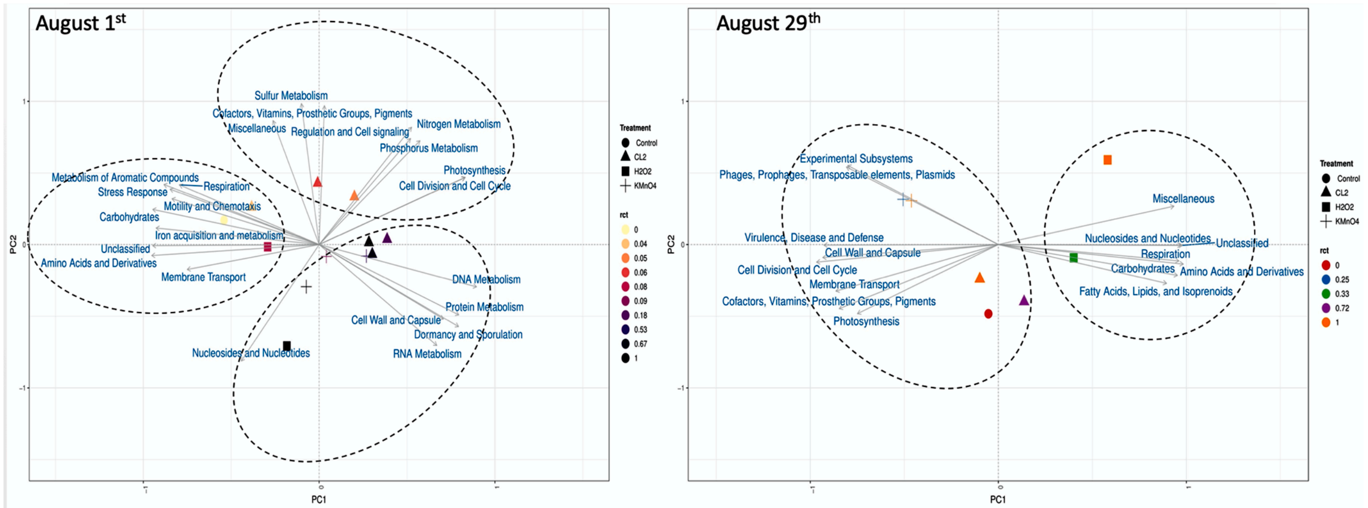

Fig. 2. PCA of the functional structure following Cl_2, $KMnO_4$ and H_2O_2 a) August 1st (Dolichospermum was the most abundant genus) b) August 29th (Microcystis was the most abundant genus).

hypothesis was investigated by comparing the functional profile, upon oxidation of bloom samples retrieved on two different days (August 1st and 29th).

To assess the impact of chemical oxidation on the community functional dynamics, principal component analysis (PCA) was conducted on cyanobacterial bloom samples before and after oxidation (Fig. 2).' The samples were those of August 1st, corresponding to *Proteobacteria* as the most abundant phyla (accounting 70% of relative abundance), and of August 29th, corresponding to *Proteobacteria, Bacteroidetes* followed by *Actinobacteria* as the most abundant phyla (Fig. S1-a). On both sampling dates, cyanobacteria accounted for 5% of the relative abundance, but exhibited different composition structure; *Dolichospermum* dominated the August 1st sample, while *Microcystis* dominated on August 29th (Fig. S1-c). Exposure to oxidant was expressed as relative CT (rCT), a normalized oxidant exposure (concentration, contact time) that was used to compare the impact of the three oxidants (Cl_2, $KMnO_4$ and H_2O_2). To compare the effects of different oxidants simultaneously, the common exposure unit (CT) is not representative due to different mechanism and mode of action of each oxidant. Normalized oxidant exposure (relative CT) is used to compare different oxidants in PCA (rCT of each oxidant; max $CT = 1$ and min $CT = 0$, the exposures in between were calculated accordingly). More details can be found in Moradinejad et al. (2020) [55].

As presented by the PCA, the differential functional structure of August 1st and August 29th bloom samples were associated with distinct microbial functional profiles after oxidation, signifying variability in microbial community fitness toward oxidation [69]. For the August 1st bloom sample, three cluster groups were detected based on the similarity of the functional composition before and after oxidation (closeness in PCA). The first group, which corresponds to control conditions and low relative CT of both Cl_2 and H_2O_2 exposure, clustered with "Metabolism of aromatic compounds", "Respiration", "Stress response", "Carbohydrates", "Iron acquisition and metabolism", "Amino acids and derivatives", "Motility and Chemotaxis" and "Membrane transport" related gene categories. The low relative CT (Cl_2 and H_2O_2) did not cause a change in the relative abundance of the genes associated with acquiring and sequestering nutrient sources such as carbohydrates. The second cluster emerged with increasing exposure to Cl_2 and was associated with the functional categories: "Sulfur metabolism", "cofactors vitamin", "Miscellaneous", "Regulation and cell signaling ", "Phosphorous metabolism ", "Nitrogen metabolism ", "Prosthetic group, Pigments". These gene-related categories correspond to the pattern of bacterial adaptation for survival in aquatic systems, encompassing various regulation and cell signaling pathways for nutrient acquisition [65].

The third group included the relatively high CT of Cl_2, $KMnO_4$ and H_2O_2 exposures, and positively correlated with "Protein, DNA and RNA metabolisms", "Dormancy and sporulation", "Cell wall and capsule" and "Nucleosides and Nucleotides" related genes (Fig. 2a). This suggests that bacterial persistence after high oxidant exposure (CT) was associated with the presence of stress response genes. Furthermore, the association of H_2O_2 with the "Nucleosides and Nucleotides" suggests a specific mode of action when compared to Cl_2 and $KMnO_4$, reflecting the differences in reactivity towards to nucleic acids and other key membrane components [70]. In the present study, the surviving bacterial community also showed a strong association with dormancy and sporulation genes compared to other genes.

To better understand the shifts within the functional gene categories following oxidation for August 1st and August 29th samples, the relative CT-related changes in the functional metagenome effect is shown for each oxidant separately in Fig. S2, S3 and S4. For August 1st, "Carbohydrates", "Amino acids and derivatives" decreased with the increase of CT of Cl_2, $KMnO_4$ and H_2O_2 exposures (compared to the control), suggesting that genes involved in energy metabolism were relatively less abundant. The relative abundance of the genes related to "Protein metabolism, "RNA metabolism", "DNA metabolism", "Cell wall and capsule" increased with increasing the exposure of Cl_2, $KMnO_4$ and H_2O_2. These functional profiles (August 1st) are in agreement with several studies showing that bacteria must synthesize stress response-related factors or metabolize excess nutrients in the environment to survive from harsh conditions imposed on the ecosystem [71, 72]. This means that bacteria must increase protein synthesis and, in consequence, increase RNA and DNA synthesis [73, 74]. Our results demonstrated that metagenomes have more abundant housekeeping genes (protein, DNA and RNA metabolisms) at a high CT of Cl_2, $KMnO_4$ and H_2O_2 compared to the more permissive oxidation strategies (relatively mid/low CT).

The PCA of functional response following oxidation on August 29th showed different functional profiles compared to August 1st (Fig. 2b). Some of the functions such as "Nitrogen metabolism", "DNA and RNA metabolism" were not identified in the PCA analysis (results of permutation test) following oxidation on the August 29th sample. We distinguished two cluster groups in the PCA of August 29th. The first cluster group encompassed control condition and the low relative CT of Cl_2 oxidation. The cluster was associated with "Membrane transport", "Cell wall and capsule", "Photosynthesis" and "Cell division and cell cycle" categories. Also, both $KMnO_4$ exposures (CT) were closely grouped and showed a strong association with the genes related to the "Prophage, transposable elements, plasmids" (Fig. 2b). The second cluster group includes H_2O_2 oxidized samples and functional categories related to "Carbohydrates", "Amino acids and derivatives", "Nucleosides and Nucleotides", "Fatty acids, lipids and Isoprenoids", "Miscellaneous, and "Respiration". Again, exposure to H_2O_2 resulted in distinct shifts in the functional related gene categories supporting the hypothesis of non-selective action of this oxidant [55].

According to increasing relative CTs, the changes in functional profiles revealed no general trends between the control conditions and the oxidized samples on August 29th (Fig. S2, S3 and S4). The August 29th bacterial communities were more resilient to oxidation with Cl_2, $KMnO_4$ and H_2O_2 and demonstrated capabilities for active nutrient uptake, growth and survival to the stressful conditions in comparison with the communities inhabiting the August 1st bloom. This is coherent with our observations of a lower Shannon index on August 1st compared to the August 29th (Fig. S5).

3.3. *Correlation between functional subsystems and microbial taxonomic profile following chlorination*

The correlation analysis was performed between functional categories (Level 1) and the taxonomic profile identified at the phylum level (~95% of the relative abundance). The correlation of different phyla with the five days of bloom samples' functional profiles before oxidation is presented in Fig. S6. This Figure shows that cyanobacteria had a significant positive correlation with "Cell division and cell cycle" in the bloom samples ($R = 0.9$). Notably, there were no correlation with "Photosynthesis", "Nitrogen" and "Phosphorus" metabolisms. Photosynthesis was driven mainly by cyanobacteria as the relative abundance of only detected photosynthetic phytoplankton, Chlorophyta, was negligible with less percentage reads ($< 10^{-5}$%).

Correlations were also evaluated between functional and taxonomic profiles observed in August 1st water samples after oxidation with Cl_2, (Fig. 3). On that date, significant positive correlations were identified between Proteobacteria and "Carbohydrates" ($R = 0.92$), "Iron acquisition and metabolism" ($R = 0.95$), "Metabolism of aromatic compounds" ($R = 0.93$), "Motility and chemotaxis" ($R = 0.95$), and "Stress response"($R = 0.94$) functions. The results indicated that members of the Proteobacteria phylum had the potential to adapt to the conditions imposed by chemical oxidation and the ability for energy acquisition (e.g. carbohydrate, iron) or usage (e.g. motility), as indicated by the abundance of reads related to nutrient and energy metabolisms. Strong support of this finding was also provided by the differential heat trees (nonparametric test where it applies the Wilcoxon rank-sum test to find

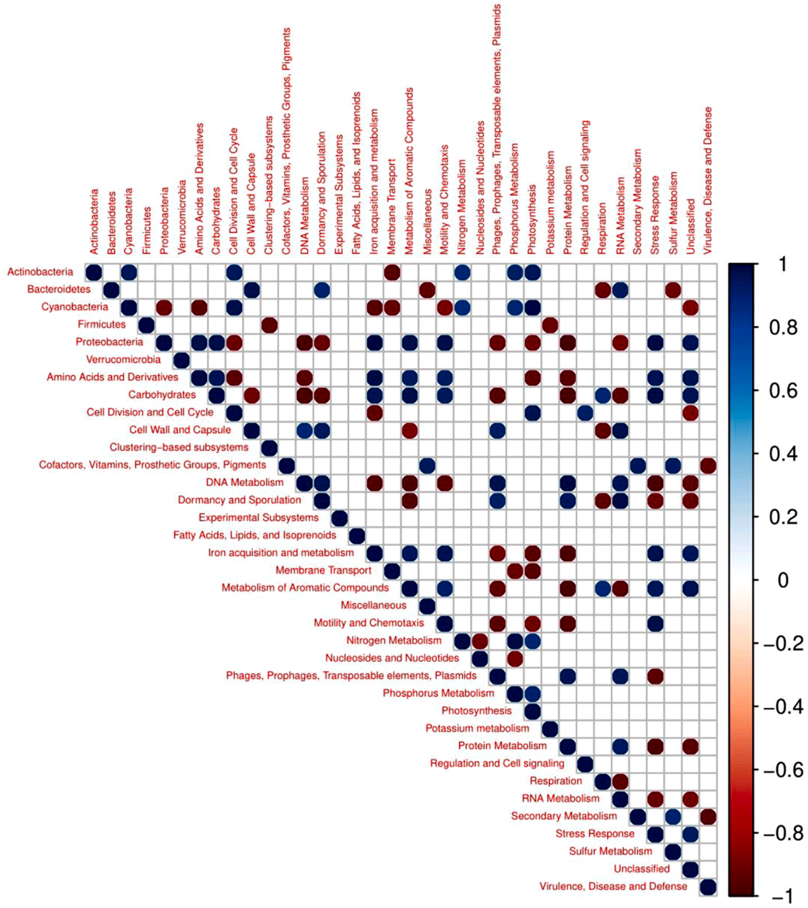

Fig. 3. Analysis of correlations between functional and taxonomic profiles identified in the August 1st 2018 water samples after oxidation with chlorine (Cl_2). Colors represent the strength of correlations with their corresponding correlation coefficients.

differences by comparison of abundances) using MetacodeR (Fig. 4), showing the effect of Cl_2 on class abundance. *Gammaproteobacteria* was the major class within *Proteobacteria* that persisted after oxidation with Cl_2 (Fig. 4). *Gammaproteobacteria* was shown in other studies to be able to win the competition for nutrients and to grow faster than the average bacterioplankton in lakes [75, 76]. Moreover, it has been often reported that iron can promote rapid growth of bacteria [77]. In our study, the genes involved in iron acquisition and metabolism may have been triggered to supply enough iron for continued bacterial growth in the August 1st samples after exposure to Cl_2.

Significant positive correlations were identified between *Bacteroidetes* and functional categories affiliated to “Dormancy and sporulation” ($R = 0.92$), “Cell wall and capsule” ($R = 0.8$) and housekeeping pathways such “RNA metabolism” ($R = 0.85$) (Fig. 3). *Bacteroidetes* was able to survive in a dormant stage after Cl_2 oxidation. Other studies have revealed that the. synthesis of stress response-associated functions or reorganization of the gene expression programs for survival under similar imposed oxidation may increase protein metabolism, thereby increasing RNA synthesis [73, 74].

Significant positive correlations between cyanobacteria and functional categories related to “Cell division and cell cycle” ($R = 0.95$), “Photosynthesis” ($R = 0.9$), Nitrogen metabolism” ($R = 0.7$), “Phosphorous metabolism” ($R = 0.7$) were also observed (Fig. 3). The ability of cyanobacteria to adapt to the presence of Cl_2 was revealed through the presence of genes involved in “Cell division and cell cycle” before and after oxidation., This ability became apparent on August 1st, because members belonging to the Cyanobacteria class persisted after oxidation with Cl_2 as compared to the *Betaproteobacteria* and *Alphaproteobacteria* (Fig. 4). Positive correlations between “Photosynthesis” related genes were identified with both *Actinobacteria* and Cyanobacteria. It seems that oxidation had an impact on Cyanobacteria to acquire energy by degrading accessible amino acids substrates and performing its associated pathways related to “Iron acquisition and metabolism” as well as” Membrane transport”.

A significant negative correlation between cyanobacterial and proteobacterial communities was identified on August 1st after chlorination. Previous studies have reported Proteobacteria’s antagonistic activity against various Cyanobacteria [78]. *Rhizobiales* belonging to the *Alphaproteobacteria* phylum have been recognized to contribute to the nitrogen cycle through their contribution to the fixation of atmospheric nitrogen by plants [79]. In our study, the relative abundance of *Alphaproteobacteria* dramatically declined after oxidation (Fig. 4). Meanwhile, Cyanobacteria, which is dominated by nitrogen-fixing cyanobacteria such as *Dolichospermum*, exhibited higher relative abundance after oxidation with Cl_2 (Fig. 4)., This suggests that cyanobacteria harboring nitrogen-related genes may be better adapted to chemical oxidation and that it involved a stress response mechanism during Cl_2 exposure.

3.4. Oxidation impact on selected cyanobacterial biomarkers

In order to track the functional cyanobacteria footsteps in response to oxidation stress, three cyanobacterial biomarkers (level 3 subsystem) where selected: “Cyanobacterial circadian clock”, “Heterocyst formation in Cyanobacteria” and “Transcription factors cyanobacterial *rpoD*-Like sigma factors”, (referred in this paper as *rpoD*-Like). Cyanobacterial circadian clock and Heterocyst formation are two well documented cyanobacterial biomarkers [44, 80-82]. The circadian clock controls

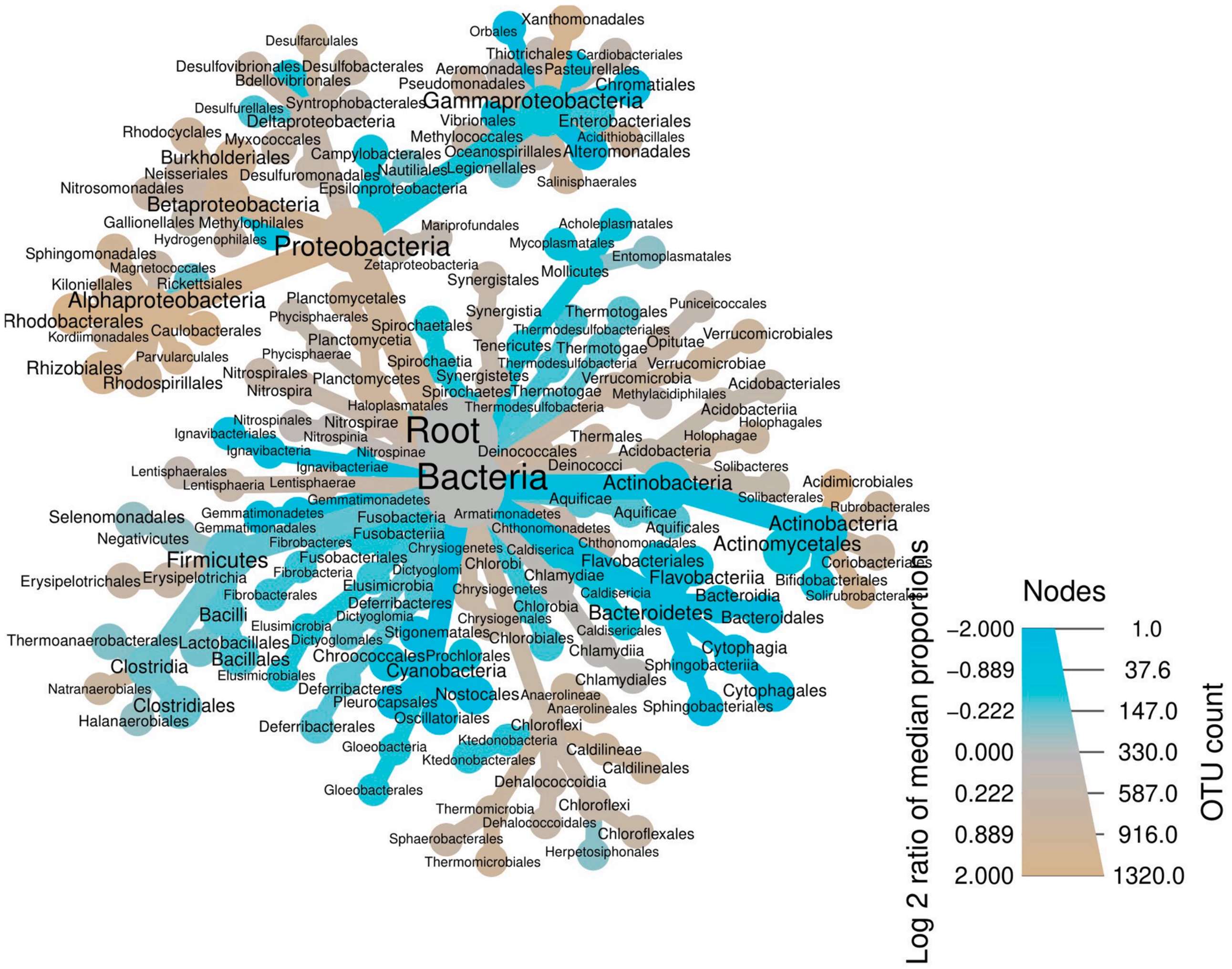

Fig. 4. Differential heat tree demonstrating changes in taxonomic profiles the class level in the August 1st 2018 water samples after oxidation with chlorine at a concentration of 0.2 mg/L. T_0/T_{10} (August 1st), T_0=control, T_{10}= after 10 min contact time. Log 2 ratio of median proportions of abundances.

gene expression at the genome-scale and plays a role in stress response and adaptability in competitive environments [41]. Heterocyst formation have been associated with a wide range of roles in cyanobacteria, such as nitrogen regulation (especially during N-limited condition), cell cycle, cell size and cell division regulation [80, 82]. The *rpoD*-Like factor was previously identified as a biomarker and a regulator to acclimate cyanobacteria and protect them during chemical oxidative stress [83]. Other studies have shown that mutated *Synechocystis* cells with only *rpoD* gene have higher resistance capability to (photo)oxidative stress in comparison with the standard strain [84].

On August 1st, the relative abundance of the three biomarkers increased following Cl_2 oxidation, with a peak at 0.2 mg.min/L (Fig. S7a and S8a). However, on August 29th such trends were not observed most probably because of the very low relative abundance of these biomarkers (Fig. S7b-S8b). Exposure to $KMnO_4$ on August 1st, was associated with an increase in the relative abundance of *rpoD*-like circadian clock and heterocyst formation which peaked at 49.7 and 287.8 mg. min/L, respectively (Fig. S9a-S10a). At higher exposure (CT=545.9 mg/L), the relative abundance of those biomarkers returned to the initial level. A slight increase was also recorded for the relative abundance of the cyanobacterial biomarkers categories on August 29th where *Microcystis* was the most abundant genus (Fig. S9b-S10b).

Oxidation with H_2O_2 on August 1st and 29th revealed that the relative abundance of the three biomarkers declined markedly at high H_2O_2 exposure (Fig. S11-S12). These results are supported by the decrease of relative abundance of cyanobacterial taxa after H_2O_2 oxidation, from our previous study [55].

3.5. ddPCR (mcyD gene) versus MC concentrations

A quick response ddPCR test could provide a tool for operators to assess potential toxicity in raw water incoming to the water treatment plant and in treated water after oxidation. To be informative, the test should be predictive of toxin concentration in raw water and conservatively predict the elimination of toxins by oxidation. Correlation analyses (p-value<0.05) for the *mcyD* gene copy numbers, assessed by ddPCR, and MC total concentrations in Lake Champlain are presented in Fig. 5. Results for dissolved and cell-bound total MC before and after oxidation are summarized in Fig. S13. For all oxidants (Cl_2, $KMnO_4$, H_2O_2), only partial removal of microcystins was observed ranging from 18% to 36%. Results for our raw water samples revealed a significant positive correlation between the *mcyD* gene copy numbers and extracellular MC total ($R = 0.97$) and intracellular MC total ($R = 0.95$). Also, a significant positive correlation ($R = 0.95$) between the *mcyD* gene copies and the intracellular MC-LR was observed for the raw water samples. Our results are in accordance with previous results in Lake Champlain, in which the *mcyD* gene copies assessed by qPCR were significantly correlated ($R = 0.93$) with the measured MC via enzyme-linked immunosorbent assay (ELISA) [37]. Similar correlation between the *mcyD* gene and MCs were also reported on other sites [51, 52, 85]. Other

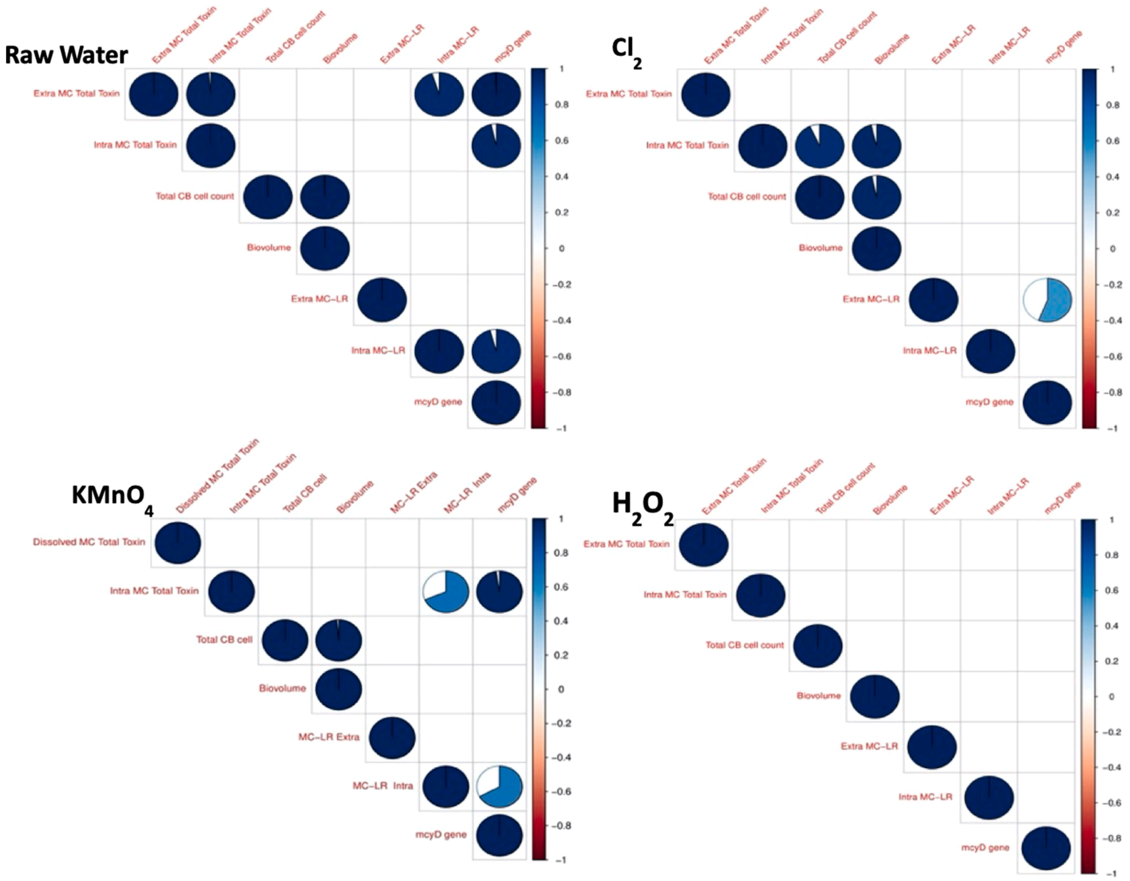

Fig. 5. Correlation analyses of the of mcyD gene copy numbers with microcystin concentrations before and after oxidation with Cl_2, $KMnO_4$ and H_2O_2. Colors represent the strength (blue-positive, red-negative) of correlations with their corresponding correlation coefficients. A complete circle represents the correlation coefficient equals one.

studies did not report any correlation between the *mcyD* gene and MC concentration [50, 86]. The complexity of *mcy* cluster sequences, that could be partially deleted, rearranged or mutated [87], and challenges associated with DNA recovery from complex samples [88] can be issues for ddPCR analyses.

The correlation of *mcyD* gene with the detected MC was also analyzed for the oxidized samples from Lake Champlain. For chlorinated samples, a significant positive correlation ($R = 0.6$) was observed between *mcyD* gene copies and the extracellular MC-LR variant. The KMnO4 oxidant was associated with stronger correlations between the *mcyD* gene copy numbers and total intracellular MC ($R = 0.98$) as well as the intracellular MC-LR variant ($R = 0.65$) (Fig. 5). As for H_2O_2, no correlation was identified between the *mcyD* gene copies and MC concentrations. Several factors may explain these different trends in correlations. First, the mode of oxidation and reactivity of the three oxidants studied varies in terms of their impact on cell components, including nucleic acids, and toxins. Secondly, detection by ddPCR relies on the ability to detect nucleic acids while the measurements of toxins relies on the ability to detect functional groups of various analogs.

Several prior studies have documented the high reactivity of microcystin with $KMnO_4$, Cl_2 and H_2O_2 [16, 17, 20, 89]. Yet, in our study, limited removal (up to 36%) of microcystin was observed at the highest CTs (Fig. S13). However, it must be noted that relatively low concentrations of toxins were present raising potential issues with the quantification of cell-bound and free toxins. This apparent low treatment performance could be related to different water quality parameters such as lower organic matter and microcystin concentrations as compared to the previous studies.

While the reactivity of toxins with the oxidants used has been widely investigated, the reactivity of Cl_2 and $KMnO_4$ with RNA integrity is not well documented. RNA is composed of ribonucleotides (ribose and one of four nitrogenous bases) linked by phosphodiester bonds. While the oxidants react poorly with ribose, they may react readily with nucleosides [90]. Although chlorine reacts slowly with Cytidine-5′-Monophosphate (CMP) (K=negligible) and Adenosine-5′-monophosphate (AMP) ($K = 66\ M^{-1}S^{-1}$), it quickly reacts with Guanosine-5′-monophosphate (GMP) ($K = 2.1 \times 10^4\ M^{-1}S^{-1}$).) and Uridine-5′-monophosphate (UMP) ($K = 5.5 \times 10^3\ M^{-1}S^{-1}$) [90]. Fig. 6 shows the impact of oxidation on the integrity of RNA extracted from a mix of cultured *Microcystis Aeruginosa* strain CPCC 300 and *Dolichospermum* strain CPCC 544. RNA was poorly degraded after exposure to $KMnO_4$ ($K = 1.8 \times 10^{-3}\ M^{-1}S^{-1}$), but Cl_2 oxidized RNA at all CTs ($K = 9.6 \times 10^{-1}\ M^{-1}S^{-1}$). These results are coherent with the recent demonstration of the high reactivity of cytosine and thymine bases with chlorine showing nearly complete oxidation at 20 μM Cl_2 [91]. Our results shows that oxidation constants (especially $KMnO_4$) for nucleic acids (RNA) is lower than documented rate constants for MC concertation (118 $M^{-1}S^{-1}$ for the most resistant MC variant) [92] which may explain different trends in the correlations (*mcyD* vs MC) after oxidation. Although not tested, H_2O_2 is known to be highly reactive with all cell components and to degrade RNases, a family of enzymes present in virtually all living cells [93]. H_2O_2 has also been shown to play a role in the degradation of bacterial DNA [94-96]. Therefore, some oxidants are likely to rapidly react free nucleic acids while others will not react at typical treatment dosages.

We attempted to correlate the presence of *mcyD* to the persistence of cell bound and free toxins for chlorine (0.2 and 0.6 mg/L), permanganate (5 mg/L) and hydrogen peroxide (10 mg/L). Because of the discrepancies in the kinetics of oxidation of RNA and toxins depending on

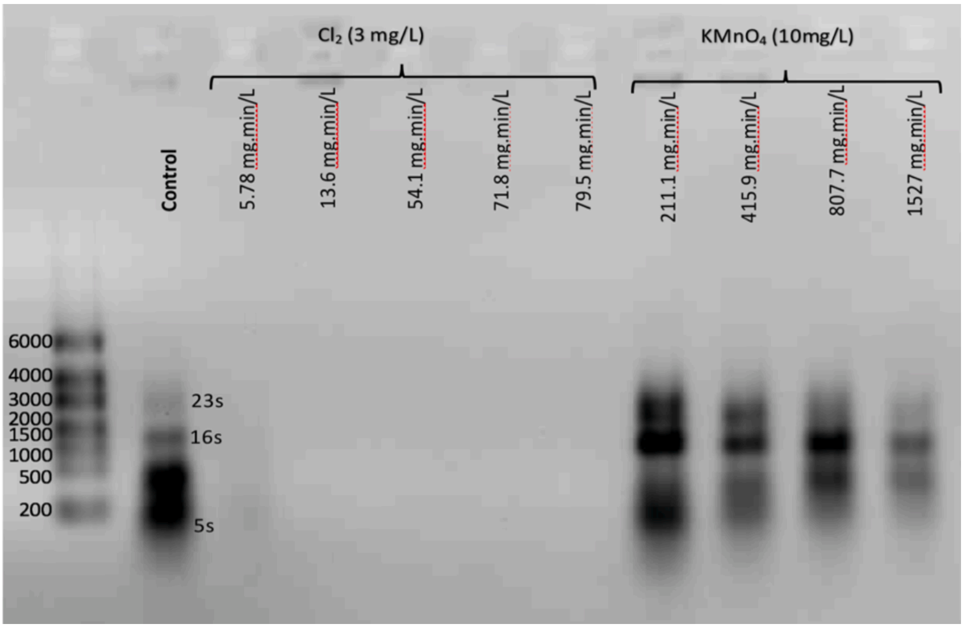

Fig. 6. Gel electrophoresis of RNA extracted from river water spiked with a mixture of Microcystis and Dolichospermum cells as a function of CT exposure to Cl_2 and $KMnO_4$.

the oxidant considered, the use of *mcyD* based toxicity test would best justified to estimate the potential for toxin presence in raw water prior to exposure to oxidant agents. The use of the ddPCR method as a quick response tool to predict toxin removal after oxidation is therefore not recommended.

Conclusion

- Distinct functional assemblages were observed in bloom samples at the intake of the drinking water production plant during the month of August (August 1st to August 29th). On August 1st, the most abundant function identified in bloom samples was associated with the “Carbohydrates” category. A shift toward “Protein metabolism” was observed on August 13th which remained abundant until the end of our sampling campaign.
- Different functional clusters were observed in our water samples following oxidation with Cl_2, $KMnO_4$ and H_2O_2. On August 1st, low oxidant exposures clustered with “Carbohydrates” and “Iron acquisition” metabolic activities and higher exposure clustered with stress response related functions such as DNA metabolism and sporulation.
- The impact of oxidation was revealed through correlation analyses between different phyla and functional profiles. Positive correlations between cyanobacteria and nitrogen as well as phosphorous metabolisms showed the impact of Cl_2 since no correlation was observed in bloom samples before oxidation. The potential of cyanobacteria to survive to Cl_2 exposure was also assessed through a positive correlation with cell division and cell cycle.
- Limited variation of cyanobacterial biomarkers such as “Cyanobacterial circadian clock”, “Heterocyst formation in Cyanobacteria” and “*rpoD*-Like” was identified following Cl_2 and $KMnO_4$ oxidation on August 29th where *Microcystis* was the most abundant genus. High exposure to H_2O_2 caused a dramatic decrease in cyanobacterial biomarkers, regardless of the dominant genus. Those results confirmed the effectiveness of H_2O_2 as a pre-oxidant in cyanobacterial management of drinking water resources.
- Quick response ddPCR tests could be used to evaluate cyanobacterial bloom toxicity in drinking water resources prior to the pre-oxidation treatment. Correlation results between the *mcyD* gene and microcystin concentrations following exposure to Cl_2, $KMnO_4$ and H_2O_2 oxidants suggest that the ddPCR method or any genomic based measurement would not be appropriate to predict the removal of microcystins.
- Pre-oxidation modified the bacterial community and cyanobacteria survived after exposure to Cl_2 and $KMnO_4$ as demonstrated by functional and community composition structure. However, pre-oxidation with Cl_2, $KMnO_4$ and H_2O_2 was partially efficient in eliminating microcystins, raising the need for optimizing pre-oxidation dosages and insuring that other down-flow processes are capable of removing any toxins that would ultimately be released in potable water.

Declaration of Competing Interest

The authors declare no competing financial interest.

Acknowledgments

The authors are sincerely acknowledging the financial support from the Algal Blooms, Treatment, Risk Assessment, Prediction and Prevention through Genomics (ATRAPP), Génome Québec and Génome Canada grant. The authors thank the staff at NSERC Industrial Chair on Drinking Water at Polytechnique Montréal, Shapiro lab, Sung Vo Duy (University of Montréal), Irina Moukhina (Université du Québec à Montréal), Sukriye Aydin Celikkol (McGill University) and Stephanie Messina Pacheco (National Research Council) for their valuable contributions to this research.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ceja.2021.100151.

References

[1] M.L. Wells, V.L. Trainer, T.J. Smayda, B.S.O. Karlson, C.G. Trick, R.M. Kudela, A. Ishikawa, S. Bernard, A. Wulff, D.M. Anderson, et al., Harmful algal blooms and climate change: learning from the past and present to forecast the future, Harmful Algae 49 (2015) 68–93, https://doi.org/10.1016/j.hal.2015.07.009.

[2] H.W. Paerl, V.J. Paul, Climate change: links to global expansion of harmful cyanobacteria, Water Res. 46 (2012) 1349–1363, https://doi.org/10.1016/j.watres.2011.08.002.

[3] J.A. Westrick, D.C. Szlag, B.J. Southwell, J. Sinclair, A review of cyanobacteria and cyanotoxins removal/inactivation in drinking water treatment, Anal. Bioanal. Chem. 397 (2010) 1705–1714, https://doi.org/10.1007/s00216-010-3709-5.

[4] M.A. Al-Sammak, K.D. Hoagland, D. Cassada, D.D. Snow, Co-occurrence of the cyanotoxins BMAA, DABA and anatoxin-a in Nebraska reservoirs, fish, and aquatic plants, Toxins (Basel) 6 (2014) 488–508, https://doi.org/10.3390/toxins6020488.

[5] M.C. Casero, D. Velázquez, M. Medina-Cobo, A. Quesada, S. Cirés, Unmasking the identity of toxigenic cyanobacteria driving a multi-toxin bloom by high-throughput sequencing of cyanotoxins genes and 16S rRNA metabarcoding, Sci. Total Environ. 665 (2019) 367–378, https://doi.org/10.1016/j.scitotenv.2019.02.083.

[6] L.C. Backer, J.H. Landsberg, M. Miller, K. Keel, T.K. Taylor, Canine cyanotoxin poisonings in the United States (1920s–2012): review of suspected and confirmed cases from three data sources, Toxins (Basel) 5 (2013) 1597–1628.

[7] P.A. Bukaveckas, J. Lesutienė, Z.R. Gasiūnaitė, L. Ložys, I. Olenina, R. Pilkaitytė, Ž. Pūtys, S. Tassone, J. Wood, Microcystin in aquatic food webs of the Baltic and Chesapeake Bay regions, Estuar. Coast. Shelf Sci. 191 (2017) 50–59.

[8] E.C. Wert, J.A. Korak, R.A. Trenholm, F.L. Rosario-Ortiz, Effect of oxidant exposure on the release of intracellular microcystin, MIB, and geosmin from three cyanobacteria species, Water Res. 52 (2014) 251–259, https://doi.org/10.1016/j.watres.2013.11.001.

[9] W. Yao, Q. Qu, U. von Gunten, C. Chen, G. Yu, Y. Wang, Comparison of methylisoborneol and geosmin abatement in surface water by conventional ozonation and an electro-peroxone process, Water Res. 108 (2017) 373–382, https://doi.org/10.1016/j.watres.2016.11.014.

[10] A. Zamyadi, R. Henderson, R. Stuetz, R. Hofmann, L. Ho, G. Newcombe, Fate of geosmin and 2-methylisoborneol in full-scale water treatment plants, Water Res. 83 (2015) 171–183, https://doi.org/10.1016/j.watres.2015.06.038.

[11] A. Zamyadi, S. MacLeod, Y. Fan, N. McQuaid, S. Dorner, S. Sauvé, M. Prévost, Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: a monitoring and treatment challenge, Water Res. 46 (2012) 1511–1523, https://doi.org/10.1016/j.watres.2011.11.012.

[12] P. Pazouki, M. Prévost, N. McQuaid, B. Barbeau, M.-L. de Boutray, A. Zamyadi, S. Dorner, Breakthrough of cyanobacteria in bank filtration, Water Res. 102 (2016) 170–179, https://doi.org/10.1016/j.watres.2016.06.037.

[13] A. Zamyadi, S. Dorner, M. Ndong, D. Ellis, A. Bolduc, C. Bastien, M. Prévost, Low-risk cyanobacterial bloom sources: cell accumulation within full-scale treatment plants, J. Am. Water Works Assn. 102 (2013) E651–E663, https://doi.org/10.5942/jawwa.2013.105.0141.

[14] A. Zamyadi, L.A. Coral, B. Barbeau, M. Prévost, Pre-ozonation a solution to prevent toxic cyanobacteria cell breakthrough into water treatment plants, in: Proceedings of American Water Works Association-Water Quality Technology Conference and Exposition (WQTC), Long Beach, CA, USA, 2021, p. 22. Nov 3-7.

[15] H.C.P. Matthijs, P.M. Visser, B. Reeze, J. Meeuse, P.C. Slot, G. Wijn, R. Talens, J. Huisman, Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide, Water Res. 46 (2012) 1460–1472, https://doi.org/10.1016/j.watres.2011.11.016.

[16] S. Zhou, Y. Shao, N. Gao, Y. Deng, J. Qiao, H. Ou, J. Deng, Effects of different algaecides on the photosynthetic capacity, cell integrity and microcystin-LR release of *Microcystis aeruginosa*, Sci. Total Environ. 463 (2013) 111–119, https://doi.org/10.1016/j.scitotenv.2013.05.064.

[17] J. Fan, R. Daly, P. Hobson, L. Ho, J. Brookes, Impact of potassium permanganate on cyanobacterial cell integrity and toxin release and degradation, Chemosphere 92 (2013) 529–534, https://doi.org/10.1016/j.chemosphere.2013.03.022.

[18] J. Fan, L. Ho, P. Hobson, J. Brookes, Evaluating the effectiveness of copper sulphate, chlorine, potassium permanganate, hydrogen peroxide and ozone on cyanobacterial cell integrity, Water Res. 47 (2013) 5153–5164, https://doi.org/10.1016/j.watres.2013.05.057.

[19] J. Naceradska, M. Pivokonsky, L. Pivokonska, M. Baresova, R.K. Henderson, A. Zamyadi, V. Janda, The impact of pre-oxidation with potassium permanganate on cyanobacterial organic matter removal by coagulation, Water Res. 114 (2017) 42–49, https://doi.org/10.1016/j.watres.2017.02.029.

[20] A. Zamyadi, Y. Fan, R.I. Daly, M. Prévost, Chlorination of *Microcystis aeruginosa*: toxin release and oxidation, cellular chlorine demand and disinfection by-products formation, Water Res. 47 (2013) 1080–1090, https://doi.org/10.1016/j.watres.2012.11.031.

[21] A. Zamyadi, L.A. Coral, B. Barbeau, S. Dorner, F.R. Lapolli, M. Prévost, Fate of toxic cyanobacterial genera from natural bloom events during ozonation, Water Res. 73 (2015) 204–215, https://doi.org/10.1016/j.watres.2015.01.029.

[22] X. He, E.C. Wert, Colonial cell disaggregation and intracellular microcystin release following chlorination of naturally occurring Microcystis, Water Res. 101 (2016) 10–16, https://doi.org/10.1016/j.watres.2016.05.057.

[23] E.C. Wert, M.M. Dong, F.L. Rosario-Ortiz, Using digital flow cytometry to assess the degradation of three cyanobacteria species after oxidation processes, Water Res. 47 (2013) 3752–3761, https://doi.org/10.1016/j.watres.2013.04.038.

[24] S. Merel, D. Walker, R. Chicana, S. Snyder, E. Baures, O. Thomas, State of knowledge and concerns on cyanobacterial blooms and cyanotoxins, Environ. Int. 59 (2013) 303–327, https://doi.org/10.1016/j.envint.2013.06.013.

[25] K.H. Kim, Y. Yoon, W.-.Y. Hong, J. Kim, Y.-.C. Cho, S.-.J. Hwang, Application of metagenome analysis to characterize the molecular diversity and saxitoxin-producing potentials of a cyanobacterial community: a case study in the North Han River, Korea, Appl. Biol. Chem. 61 (2018) 153–161, https://doi.org/10.1007/s13765-017-0342-4.

[26] S.L.C. Eldridge, T.M. Wood, Annual variations in microcystin occurrence in Upper Klamath Lake, Oregon, based on high-throughput DNA sequencing, qPCR, and environmental parameters, Lake Reservoir Manag. (2019) 1–14, https://doi.org/10.1080/10402381.2019.1619112.

[27] M.C. Casero, A. Ballot, R. Agha, A. Quesada, S. Cirés, Characterization of saxitoxin production and release and phylogeny of *sxt* genes in paralytic shellfish poisoning toxin-producing *Aphanizomenon gracile*, Harmful Algae 37 (2014) 28–37, https://doi.org/10.1016/j.hal.2014.05.006.

[28] t I.S. Pessi, C. Maalouf Pde, H.D. Laughinghouse, D. Baurain, A. Wilmotte, On the use of high-throughput sequencing for the study of cyanobacterial diversity in Antarctic aquatic mats, J. Phycol. 52 (2016) 356–368, https://doi.org/10.1111/jpy.12399.

[29] A. Willis, J.N. Woodhouse, Defining cyanobacterial species: diversity and description through genomics, Crit. Rev. Plant Sci. (2020) 1–24.

[30] H. Xu, H. Pei, Y. Jin, C. Ma, Y. Wang, J. Sun, H. Li, High-throughput sequencing reveals microbial communities in drinking water treatment sludge from six geographically distributed plants, including potentially toxic cyanobacteria and pathogens, Sci. Total Environ. 634 (2018) 769–779, https://doi.org/10.1016/j.scitotenv.2018.04.008.

[31] M.W. Lusty, C.J. Gobler, The efficacy of hydrogen peroxide in mitigating cyanobacterial blooms and altering microbial communities across four lakes in NY, USA, Toxins (Basel) 12 (2020) 428, https://doi.org/10.3390/toxins12070428.

[32] M.Á. Lezcano, D. Velázquez, A. Quesada, R. El-Shehawy, Diversity and temporal shifts of the bacterial community associated with a toxic cyanobacterial bloom: an interplay between microcystin producers and degraders, Water Res. 125 (2017) 52–61, https://doi.org/10.1016/j.watres.2017.08.025.

[33] M.A. Berry, T.W. Davis, R.M. Cory, M.B. Duhaime, T.H. Johengen, G.W. Kling, J. A. Marino, P.A. Den Uyl, D. Gossiaux, G.J. Dick, et al., Cyanobacterial harmful algal blooms are a biological disturbance to Western Lake Erie bacterial communities, Environ. Microbiol. 19 (2017) 1149–1162, https://doi.org/10.1111/1462-2920.13640.

[34] J.N. Woodhouse, A.S. Kinsela, R.N. Collins, L.C. Bowling, G.L. Honeyman, J. K. Holliday, B.A. Neilan, Microbial communities reflect temporal changes in cyanobacterial composition in a shallow ephemeral freshwater lake, ISME J. 10 (2015) 1337, https://doi.org/10.1038/ismej.2015.218.

[35] H. Pei, H. Xu, J. Wang, Y. Jin, H. Xiao, C. Ma, J. Sun, H. Li, 16S rRNA Gene amplicon sequencing reveals significant changes in microbial compositions during cyanobacteria-laden drinking water sludge storage, Environ. Sci. Technol. 51 (2017) 12774–12783, https://doi.org/10.1021/acs.est.7b03085.

[36] P.I. Scherer, A.D. Millard, A. Miller, R. Schoen, U. Raeder, J. Geist, K. Zwirglmaier, Temporal dynamics of the microbial community composition with a focus on toxic cyanobacteria and toxin presence during harmful algal blooms in two South German lakes, Front. Microbiol. 8 (2017) 2387, https://doi.org/10.3389/fmicb.2017.02387.

[37] N. Fortin, R. Aranda-Rodriguez, H. Jing, F. Pick, D. Bird, C.W. Greer, Detection of microcystin-producing cyanobacteria in Missisquoi Bay, Quebec, Canada, using quantitative PCR, Appl. Environ. Microbiol. 76 (2010) 5105–5112.

[38] N. Fortin, V. Munoz-Ramos, D. Bird, B. Levesque, L.G. Whyte, C.W. Greer, Toxic cyanobacterial bloom triggers in Missisquoi bay, lake Champlain, as determined by next-generation sequencing and quantitative PCR, Life (Basel) 5 (2015) 1346–1380, https://doi.org/10.3390/life5021346.

[39] C.K. Gomez-Smith, D.T. Tan, D. Shuai, Research highlights: functions of the drinking water microbiome–from treatment to tap, Environ. Sci.: Water Res. Technol. 2 (2016) 245–249.

[40] M.J. Harke, T.W. Davis, S.B. Watson, C.J. Gobler, Nutrient-controlled niche differentiation of western Lake Erie cyanobacterial populations revealed via metatranscriptomic surveys, Environ. Sci. Technol. 50 (2015) 604–615, https://doi.org/10.1021/acs.est.5b03931.

[41] C. Hörnlein, V. Confurius-Guns, M. Grego, L.J. Stal, H. Bolhuis, Circadian clock-controlled gene expression in co-cultured, mat-forming cyanobacteria, Sci. Rep. 10 (2020) 14095, https://doi.org/10.1038/s41598-020-69294-3.

[42] H. Li, M. Barber, J. Lu, R. Goel, Microbial community successions and their dynamic functions during harmful cyanobacterial blooms in a freshwater lake, Water Res. (2020), 116292.

[43] J. Lu, B. Zhu, I. Struewing, N. Xu, S. Duan, Nitrogen–phosphorus-associated metabolic activities during the development of a cyanobacterial bloom revealed by metatranscriptomics, Sci. Rep. 9 (2019) 2480, https://doi.org/10.1038/s41598-019-38481-2.

[44] A. Taton, C. Erikson, Y. Yang, B.E. Rubin, S.A. Rifkin, J.W. Golden, S.S. Golden, The circadian clock and darkness control natural competence in cyanobacteria, Nat. Commun. 11 (2020) 1688, https://doi.org/10.1038/s41467-020-15384-9.

[45] L. Shi, Y. Huang, M. Zhang, Y. Yu, Y. Lu, F. Kong, Bacterial community dynamics and functional variation during the long-term decomposition of cyanobacterial blooms in-vitro, Sci. Total Environ. 598 (2017) 77–86.

[46] Z. Shilei, S. Yue, H. Tinglin, C. Ya, Y. Xiao, Z. Zizhen, L. Yang, L. Zaixing, C. Jiansheng, L. Xiao, Reservoir water stratification and mixing affects microbial community structure and functional community composition in a stratified drinking reservoir, J. Environ. Manag. 267 (2020), 110456.

[47] S. Zhou, Y. Sun, M. Yu, Z. Shi, H. Zhang, R. Peng, Z. Li, J. Cui, X. Luo, Linking shifts in bacterial community composition and function with changes in the dissolved organic matter pool in ice-covered Baiyangdian Lake, Northern China, Microorganisms 8 (2020) 883.

[48] K. Wang, H. Mao, X. Li, Functional characteristics and influence factors of microbial community in sewage sludge composting with inorganic bulking agent, Bioresour. Technol. 249 (2018) 527–535.

[49] Y. Li, C. Xu, W. Zhang, L. Lin, L. Wang, L. Niu, H. Zhang, P. Wang, C. Wang, Response of bacterial community in composition and function to the various DOM at river confluences in the urban area, Water Res. 169 (2020), 115293.

[50] D.V. Baxa, T. Kurobe, K.A. Ger, P.W. Lehman, S.J. Teh, Estimating the abundance of toxic *Microcystis* in the San Francisco Estuary using quantitative real-time PCR, Harmful Algae 9 (2010) 342–349, https://doi.org/10.1016/j.hal.2010.01.001.

[51] T.W. Davis, D.L. Berry, G.L. Boyer, C.J. Gobler, The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms, Harmful Algae 8 (2009) 715–725, https://doi.org/10.1016/j.hal.2009.02.004.

[52] J.M. Rinta-Kanto, E.A. Konopko, J.M. DeBruyn, R.A. Bourbonniere, G.L. Boyer, S. W. Wilhelm, Lake Erie Microcystis: relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake, Harmful Algae 8 (2009) 665–673, https://doi.org/10.1016/j.hal.2008.12.004.

[53] A. Zamyadi, S. Dorner, D. Ellis, A. Bolduc, C. Bastien, M. Prévost, Species-dependence of *cyanobacteria* removal efficiency by different drinking water treatment processes, Water Res. 47 (2013) 2689–2700, https://doi.org/10.1016/j.watres.2013.02.040.

[54] N. McQuaid, A. Zamyadi, M. Prévost, D.F. Bird, S. Dorner, Use of in vivo phycocyanin fluorescence to monitor potential microcystin-producing cyanobacterial biovolume in a drinking water source, J. Environ. Monit. 13 (2011) 455–463, https://doi.org/10.1039/C0EM00163E.

[55] S. Moradinejad, H. Trigui, J.F. Guerra Maldonado, J. Shapiro, Y. Terrat, A. Zamyadi, S. Dorner, M. Prévost, Diversity assessment of toxic cyanobacterial blooms during oxidation, Toxins (Basel) 12 (2020) 728.

[56] Z.S. Foster, T.J. Sharpton, N.J. Grunwald, Metacoder: an R package for visualization and manipulation of community taxonomic diversity data, PLoS Comput. Biol. 13 (2017), e1005404, https://doi.org/10.1371/journal.pcbi.1005404.

[57] R. Overbeek, R. Olson, G.D. Pusch, G.J. Olsen, J.J. Davis, T. Disz, R.A. Edwards, S. Gerdes, B. Parrello, M. Shukla, The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST), Nucleic Acids Res. 42 (2014) D206–D214.

[58] M. Kanehisa, S. Goto, Y. Sato, M. Furumichi, M. Tanabe, KEGG for integration and interpretation of large-scale molecular data sets, Nucleic Acids Res. 40 (2012) D109–D114.

[59] R.L. Tatusov, M.Y. Galperin, D.A. Natale, E.V. Koonin, The COG database: a tool for genome-scale analysis of protein functions and evolution, Nucleic Acids Res. 28 (2000) 33–36.

[60] K. Pearson, Mathematical contributions to the theory of evolution.—III. Regression, heredity, and panmixia, in: Philosophical Transactions of the Royal Society of London. Series A, Containing Papers of a Mathematical or Physical Character, 1896, pp. 253–318.

[61] S. Janson, J. Vegelius, Measures of ecological association, Oecologia 49 (1981) 371–376.

[62] M.L. Calle, Statistical analysis of metagenomics data, Genomics Inform. 17 (2019).

[63] G. Munoz, S. Vo Duy, A. Roy-Lachapelle, B. Husk, S. Sauve, Analysis of individual and total microcystins in surface water by on-line preconcentration and desalting coupled to liquid chromatography tandem mass spectrometry, J. Chromatogr. 1516 (2017) 9–20, https://doi.org/10.1016/j.chroma.2017.07.096.

[64] A. Roy-Lachapelle, S.V. Duy, G. Munoz, Q.T. Dinh, E. Bahl, D.F. Simon, S. Sauvé, Analysis of multiclass cyanotoxins (microcystins, anabaenopeptins, cylindrospermopsin and anatoxins) in lake waters using on-line SPE liquid chromatography high-resolution Orbitrap mass spectrometry, Anal. Methods (2019), https://doi.org/10.1039/c9ay01132c.

[65] H.-.K. Song, W. Song, M. Kim, B.M. Tripathi, H. Kim, P. Jablonski, J.M. Adams, Bacterial strategies along nutrient and time gradients, revealed by metagenomic analysis of laboratory microcosms, FEMS Microbiol. Ecol. 93 (2017) fix114.

[66] W. Gong, J. Browne, N. Hall, D. Schruth, H. Paerl, A. Marchetti, Molecular insights into a dinoflagellate bloom, ISME J. 11 (2017) 439–452.

[67] M.M. Zambrano, D.A. Siegele, M. Almiron, A. Tormo, R. Kolter, Microbial competition: escherichia coli mutants that take over stationary phase cultures, Science 259 (1993) 1757–1760.

[68] O. Zhaxybayeva, J.P. Gogarten, R.L. Charlebois, W.F. Doolittle, R.T. Papke, Phylogenetic analyses of cyanobacterial genomes: quantification of horizontal gene transfer events, Genome Res. 16 (2006) 1099–1108.

[69] N. Sharma, J. Kumar, M.M. Abedin, D. Sahoo, A. Pandey, A.K. Rai, S.P. Singh, Metagenomics revealing molecular profiling of community structure and metabolic pathways in natural hot springs of the Sikkim Himalaya, BMC Microbiol. 20 (2020) 1–17.

[70] S. Moradinejad, C. Glover, J. Mailly, T. Zadfatollah-Seighalani, S. Peldszus, B. Barbeau, S. Dorner, M. Prévost, A. Zamyadi, Using advanced spectroscopy and organic matter characterization to evaluate the impact of oxidation on cyanobacteria, Toxins (Basel) 11 (2019), https://doi.org/10.3390/toxins11050278.

[71] J. Cheriaa, M. Rouabhia, M. Maatallah, A. Bakhrouf, Phenotypic stress response of Pseudomonas aeruginosa following culture in water microcosms, J. Water Health. 10 (2012) 130–139.

[72] B. Polek, J. Godočíková, The effect of some factors of polluted environment on catalase responses and resistance of microbial isolates against toxic oxidative stress, Curr. Microbiol. 65 (2012) 345–349.

[73] M.Z. Humayun, SOS and Mayday: multiple inducible mutagenic pathways in Escherichia coli, Mol. Microbiol. 30 (1998) 905–910.

[74] R. Krämer, Bacterial stimulus perception and signal transduction: response to osmotic stress, Chem. Rec. 10 (2010) 217–229.

[75] K. Šimek, K. Horňák, J. Jezbera, J. Nedoma, J. Vrba, V. Straškrábová, M. Macek, J. R. Dolan, M.W. Hahn, Maximum growth rates and possible life strategies of different bacterioplankton groups in relation to phosphorus availability in a freshwater reservoir, Environ. Microbiol. 8 (2006) 1613–1624.

[76] J.M. Gasol, M. Comerma, J.C. García, J. Armengol, E.O. Casamayor, P. Kojecká, K. Šimek, A transplant experiment to identify the factors controlling bacterial abundance, activity, production, and community composition in a eutrophic canyon-shaped reservoir, Limnol. Oceanogr. 47 (2002) 62–77.

[77] M.J. Church, D.A. Hutchins, H.W. Ducklow, Limitation of bacterial growth by dissolved organic matter and iron in the Southern Ocean, Appl. Environ. Microbiol. 66 (2000) 455–466.

[78] S.J.L. Wright, R.J. Thompson, Bacillus volatiles antagonize cyanobacteria, FEMS Microbiol. Lett. 30 (1985) 263–267.

[79] R.J. Newton, S.E. Jones, A. Eiler, K.D. McMahon, S. Bertilsson, A guide to the natural history of freshwater lake bacteria, Microbiol. Mol. Biol. Rev. 75 (2011) 14–49.

[80] C.C. Zhang, S. Laurent, S. Sakr, L. Peng, S. Bédu, Heterocyst differentiation and pattern formation in cyanobacteria: a chorus of signals, Mol. Microbiol. 59 (2006) 367–375.

[81] N.J. Bale, R. Hennekam, E.C. Hopmans, D. Dorhout, G.-.J. Reichart, M. van der Meer, T.A. Villareal, J.S. Sinninghe Damsté, S. Schouten, Biomarker evidence for nitrogen-fixing cyanobacterial blooms in a brackish surface layer in the Nile River plume during sapropel deposition, Geology 47 (2019) 1088–1092.

[82] W. Xing, L.-r. Xie, X. Zeng, Y. Yang, C.-.C. Zhang, Functional dissection of genes encoding DNA polymerases based on conditional mutants in the heterocyst-forming cyanobacterium anabaena PCC 7120, Front. Microbiol. 11 (2020) 1108.

[83] A. Srivastava, M.L. Summers, R. Sobotka, Cyanobacterial sigma factors: current and future applications for biotechnological advances, Biotechnol. Adv. 40 (2020), 107517.

[84] K. Hakkila, D. Valev, T. Antal, E. Tyystjärvi, T. Tyystjärvi, Group 2 sigma factors are central regulators of oxidative stress acclimation in cyanobacteria, Plant Cell Physiol. 60 (2019) 436–447.

[85] S.A. Wood, A. Rueckert, D.P. Hamilton, S.C. Cary, D.R. Dietrich, Switching toxin production on and off: intermittent microcystin synthesis in a Microcystis bloom, Environ. Microbiol. Rep. 3 (2011) 118–124, https://doi.org/10.1111/j.1758-2229.2010.00196.x.

[86] A.M. Hotto, M.F. Satchwell, D.L. Berry, C.J. Gobler, G.L. Boyer, Spatial and temporal diversity of microcystins and microcystin-producing genotypes in Oneida Lake, NY, Harmful Algae 7 (2008) 671–681, https://doi.org/10.1016/j.hal.2008.02.001.

[87] A.B. Pacheco, I.A. Guedes, S.M. Azevedo, Is qPCR a reliable indicator of cyanotoxin risk in freshwater? Toxins (Basel) 8 (2016) https://doi.org/10.3390/toxins8060172.

[88] J. Kim, J. Lim, C. Lee, Quantitative real-time PCR approaches for microbial community studies in wastewater treatment systems: applications and considerations, Biotechnol. Adv. 31 (2013) 1358–1373, https://doi.org/10.1016/j.biotechadv.2013.05.010.

[89] J. Ding, H. Shi, T. Timmons, C. Adams, Release and removal of microcystins from microcystis during oxidative-, physical-, and UV-based disinfection, J. Environ. Eng. 136 (2010) 2–11.

[90] M. Deborde, U. von Gunten, Reactions of chlorine with inorganic and organic compounds during water treatment–Kinetics and mechanisms: a critical review, Water Res. 42 (2008) 13–51.

[91] S. Zhang, T. Lin, H. Chen, W. Chen, H. Xu, H. Tao, DNA pyrimidine bases in water: insights into relative reactivity, byproducts formation and combined toxicity during chlorination, Sci. Total Environ. 717 (2020), 137205.

[92] K. Piezer, L. Li, Y. Jeon, A. Kadudula, Y. Seo, The application of potassium permanganate to treat cyanobacteria-laden water, Process Saf. Environ. Prot. (2020).

[93] R.E. Farrell, RNA Methodologies, A laboratory guide for isolation and characterization, in: R.E. Farrell (Ed.), RNA Methodologies (Fourth Edition), Academic Press, San Diego, 2010, pp. 179–219, https://doi.org/10.1016/B978-0-12-374727-3.00009-7.

[94] Q. Yang, J. Xu, X. Qian, K. Zhang, X. Lei, Eliminating nucleic acids contaminants by hydrogen peroxide-induced free radicals during the preparation of proteins, Biochem. Eng. J. 29 (2006) 23–26.

[95] J.R. Wagner, J. Cadet, Oxidation reactions of cytosine DNA components by hydroxyl radical and one-electron oxidants in aerated aqueous solutions, Acc. Chem. Res. 43 (2010) 564–571.

[96] A. Latifi, M. Ruiz, C.C. Zhang, Oxidative stress in cyanobacteria, FEMS Microbiol. Rev. 33 (2009) 258–278, https://doi.org/10.1111/j.1574-6976.2008.00134.x.